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Molecular Detection of Extended Spectrum β-lactamase genes in *Escherichia coli* Isolates from Urinary Tract Infections

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بسم الله الرحمن الرحيم

(قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا
إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة الآية(32)

Certification

I certify that this thesis entitled “**Molecular Detection of Extended Spectrum B-lactamase genes in *Escherichia coli* Isolates from Urinary Tract Infections**” was prepared by **"Fatima Abbas Nasser Hussein"** under my supervision at the Department of Biology / College of Science/ University of Babylon, as a partial requirement for the degree of Master in Microbiology.

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Dedications

Who taught me the ways of progress and I carry his name with pride

my father

The soul of my heart, endless support, and the source of compassion

my mother

The best that supported and encouraged me to reach my dreams

my husband

The source of support and help to me in everything

my brothers& sisters

Fatima

Summary

Extended-spectrum-lactamases (ESBLs) are enzymes produced by Enterobacteriaceae family that mediate resistance to penicillin, third generation cephalosporins, and monobactams. ESBL-producing *Escherichia coli* is a major multidrug-resistant pathogen involved in serious hospital and community-acquired infections worldwide, resulting in expanded mortality morbidity, and treatment costs. The current study was conducted to collect *E. coli* isolates from UTI's patients using selective media; to determine antibiotic susceptibility patterns, phylogenetic groups, and biofilm formation among the isolates of *E. coli* isolates; To identify the genes encoding ESBL using PCR technique.

A total of 204 urine specimens collected from patients diagnosed with Urinary tract infection (UTIs) from clinic in Al-Hillah hospitals during a period extended from October, 2021 to April, 2022. The isolates were isolated and identified by Eosin Methylene blue, MacConkey's agar and VITEK-2 compact system, then tested for Biofilm formation and ESBL-producing phenotypically and genotypically. The ESBL-Producing *E. coli* isolates were typed phylogenetically by rapid PCR-based analyses.

Among 204 urine specimens, 73.5% (150/204) of these specimens are *E. coli* and others distributed among *Klebsiella pneumoniae* 26.5% (54/204). *Escherichia coli* isolates were detected by colonial morphology on Eosin methylene blue and MacConkey's agar. Isolates were further identified and confirmed as presumptive *E. coli* using Vitek-2 compact system.

The results of phenotypic detection of ESBL-producing of *E. coli* isolates showed that 72.7% (109/150) of *E. coli* isolates were positive for production of ESBL, whereas, 27.3% (41/150) of *E. coli* isolates were negative for the production of ESBL. A total of 109 isolates, high resistance was 100% for Ceftazidime/Clavulantae (CZC), Cefotaxime/Clavulantae (CTC); 78.66% for Ceftazidime (CAZ); 86.66% for Cefotaxime (CTX); 84% for Aztreonam (ATM); 87.33% for Ceftriaxone (CRO); 83.33% for Cefpodoxime (CPD).

Biofilm formation was detected by Congo-red agar (CRA) the results were showed that a total of 100 UPEC isolates, 71% (71/100 isolates) was found to be biofilm producers while 29% (29/100) isolates were non-biofilm producers.

When used polymerase chain reaction technique (Multiplex PCR), to detect Phylogenetic groups and subgroups of *E. coli* isolates by using the *chuA*, *yjaA*, and TspE4.C2 DNA fragment. The results showed that the majority of isolates were in phylogenetic group B2 91% (91/100) and subgroup $B2_3$ 75% (75/100), followed by group D 6% (6/100) and subgroup D_1 4% (41/100), the least prevalence isolates was in phylogenetic group A 1% (1/100) and subgroup A_1 1%, (1/100), followed by B1 2% (2/100).

Genotypic detection of the ESBL genes ($bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV}) were performed by using PCR. The current study revealed a high rate 84% (126/150) of ESBL-producing UPEC isolates among patients in Al-Hillah city. The results displayed that $bla_{\text{CTX-M}}$ genotype was the high prevalent

ESBL-encoding gene. It was found in almost 76% (76/100) of the ESBL-producing *E. coli*, while *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ were found in 75% (75/100) and 73% (73/100) of isolates respectively. Phylogenetic finding analysis of ESBL CTX-M-producing *E. coli* isolates affiliated to the ExPEC Phylogenetic group B2.

The results of the current study suggest that the UTIs specimens analyzed may serve as an essential source for prevalence of ESBL-producing and pathogenic *E. coli*, which is important for understanding the current conditions of the people residing in the Al-Hillah, as the data displayed a high prevalence of ESBL among *E. coli* isolates.

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List of Abbreviations

Sequence	**Abbr.**	**meaning**
1	BHIB	Brain heart infusion broth
2	CLSI	Clinical and Laboratory Standards Institute
3	CRA	Congo red agar
4	CTX-M	Cefotaximase
5	DDH_2O	Double deionized distilled water
6	DNA	Deoxyribonucleic Acids
7	D.W	Distillated water
8	*E. coli*	*Escherichia coli*
9	EMB	Eosin methylene blue

10	EPS	Extraintestinal polymeric substance
11	ESBL	Extended spectrum β-lactamases
12	ESBL-EC	ESBL-producing *Escherichia coli*
13	F	Forward
14	g	Gram
15	G-ve	gram-negative
16	h	Hours
17	InPEC	Intestinal pathogenic *E. coli*
18	LPS	lipopolysaccharide
19	MDR	Multi drug resistance
20	MHA	Mueller Hinton agar
21	µg	Microgram
22	µl	Microliter
23	NA	Nutrient agar
24	NB	Nutrient broth
25	PCR	Polymerase chain reaction
26	Sec	Second
27	SHV	Sulfhydryl variable
28	TBE	Tris-Borate-EDTA
29	TEM	Temoniera
30	UPEC	Uropathogenic *E. coli*
31	UTI	Urinary infections tract

32	R	Reverse
31	VFs	Virulence factors

Chapter One

Introduction

1.1. Introduction

Beta-lactams (β-lactams) are among the most commonly prescribed antibiotics in Iraq as well as world-wide. The group is distinguished by a β-lactam ring in their molecular structure. They include penicillins, cephalosporins, carbapenems, monobactams, and β-lactamase inhibitors These antibiotics have activity against gram-positive and gram-negative bacteria (e.g., many Enterobacteriaceae) (Pandey and Cascella, 2021).

The global emergence and spread of extended spectrum βlactamases (ESBLs) producing Enterobacteriaceae have been threatening the ability to treat an infection (Teklu *et al*., 2019). ESBL-producing bacteria are capable of hydrolyzing penicillin, broad-spectrum cephalosporins, and monobactams, but they do not affect the cephamycins or carbapenems, and their activity is inhibited by β-lactam inhibitors such as clavulanic acid (Paterson and Bonomo, 2005; Bush and Fisher, 2011).

In addition, ESBLs are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-acquired infections (Chandel *et al*., 2011). Infections caused by ESBL producers range from uncomplicated urinary tract infections to lifethreatening sepsis (Teklu *et al*., 2019). There is a worrying global public health issue as infections caused by

ESBL-producing bacteria which may present an increasing risk of transmission of resistant strains in humans and animals (Abayneh *et al*., 2018). The problem is clearly severe in developing countries such as Iraq, where the drugs can be obtained without prescription due to the lack of drug regulation (Alwash and Al-Rafyai., 2019).

In addition, only two studies by Abbas(2016) and Abbas (2019) studies have reported on ESBL-producing Enterobacteriaceae from lower respiratory tract infection patients and hospital teaching hospital environment respectively in AL-Hillah city, Iraq. Expanding and building on 3 the previous studies, this study will focus on the isolation, identification of both ESBL-producing *E. coli* from the larger number of specimens in UTI's patients. Therefore, this study will be conducted to determine the prevalence and antibiotic resistance pattern of ESBL-producing *E. coli* isolates from UTI's patients in Al-Hillah city, Iraq.

1.2. Aim of the study

The main aim of this study was be to determine the prevalence of ESBL-producing *E. coli*, including the resistance types among UTI's patients attending the hospitals and some clinic laboratories.

Specific objectives of this project are :-

1. Collection of *E. coli* isolates from UTI's patients using selective media.
2. Determination of antibiotic susceptibility patterns, phylogenetic groups, ESBL production, and biofilm formation among the isolates of *E. coli* isolates.
3. Detection of the genes encoding ESBL using Conventional PCR technique.

Chapter Two

Literature Review

2. Literature Review

2.1. Characteristics of *Escherichia coli*

Escherichia coli (*E. coli*) is a gram-negative, a non-sporulating, motile, lactose-fermenting, facultative anaerobic bacteria that belongs to the Enterobacteriaceae family. As a normal inhabitant of the mucus layer of the colon, it is a very adaptable type of bacteria that can cause diseases in both the digestive tract and other parts of the body (Foster-Nyarko & Pallen, 2022).

Pathogenic *E. coli* isolates can be categorized into two main groups based on where they cause disease and how they spread Extraintestinal pathogenic *E. coli* (ExPEC) which causes infections in organs other than the gut (ExPEC), can colonize and cause disease in the human. The most common infections are urinary tract infections (UTIs), abdominal sepsis, septicemia and neonatal meningitis (Gultekin *et al*., 2022).

The ExPEC infections are associated with a high rate of both human and animal morbidity and mortality and they are very costly for the human health care system as well as for animal owners(Santona *et al*., 2022). Intestinal pathogenic *E. coli* (InPEC) causes gastroenteritis or colitis when ingested (Rojas-Lopez *et al*., 2018).

The majority of UTIs are caused by a well-known group of ExPEC called uropathogenic *E. coli* (UPEC) which far the most common cause of UTIs. That divert from their commensal status an intestinal flora, grows and persists in the urinary tract , exhibits diverse array of virulence factors and strategies, which allow them to infect and cause diseases in the urinary tract (Shah *et al*., 2019).

The different phylogenetic groups of *E. coli* differ in ecological niche virulence factors and ability to cause disease. There are present *E. coli* phylogenetic groups (A, B1, B2, D). Lin *et al* reported that UPEC Isolates belong mainly to phylogenetic groups B2 and D whereas commensal, colonizing strains were associated with phylogenetic groups A and B1 (Lin *et al*., 2022).

2.1. Clinical infections caused by *E. coli*

Escherichia coli is a normal resident of the human digestive system, certain isolates have acquired virulence genes that allow them to cause infections in other parts of the body (Khairy *et al*., 2019).These include gastrointestinal illnesses like diarrhea and urinary tract infections, *E. coli* is divided into pathotypes and pathovars based on which organ systems they affect (Denamur *et al*., 2021).

Extraintestinal infections in humans have a high incidence and ExPEC is the most common gram negative extraintestinal pathogen. The most frequent infection is UTI. Several presumed virulence genes were linked to the pathogenicity of ExPEC, enabling them to invade almost any extraintestinal tissue (Baloch, 2021).

Bacteria causing UTI originate from the normal fecal flora. Infection occurs when colonizing flora ascend from the periurethral area or the vagina up the urethra to the bladder, ureters and sometimes to the kidneys. Urinary tract infections (UTIs) are the most prevalent microbial infections in humans, which can be either hospital- or community-acquired (Naziri *et al.*, 2021).

3.2. Pathogenicity of *E. coli* (Biofilm formation)

Pathogenicity is improved by *E. coli* ability to create several virulence factors (VFs) that can cause several infections including urinary tract infection. Bacteria create and secrete virulence factors, which are specialized molecules, mostly proteins, synthesized and secreted by the microorganisms. Genes on the chromosome or in mobile genetic elements (e.g., plasmids or transposons) encode these components in bacterial pathogens (Pakbin *et al.*, 2021).

There is a great diversity in the repertoire of VFs in ExPEC and no exact distinguishing subset of VFs has been associated with disease (Ejrnaes *et al.*, 2011). The pathogenesis of ExPEC has been attributed to theories; the special pathogenicity theory, based on the assumption that ExPEC isolates are more virulent than fecal isolates, and the prevalence definition; based on the observation that ExPEC isolates dominate over colonizing strains in the fecal flora prior to infection. The two theories are probably not mutually exclusive (Lindbloom, 2020).

Examples of important and well-characterized VFs in *E. coli* according to their functional category, are adhesions, toxins, lipopolysaccharide, iron acquisition systems and biofilm formation (Sora *et al*., 2021).

Biofilm is an organized accumulation of microorganisms and associated with a self-produced hydrated matrix of extracellular polymeric substances (EPSs) and are comprised of polysaccharides, proteins, lipids, and extracellular DNA (e DNA), which adhere to each other and adhere to the surface of an object (Jamal *et al.,* 2018).

Biofilm formation is a significant virulence mechanism in the pathogenesis ability of UPEC which protects them from antibiotic treatment and is the most important element in the persistence and recurrence of UTIs (Naziri *et al*., 2021). Production and development of bacterial biofilms are known to be dynamic and complex processes regulated by intrinsic biological properties and also by many environmental factors, since changes in the environment often trigger the formation of biofilm (Cowle *et al*., 2019).

Biofilm formation is complex, and occurs in four steps :-

(i) Initial attachment to the surface: in this step of biofilm formation, microbial cells attach to the surface through their appendages like pilli and flagella and may also be attached through other physical forces like van der Waal's forces, electrostatic interactions and others enable the bacteria to swim and approach the surface that help the cell to overcome the effects of repulsive forces between the bacteria and the surface (such as hydrodynamic) and helping the bacteria to reach and adhere to the surface (Ballén *et al*., 2022).

(ii) Micro-colony formation: after an attachment of microorganisms to a biotic or an abiotic surface occurs, this attachment becomes stable besides a process of multiplication and division of microbial cells starts, it is initiated through particular chemical signaling within the EPS. This process then leads to the formation of micro-colonies (Jamal *et al*., 2018).

(iii) Maturation and architecture: in this stage of biofilm formation microbial cells communicate with one another through auto-inducer signals. Cell-to-cell communication is an important process, this leads to the secretion of signaling molecules, known as auto-inducers. These auto inducers facilitate quorum sensing. At this stage of maturation certain gene products are expressed, that are considered important for the formation of EPS (Alotaibi & Bukhari, 2021).

(iv) Detachment of biofilm: in this phase, microbial cells within the biofilm perform quick multiplication and dispersion in order to convert from sessile into motile form. Detachment then occurs in a natural pattern. However, some types of bacteria do not produce extracellular polysaccharide and the bacterial cells disperse directly into the environment, but sometimes mechanical stress may also be involved in this process. During the detachment process microbial communities within the biofilm produce different saccharolytic enzymes that help to release the surface of the microbes into a new area for colonization (Jamal *et al*., 2018).

2.4. Antibiotic resistance in *E. coli*

Antibiotic resistance occurs when a drug loses its cytotoxic activity on bacteria, meaning that bacteria survive and multiply in the presence of the drug whose activity is lost (Al-Mir, 2020). There are two types of resistance, including intrinsic and acquired. Intrinsic (or natural) resistance is not affected by antibiotic treatment, is present in all members of a given bacterial species or genus, and is unrelated to horizontal gene transfer (Arbab *et al*., 2022).

One typical example of intrinsic resistance is the natural efflux pump activity that reduces the permeability of the outer membrane to molecules like lipopolysaccharide (LPS), which is present in gram-negative bacteria (Reygaert, 2018). Acquired resistance is conferred either by modification of the existing bacterial genetic material or by acquisition of exogenous genetic material (Al-Mir, 2020).

Generally, there are five main methods by which gram-negative bacteria develop resistance.(1) alternate metabolic pathways can by-pass paths inhibited by antibiotics (2) mutations can occur in the genes for binding sites for antibiotics changing the specific target or its function.(3) alterations of the membrane porins result in reduced permeability. (4) bacteria can express efflux pumps to actively transport antibiotics out of the cell and finally, (5) bacteria can carry genes coding for enzymes, such as beta-lactamases (ß-lactamases), hydrolyzing and inactivating ß-lactam antibiotics (Ogawara, 2019; Peterson & Kaur, 2018).

The predominant mechanism of resistance is the hydrolysis of the antibiotic by ß-lactamases that have the ability to produce these enzymes, including extended spectrum ß-lactamase (ESBL). ESBL is frequently acquired through large plasmids holding many different genes coding resistance against other antibiotic classes, contributing to multidrug resistance (MDR) (Tooke *et al.*, 2019).

The prevalence of ESBL increased among patients infected with bacterial pathogens treated heavily with β-lactams such as extra-intestinal infections caused by Enterobacteriaceae (Talan *et al.*, 2021). *Escherichia coli* is one of the organisms most frequently found harboring ESBL-genes and MDR in ESBL-producing Enterobacteriaceae is rapidly becoming a threat to the medical community (Martak *et al.*, 2022).

Enzymes that inactivate the ß-lactam antibiotics have been known since the 1940s and it is the most important resistance mechanism in gram-negative gut bacteria (Lindbloom, 2020). The ß-lactamases are found in the bacterial periplasmic space where they inactivate the ß-lactam-ring by hydrolysis. Theses enzymes include the penicillinases (degrading penicillins), the cephalosporinases (degrading penicillins and cephalosporins) (Abbas *et al.*, 2022).

2.5. Extended spectrum β-lactamases

The emergence of ESBL-producing *E. coli* isolates is becoming a critical issue globally (Chotinantakul *et al*., 2021). Tschudin-Sutter *et al* reported that resistant Enterobacteriaceae isolates could be easily transmitted between hospital and community settings (Tschudin-Sutter *et al*., 2017).

The classification in which ß-lactamases are grouped into molecular classes based on their amino acid similarities (Ambler scheme A to D) or into groups by functional similarities (Bush-Jacoby Medeiros Groups 1-4). The presence of a serine radical would classify them as serine ß-lactamases, while the presence of a zinc ion at the active site of the enzyme would classify them as metallo-ß-lactamases (Akpaka *et al*., 2021). Ambler class A, C and D are serine- β-lactamases and class B are metallo-β-lactamases (Reid *et al*., 2019).

The ESBLs are derived from genes for the narrow spectrum ß-lactamases. The most common ESBL types include TEM (named Temoniera in Greece), SHV (sulphydral variable), and CTX-M (reference to its preferential hydrolytic activity against cefotaxime, CTX as its acronym, M for Munich) (Akpaka *et al* , 2021).

The first plasmid-mediated ß-lactamase in gram-negative bacteria is TEM which was described in the early 1960s. This genotype has spread worldwide and is now found in many different species of the family Enterobacteriaceae (Lindbloom, 2020). Years later, the SHV genotype derived from the *Klebsiella. pneumonia* chromosome and mobilized onto plasmids was discovered in and had the ability to hydrolyze broad-spectrum cephalosporin’s and monobactams (Nzima *et al*., 2020).

In the mid 1980’s first reports of the new CTX-M enzymes and isolated in Munich. The CTX-M genes have been mobilized from the chromosome of members of the *Kluveyra* genus, a member of the Enterobacteriaceae family and an opportunist that seldom causes disease (Lindbloom, 2020).

The CTX-M enzymes were observed to hydrolyze cefotaxime more thoroughly than ceftazidime and were unrelated to the previously identified TEM and SHV genes (Akpaka *et al*., 2021). The emergence of CTX-M ESBLs, community-onset ESBL infections have become an important public health issue, primarily caused by *E. coli* producing CTX-M type ESBL (Castanheira *et al*., 2021).

6.2. Prevalence of ESBL-producing UPEC isolates

Since the year 2000, there has been a global increase in the prevalence of ESBL-producing *Escherichia coli* (ESBL-EC) in healthcare-acquired UTIs despite the fact that there are huge differences in frequency depending on geographical location (Larramendy *et al*., 2021). Several studies have shown the prevalence of ESBL production in Iraq, a study conducted by Majeed and Aljanaby (2019) in Al-Najaf city reported that 38.9% *E. coli* isolates producing ESBL genes (Majeed and Aljanaby, 2019).

The results of Lhwak and Abbas study in Al-Nassiryiah city detected the production of ESBL genes (28.9%) from *E. coli* isolates (Lhwak and Abbas, 2018) Study from Zakho conducted by Polse *et al*. (2016) reported that CTX-M (87.2%) was the most dominant for ESBL production. In Duhok, Michael and Saadi noted that the prevalence of TEM (80.9%) was higher than CTX-M (72.3%) and SHV (4.3%) (Michael and Saadi, 2018).

Hajj *et al*. showed variable results of prevalence of ESBL genes in Lebanon and Jordan. Reports of ESBL rates for UPEC were significantly higher in Jordan (58%) compared with Lebanon (33%) (Hajj *et al*., 2018).In another study from Jordan, Al-Tamimi *et al*. reported that high resistance rates (≥95%) were showed by UPEC isolated towards third-generation cephalosporin's (Al-Tamimi *et al*., 2019).

The prevalence of ESBL genes have also been mentioned in Iran and Turkey with rate accounting 63% and 20.6% respectively (Jubrael , 2016).In Mashad city Iran the prevalence of ESBL-producing *E. coli* isolates was 51.6% (Moghanni *et al*., 2018). This result similar to study in Iran reported 46.6 % of *E. coli* isolates done by (Barzegar *et al*., 2022).

7.2. Genotyping of *E. coli* isolates (phylogenetic groups and ESBL genes).

Escherichia coli isolates mainly fall into four phylogenetic groups A, B1, B2, and D, based on combinations of three genetic markers: (i) chuA, a gene, that is responsible for haeme transport in enterohaemorrhagic O157 :

H7 *E. coli*; (ii) yjaA, a gene of unknown function that was identified in the recent complete genome sequence of *E. coli* K-12; and (iii) TspE4.C2, an anonymously designated DNA fragment of a noncoding region in *E. coli* isolates (Alwash& Al-Rafyai, 2019).

The first described PCR protocol was a triplex PCR developed by Clermont and colleagues. The Clermont method was originally designed to assign *E. coli* isolates to four phylogenetic groups (A, B1, B2 or D) (Clermont *et al*., 2000). Barzan *et al*.(2016) have shown the association between phylogeny and pathogenicity of *E.coli* isolates (Barzan *et al*., 2016).

Commensal *E. coli* isolates are commonly associated with phylogenetic groups A and B1, while the extraintestinal (noncommensal) pathogenic *E. coli* isolates belong mostly to the B2 phylogenetic group and (to a minor extent) to group D. Commensal *E. coli* isolates do not normally carry any known virulence factors (Alwash& Al-Rafyai 2019).

PCR technique depends on a triplex PCR using a combination of two genes (*chuA* and *yjaA*) and DNA fragment designated (TspE4.C2) this method whose results strongly correlate with those acquired by other standard strategies , is an excellent technique for fast and cheap assigning of *E. coli* isolates in various phylogenetic groups (Yılmaz & Aslantaş, 2020).

All ESBL-producing *E. coli* isolates were subjected to PCR to detect ESBL genes including bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$ (Adekanmbi *et al*.,2021). Amplified PCR-products for gene bla_{SHV} was about 481bp using SHV-F and

SHV-R primers. The amplified PCR-products for the gene *bla*$_{CTX\text{-}M}$ gave a molecular weight about 599bp using CTX-M-F and CTX-M-R primers. and amplified PCR-products for *bla*$_{TEM}$ gene using the TEM-F and TEM-R primers gave an expected band of 500bp (Ajuga *et al*., 2021).

Chapter Three

Materials &Methods

3.1. Materials

3.1.1. Laboratory Instruments, Equipment, and Disposables

All Laboratory instruments, equipment, and disposables used in the study are listed in Table (3-1).

Table (3-1): List of laboratory instruments, equipment, and disposables

No.	**Laboratory Instruments and Equipment**	**Manufacturer/ Origin**
1	Autoclave	Stermite (Japan)
2	Benson burner	Eppendorf (Germany)
3	Centrifuge	GFL (Germany)
4	Deep freezer	Almateen (China)
5	Digital camera	Samsung (Japan)
6	Distillatory	GFL (Germany)
7	Electric oven	Memmert (Germany)
8	Eppendorf tubes	Sigma (UK)
9	Flask	Chemical-Lab (China)
10	Gel electrophoresis	Clever (USA)
11	Hood	Lab o gene (Denmark)
12	Incubator	Memmert (Germany)
13	Latex gloves	Broche (Malaysia)
14	Micropipette 100-1000µI	Sartorius (Germany)
15	Micropipette 1-10µI	Sartorius (Germany)
16	Micropipette 1-200µI	Sartorius (Germany)

17	Microwave	Argos (Germany)
18	Nano drop	Memmert (Germany)
19	Para film	HDA (China)
20	PCR system	Biometra (Germany)
21	PCR tube 1.5ml	Biobasic (Canada)
22	PCR tubes 200µl	Biobasic (Canada)
23	Petri dishes	Afco-Dispo (Jordan)
24	Plain tube 10ml	Afco-Dispo (Jordan)
25	Platinum wire loop	Himedia (India)
26	Rack	HDA (China)
27	Senstive electric balance	Metter (Switzerland)
28	Sterilized cotton swabs	Afco-Dispo (Jordan)
29	UV-transilluminator	Quantum (France)
30	Vortex mixer	Gemmy (Taiwan)
31	Water bath	Memmert (Germany)
32	Wooden sticks	Supreme (China)

3.1.2. Biological and Chemical Materials

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

Table (3-2): List of biological and chemical materials

No.	Biological and chemical materials	Manufacturer/Origin
1	Glycerol	Oxoid (UK)
2	Ethanol (70%)	Fluka chemika(Switzerland)
3	Ethidium bromide	Promega (USA)
4	Agarose	Pronadisa (Spain)

5	Nuclease free water	Cyntol (Russia)
6	Favor Prep Genomic DNA Mini Kit	Favorgen (Taiwan)
7	EDTA	Thomas baker (India)
8	DNA loading dye	Promega (USA)
9	$Mgcl_2$	Cyntol (Russia)
10	PCR master mix	Cyntol (Russia)
11	Normal Saline	Mehico (India)
12	Primer Pairs	Bioneer (Korea)
13	DNA ladder 100bp	Cyntol (Russia)

3.1.3 Cultures Media

The Culture Media used in this study were listed in Table 3-3.

Table (3-3): Cultures Media

No.	**Culture media (Medium)**	**Manufacturer/Origin**
1	MacConkey's agar	Himedia (India)
2	Brain heart infusion broth (BHI)	Himedia (India)
3	Brain heart infusion agar	Liofilchem (Italy)
4	Eosin methylene blue agar (EMB)	Oxoid (USA)
5	Mueller-Hinton Agar	Liofilchem (Italy)
6	Congo Red agar	Himedia (India)
7	Nutrient agar	Himedia (India)
8	Nutrient broth	Himedia (India)

3.1.4 Antibiotic Disks

The antibiotic disks used in this study are listed in Table 3-4.

Table (3-4): Antibiotic disks, symbol, and potency

No	Antibiotics	Assembly	Potency (µg)	Manufacturer/Origin
1	Aztreonam	ATM	30	Bioanalyse (Turkey)
2	Ceftriaxone	CRO	30	Bioanalyse (Turkey)
3	Ceftazidime	CAZ	30	Liofilchem (Italy)
4	Cefotaxime	CTX	30	Liofilchem (Italy)
5	Cefpodoxime	CPD	10	Biomaxima (Poland)
6	Ceftazidime/Clavulantae	CZC	30/10	Biomaxima (Poland)
7	Cefotaxime/Clavulantae	CTC	30/10	Biomaxima (Poland)

Table (3-5): Favor Prep™ genomic DNA mini kit

FATG buffer	30 ml
FABG buffer	40 ml
W1 buffer	45 ml
Wash buffer	25 ml
Elution buffer	30 ml
FABG column	100 pcs
Collection tube (2 ml)	200 pcs

3.2. Methods

3.2.1. Study design

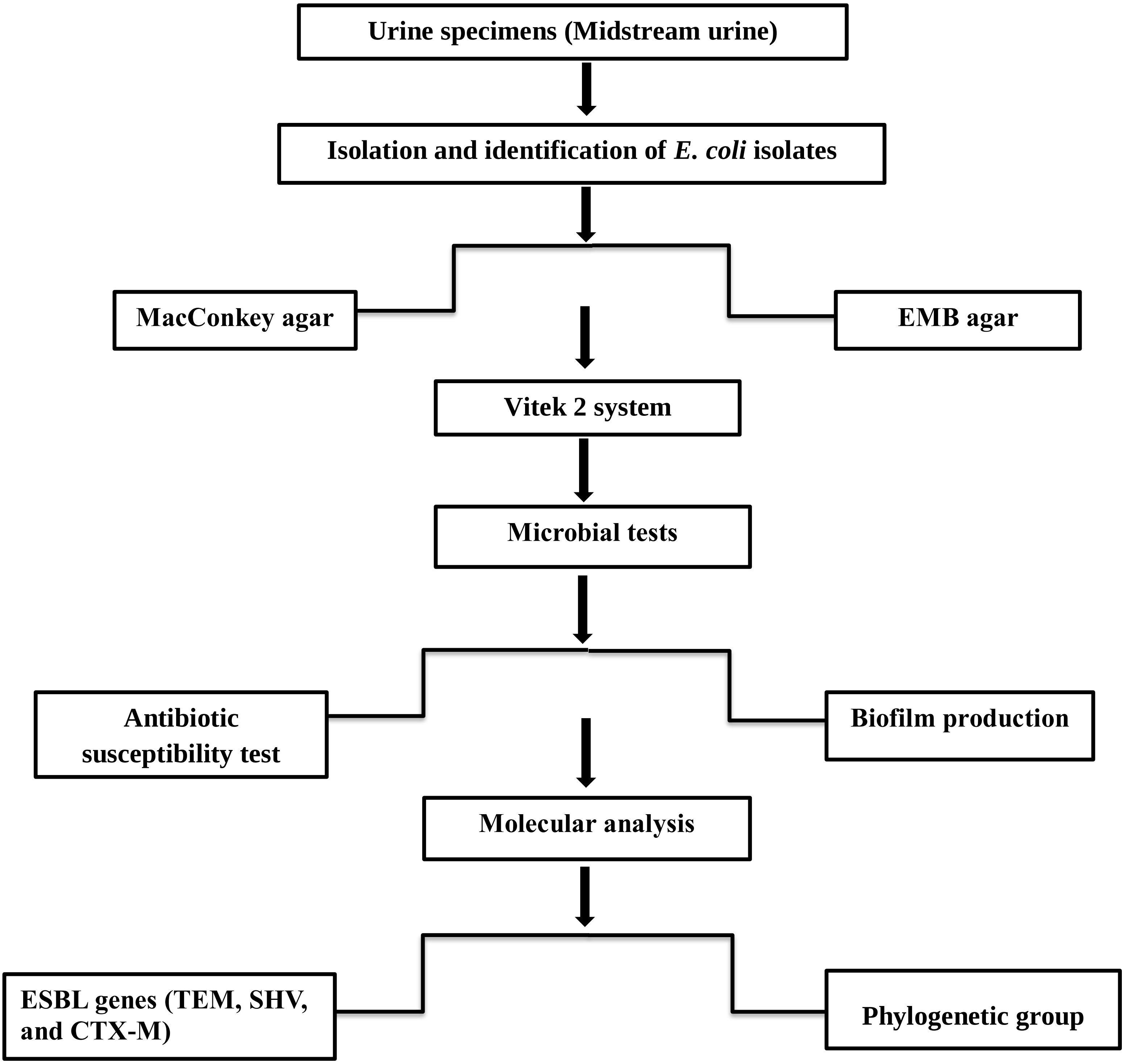

Figure (3-1): Study design

3.2.2. Preparation of solutions used in the present study

3.2.2.1. McFarland standard solution

The 0.5 McFarland**'s** standard (1.5×10^8 CFU/ml) was prepared by adding 0.5 ml of 1.175% barium chloride [$BaCl_2.2H_2O$: H2O (w/v, 1.175g: 98.825 ml)] to 1% sulfuric acid [H_2SO_4: H_2O (v/v, 1 ml: 99 ml)] in order to obtain a barium sulfate precipitate. The solution was used to visually compare the turbidity of a suspension bacteria with the turbidity of the 0.5 McFarland**'s** standard. The McFarland**'s** standard tubes were sealed with parafilm to prevent evaporation and stored in the dark for up to 6 months at room temperature. The accuracy of a prepared 0.5 McFarland's standard was checked by using a spectrophotometer. The optical density was measured at 625 nm that should be between 0.08 and 0.1 (CLSI, 2021).

3.2.2.2. Preparation of TBE buffer (Tris-Borate-EDTA)

Tris-Borate-EDTA(TBE) running buffer is the most commonly used buffer for DNA and RNA gel electrophoresis. TBE was prepared and stored as a 10× stock solution. The 10× working solution was prepared by dissolving 108 g of Tris base, 55 g of boric acid, and 40 ml of 0.5 M EDTA in 1000 ml of D.W. However, final concentration of 1× TBE solution was prepared by adding 100 ml of 10× TBE buffer to 900 ml of sterile D.W. (Sambrook and Russel, 2001).

3.2.2.3. Preparation of 6× DNA loading buffer blue

Loading buffer is provided in a premixed, ready-to-use form, containing [100 mM Tris-HCl (pH 8.0), mix of dyes (Xylene Cyanol and Bromophenol Blue), and 30% glycerol, 100 mM EDTA (pH 8.0)]. DNA loading buffer blue (6×) is designated to prepare samples for DNA gel electrophoresis. The glycerol content increases the density of the sample and ensures that DNA forms a layer at the bottom of the well. EDTA is known for inhibition of metal dependent nucleases. Tracking dyes permit monitoring the progress of electrophoresis. Only 1µl of loading dye was mixed with 5 µl of DNA sample to check DNA after extraction (Sambrook and Russel, 2001).

3.2.3. Preparation of culture media:

Growth media used in the present study were prepared following manufacturer's protocol. The media were sterilized by the autoclave at 121°C for 15 minutes (min).

3.2.3.1. MacConkey's agar medium

MacConkey (MA) agar is a selective and differential culture medium for bacteria designed to selectively isolate gram-negative (G-ve) bacteria enteric rods and differentiate them based on lactose fermentation (Jung &Hoilat, 2022). The MA was prepared by adding 49.5 of MA to 1000 ml D.W a selective and differentiating culture media, MA exclusively supports the growth of G-ve bacteria.

3.2.3.2 Eosin methylene blue

Eosin methylene blue (EMB) is used for the isolation and differentiation of G-ve enteric bacteria from clinical and nonclinical specimens (Murray *et al.*, 2020). The EMB was prepared by dissolving 36 g in 1000 ml D.W.

3.2.3.3. Brain heart infusion broth

Brain heart infusion broth (BHIB) is a highly nutritious general-purpose growth medium recommended for the cultivation (McFadden, 2000). The BHI Agar was prepared by dissolving 37 g in 1000 ml D.W.

3.2.3.4. Mueller Hinton agar

Mueller Hinton agar (MHA) is used to determine the susceptibility of microorganisms to antimicrobial agents by either the disk diffusion or well diffusion methods (McFadden, 2000). The MHA was prepared by dissolving 38 g in 1000 ml D.W.

3.2.3.5. Nutrient agar

Nutrient agar (NA) is a general purpose medium which can be used for cultivation of non-highly nutritious bacteria (McFadden, 2000). The NA was prepared by dissolving 28 g in 1000 ml.

3.2.3.6. Nutrient broth

Nutrient broth (NB) is a general purpose medium which supports the growth of bacteria that are not very nutritionally demanding (McFadden, 2000). The NB was prepared by dissolving 25 g in 1000 ml D.W.

3.2.3.7. Congo red agar

For all *Escherichia.coli* isolates, biofilm-forming capacity was assessed by Congo red agar (CRA) modified method as described by Freeman *et al.* 1989 (Jain and Agarwal 2009). CRA was prepared by mixing brain heart infusion broth (37 g/L), sucrose (50 g/L), agar No. 1 (10 g/L), and Congo red indicator (8 g/L). The aqueous solution of Congo red was prepared separately, autoclaved at 121°C for 15 min, and added to the autoclaved brain heart infusion agar with sucrose when cooled down at 55°C. CRA plates were later inoculated with UPEC isolates and incubated at 37°C/24 h aerobically. A positive result was indicated by black colonies with a dry crystalline consistency, while non-biofilm producers usually remained pink crystalline colonies (Jain and Agarwal 2009).

3.2.4 Sample collection

The current study was performed from October 2021 to April 2022 in selected health facilities of Al-Hillah, Babylon province, Iraq; namely Al Hillah Teaching Hospital, Al Hashimiya General Hospital, Babel Maternity and Children, Merjan Medical City for Internal Medicine and Cardiology, and Al-Hillah laboratory for microbiological analysis. 204 Mid-stream urine specimens were collected by using a sterile container from patients (with recurrent and complicated UTIs) with bacterial count $\geq 10^5$ CFU/mL and white blood cell count $\geq 10^4$ leukocyte/mL of urine. *Escherichia coli* isolates were detected by colonial morphology on Eosin Methylene Blue and MacConkey's agar after incubation at 37°C. Isolates were further identified confirmed as presumptive *E. coli* using VITEK® 2 Compact according to the manufacturer’s instructions. The presumptive *E. coli* isolates were stored in the Brain Heart Infusion Broth with 15% glycerol at 20°C for further analysis (LeBouguenec *et al.*, 1992).

3.2.5. Antibiotic Susceptibility test (AST)

The *in vitro* susceptibility of 150 *E. coli* isolates to 7 antibiotics was tested via disk diffusion method according to Clinical and Laboratory

Standards Institute instructions (CLSI, 2021). The bacterial isolates were cultured in a MacCkonkey's agar and then sub cultured in brain heart infusion broth for 24 h at 37ºC. The turbidity was adjusted in 0.85% sterile normal saline solution to 0.5 McFarland’s standard (10^8 CFU/mL) and then cultured on Mueller Hinton agar with a cotton swab. Antibiotic disks were put onto MHA by using a sterile forceps, then, pressed down to ensure complete contact with the agar. The plates were incubated for 18 h at 37°C and the antibacterial activity was expressed as mean of the inhibition zone diameter in millimeters (mm). The zone size was measured the next day and classified as sensitive or resistant by compared to standard zone.

3.2.6 Molecular study *of E. coli*:

3.2.6.1. Extraction of genomic DNA

Favor Prep^{TM} Genomic DNA Mini Kit was utilized to extract DNA from UPEC isolates following the manufacturer's instructions. Inocula of *E. coli* were prepared at density up to 10^9. Transfer the suitable amount of bacterial cells (up to 1×10^9) to a 1.5mL micro centrifuge tube and then centrifuged at 7.500 rpm for 10min. Bacterial pellets were harvested after 10min. The harvested cells were resuspended thoroughly in 200µL of FATG Buffer and then, incubated for 5 min at room temperature. Buffer FABG (200µl) was added respectively to the bacterial cells. The resulting homogeneous cells suspension was incubated for 10min at 70ºC and vortexed for 10s (second) every 3min. until the sample lysate is clear.

DNA was extracted from the suspension by the addition of 96~100% of 200 µL and then transferred to the FABG column assembled inside a 2 mL collection tube, and centrifuged for 1 min at 14000 rpm. The flow through was discarded. The FABG column was assembled in a new 2 mL collection tube. W1 Buffer (400 µL) and Wash Buffer (750 µL) were then added respectively to the FABG column assembled inside a new 2 mL collection tube, and centrifuged for 1min at 14000 rpm to wash column membrane. The flow through was discarded.

The FABG Column was dried via further centrifugation at 14000 rpm for 3 min over a new 2 mL column collection tube to remove any residual ethanol solution. The dry FABG Column was then transferred to a new 1.5 ml micro centrifuge tube, and 100µl of Preheated Elution Buffer or TE was added directly to the FABG column membrane for 3~5 min, followed by centrifugation for 1 min at 14000 rpm to elute DNA. DNA quality and quantity were determined using a NanoDrop 2000 spectrophotometer. The eluted genomic DNA was stored at -20ºC until use.

3.2.6.2. DNA concentration and purity testing:

The concentration and purity of the sample were measured by using a conventional UV-V is spectrophotometer called Nano Drop. This optical spectrometer can interpret optical signals with wavelengths ranging from 190 nm to 840 nm with high accuracy and the ratio of sample absorbance at 260 and 280 nm was used to determine DNA purity (Minhas *et al.*, 2021).

3.2.6.3. Agarose gel electrophoresis

Agarose gel electrophoresis is the most effective way of separating DNA fragments. The concentration of agarose in a gel depends on the sizes of the DNA fragments that need to be separated, ranging between 0.5%-2% (Lee *et al.*, 2012). A 0.7% gel was used to obtain good separation of genomic DNA (5-10 kb) after extraction while 1.5% was used to gain good resolution for small fragments of PCR product (0.2-1 kb). However, the specific weight of agarose was added to 100 ml of 1×TBE buffer and then melted in microwave until the solution becomes clear. Once the agarose was cooled to 50-55°C, 5µl of ethidium bromide dye (10 mg/ml) was added to 100 ml of melting agarose gel to get final concentration 0.5µg/ml (Sambrook and Russel, 2001). The agarose was poured in the gel tray with sealed ends, comb placed properly, and then left to dry. The samples were loaded in a separate well of the gel, with marker in one well. Electrodes were connected correctly and the run was applied according to the gel percentage and size of gel.

3.2.6.4. Primer pairs

All primer pairs used in this study were dissolved using sterile ddH_2O. Stock solution (100 pmol/µL) was prepared by adding ddH_2O to the vial containing lyophilized primer while working stock of 10 pmol/µL was made by mixing 10 µL of the stock primer and 90 µL of ddH_2O.

3.2.6.5 Detection of phylogenetic groups

The UPEC isolates were typed to one of the four major *E. coli* phylogenetic groups (A, B1, B2, and D) using the Clermont *et al.* method (Clermont *et al.* 2000) via a triplex polymerase chain reaction assay (PCR) that evaluated the genetic markers *chuA* and *yjaA* and the TspE4.C2 DNA fragment (Table 3-6). The reaction mixture (20 µL) contained 8µL of Master Mix, 7.5µL of $MgCl_{2,}$, 2µL of 10 pmole/µL forward and reverse primers, 0.5µL of Nuclease-free water, and 2µL of

Genomic DNA. The thermal cycling conditions were conducted in an Eppendorf thermal cycler at an initial denaturation of 94°C for 4 min, followed by 30 denaturation cycles of 20s at 94°C; 30 annealing cycles of 30s at 59°C and 30 extension cycles of 1 min at 72°C. The amplification products were separated in 1.5% agarose gel containing ethidium bromide. Following electrophoresis, the gel was visualized and photographed under UV light, and the UPEC isolates were assigned to the phylotypes A (*chuA*–, TspE4.C2–), B1 (*chuA−,* TspE4.C2+), B2 (*chuA*+, *yjaA*+), and D (*chuA*+, *yjaA*−). To increase the resolution of the isolate discrimination, subgroups were determined as follows: subgroup A_0 (group A), *chuA*−, *yjaA*−, TspE4.C2–; subgroup A_1 (group A), *chuA*−, *yjaA*+ TspE4.C2−; group B1, *chuA*− , *yjaA*− , TspE4.C2+; subgroup $B2_2$ (group B2), *chuA*+, *yjaA*+, TspE4.C2−; subgroup $B2_3$ (group B2), *chuA*+, *yjaA*+, TspE4.C2+; subgroup D1 (group D), *chuA*+, *yjaA*–, TspE4.C2–; and subgroup D2 (group D), *chuA*+, *yjaA*–, TspE4.C2+ (Escobar-Páramo *et al*. 2004).

Table (3-6): Phylogenetic primers for *Escherichia coli*

Primer ID		**Target primer sequence**	**Amplicon size (bp)**	**References**
ChuA	F	5′-ATGGTACCGGACGAACCAAC-3′	288	Bajaj *et al.* (2015)
	R	5′-TGCCGCCACTACCAAAGACA-3′		
yjaA	F	5′-CAAACGTGAAGTGTCAGGAG-3′	211	Bajaj *et al.* (2015)
	R	5′-AATGCGTTCCTCAACCTGTG-3′		
TspE4C2	F	5′-CACTATTCGTAAGGTCATCC-3′	152	Bajaj *et al.* (2015)
	R	5′-AGTTTATCGCTGCGGGTCGC-3′		

3.2.6.6. Genotypic detection of the ESBL genes

Genotypic detection of *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ were performed using polymerase chain reaction (PCR). The primer used and their amplicon sizes are listed in Table 3-7. The PCR amplification was conducted in 20 µL reaction mixture containing the Master mix (8µL), $MgCl_2$ (7.5µL), 10 pmole/µL of forward primer (1µL), 10 pmole/µL of reverse primer (1µL), template DNA (2µL), and sterile nuclease-free water (0.5µL) was used to make up the volume of the reaction mixture. The thermal cycling conditions are listed in Table 3-8.

Table (3-7): List of Primer Pairs, Sequences, and Amplicon Size

Primers		**5'-sequence-3'**	Amplicon size (bp)	References
CTX	F	5'-ACAGCAGATAATTCGCAA-3'	599	Bajaj *et al.* (2015)
	R	5'-AACCAGATCACCGCGATA-3'		
TEM	F	5'-TCAACAGCGGTAAGATCCTTGA-3'	500	Bajaj *et al.* (2015)
	R	5'-TGCAACTTTATCCGCCTCCA-3'		
SHV	F	5'-AAATGGATCTGGCCAGCG-3'	481	Bajaj *et al.* (2015)
	R	5'-AGCAGCTGCCGTTGCGAA-3'		

3.2.6.7 Accession number

Nucleotide sequences of triplicate *bla*$_{\text{CTX-M-1}}$ gene of UPEC isolates have been submitted Gene Bank under the accession number (CP098207.1).

3.2.7. Ethical approval

The present research was reviewed and approved by the committee of University of Babylon, College of Sciences along with Al-Hilla Hospitals (Project No. M220602). The approval form is available in its original version from the corresponding author on request..6.

Table (3-8): PCR Thermal cycling Conditions

Genes	**Thermocycling conditions**										**No. of Cycles**
	Initial denaturation		**Denaturation**		**Annealing**		**Extension**		**Final extension**		
	Temp (ºC)	**Time**	**Temp (ºC)**	**Time**	**Temp (ºC)**	**Time**	**Temp (ºC)**	**Time**	**Temp (ºC)**	**Time**	
bla**CTX**	94	4 min.	94	20 sec.	63	30 sec.	72	1 min.	72	5 min.	30
bla**TEM**	94	4 min.	94	20 sec.	58	30 sec.	72	1 min.	72	5 min.	30
blaSHV	94	4 min.	94	20 sec.	63	30 sec.	72	1 min.	72	5 min.	30

Chapter Four

Results & Discussion

4. Results and discussion

4.1. Isolation and identification of *Escherichia coli* isolates

This study included 204 specimens, (150/204) 73.5% of these specimens are *E. coli* and others distributed among *Klebsiella pneumoniae* (54/204) 26.5% showed in (Table 4-1). *Escherichia coli* isolates were detected by colonial morphology on EMB and MA (Figure 4-1).

Laboratory diagnosis procedure depending on the steps recommended by (MaCfadden, 2000) for diagnosis. Isolates were further identified confirmed as presumptive *E. coli* using Vitek-2 system according to the manufacturer's instructions (Figure 4-1). *Escherichia coli* on MA showed pink colonies due to the fermentation of lactose, leading to the production of acids, which decrease the pH and turn the colonies to pink (Murray *et al*., 2020).

Escherichia coli isolates when cultured on EMB agar produced a green metallic sheen colonies due to the fact that under acidic conditions the dyes produce a dark purple complex which is usually associated with a green metallic sheen. This metallic green sheen is an indicator of vigorous lactose and/or sucrose fermentation ability typical of fecal coliforms (Lal & Cheeptham., 2007).

Table 4-1: Gram negative bacterial species isolates from patients diagnosed with urinary tract infections (UTIs).

Bacterial isolates	No.	Percentage %
Escherichia coli	150	73.5%
Klebsiella pneumoniae	54	26.5%
Total	204	100%

bioMérieux Customer: Microbiology Chart Report Printed February 20, 2022 11:23:05 AM CST

Patient Name: h. fatima
Location:
Lab ID: 3320221

Patient ID: 3320221
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.35 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Escherichia coli** 0405610550526610		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Figure 4-1: *E. coli* isolates growth on (A) MacConkey's agar, (B) EMB agar, and (C) Vitek 2 system result **.**

The current result showed that *E.coli* is the most causative pathogen for UTI (73.5%) Table (4-1). In parallel with current study, in Iraq, Al-Gasha'a *et al*. (2020) who reported the most prevalent bacterial uropathogen was *E. coli* (33.33%), followed by *K. pneumoniae* (20.63%) in Baghdad city.

In contrast with present findings, Al-Tulaibawi (2019) documented that *E. coli* was the most common bacteria causes UTI (52.9%), followed by *K. pneumonia* (15.7%) in Misan city. In addition, Abdullah (2019) in Duhok city who noticed that the most prevalent causes of UTI was *Staphylococcus aureus* (40.4%), the second most prevalent isolate was *E. coli* (31.8%), followed by *K. pneumoniae* (10.6%). these results disagreed with our results. Al-Hamdany (2018) who recorded that (55%) of UTI cases caused by *E. coli* in Al-Hillah city.

In parallel with present study. Yenehun Worku *et al.* (2021) reported UPEC isolates was the most frequently uropathogenic isolates (63.6%), followed by *K. pneumoniae* (13.7%) in Ethiopia. Klein & Hultgren (2020) reported that *E. coli* was isolated from 80% of midstream urine specimens with UTIs in USA. Al-shabi *et al.* (2019) documented that *E. coli* isolates was the most prevalent causes of UTI in the rate (73.68%) in Saudi Arabia.

Mohammed & Abass (2019) reported that UPEC isolates showed high prevalence among all clinically significant urinary isolates (66.6%) in Pakistan. Odoki *et al.* (2019) who explained that the most prevalent bacterial uropathogen was *E. coli* (41.9%) followed by *S. aureus* (31.4%) in Uganda. Sharma *et al.*(2016) recorded that UPEC isolates showed high prevalence among all clinically significant urinary isolates (68%), followed by *K pneumoniae* (14%) in India.

ExPEC's dominance among UTI when compared to other gram negative bacteria can be explained by their development of a number of virulence factors that increase their ability to colonize the urinary tract and cause an infection. The most important virulence factors include surface- associated factors (flagella, outer membrane vesicles, pili, curli, non-pilus adhesins, polysaccharide capsules, outer membrane proteins, and lipopolysaccharide, toxins (α-hemolysin and cytotoxic necrotizing factor type 1), and iron-acquisition systems (Terlizzi *et al*., 2017).

4.2 Antibiotic susceptibility profile

Antibiotic susceptibility test was carried out following the method outlined by the CLSI (CLSI, 2021). Detection of ESBL-producing *E. coli* isolates (150) was first screened by the phenotypic method and then it was confirmed by the phenotypic confirmatory test as prescribed in CLSI guidelines (CLSI, 2021). The results showed that (109/150) 72.7% of *E. coli* isolates showed positive results for production of ESBL (Figure 4-2), whereas, (41/150) 27.3% *E. coli* isolates showed negative results for the production of ESBL. A total of 109 isolates, the high resistance was 100% for CZC, CTC; 78.66% for CAZ; 86.66% for CTX, 84% for ATM; 87.33% for CRO; 83.33% for CPD (Table 4-2).

Table 4-2 Resistance profiles of phenotypic ESBL-producing *Escherichia coli* isolates.

Antibiotics name	No. of resistant isolates	Resistance%
Aztreonam (ATM)	126	84%
Ceftriaxone (CRO)	131	87.33%
Cefpodoxime (CPD)	125	83.33%
Ceftazidime (CAZ)	118	78.66%
Ceftazidime-Clavulantae (CZC)	150	100%

Cefotaxime (CTX)	130	86.66%
Cefotaxime-Clavulantae(CTC)	150	100%

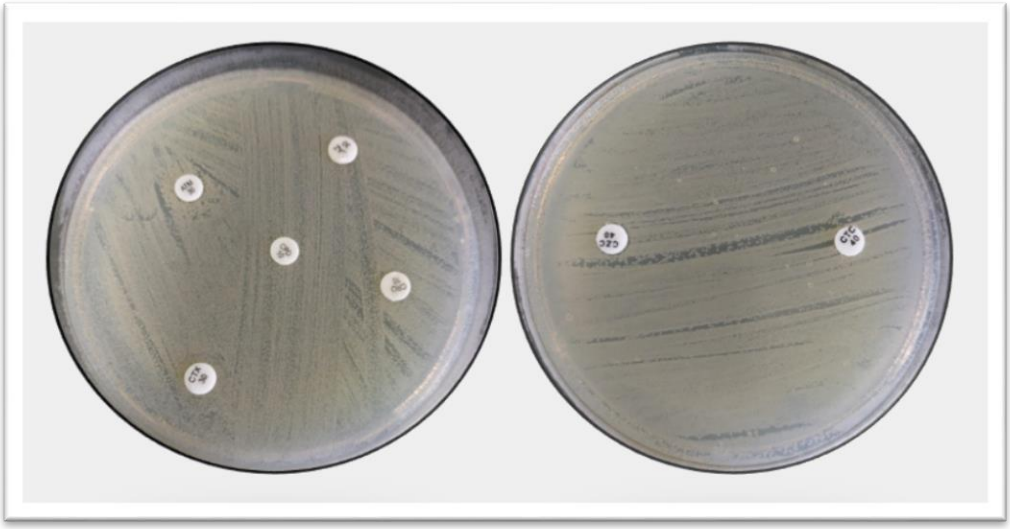

Figure 4-2 ESBL-producing in *E. coli* isolates

The antibiotic susceptibility results in Table (4-2) showed that the majority of *E. coli* isolates were resistant to several antibiotics because of the increased use of antibiotics including third-generation cephalosporin. The emergence and dissemination of ESBL-producing Enterobacteriaceae has been reported in the world (Mitman *et al*., 2022). The current findings are in an agreement with Pishtiwan & Khadija (2019) recorded that (77%) of *E. coli* isolates are resistance to ESBL in Erbil city.

Majeed and Aljanaby, (2019) documented (38.9%) *E. coli* isolates producing-ESBL genes in Al-Najaf city. Alkhudhairy & Alshammari (2019) reported that 23.7% of UPEC isolates were positive for ESBL producers while 76.3% of UPEC isolates were negative results for ESBL this result disagrees with our results in Al-Najaf city.

Al-Tamimi *et al.* (2022) in Jordan reported the ESBL screening test was positive in (81.3%) of *E. coli* isolates. In Iran, Aghamohammad *et al.* (2022) reported that 60% of UPEC isolates were ESBL-producers. Ghaderi *et al.* (2020) recorded the prevalence of UPEC producing ESBL in Iran (75%). Al-Tamimi *et al.* (2019) in Jordan who reported that 87% of *E. coli* isolates producing ESBL from out and in patients.

Al-baramki *et al.* (2019) in Jordan who noted that 81.8% of *E. coli* isolates are resistance for ESBL-producing. Al-Jamei *et al.* (2019) recorded (62%) of the tested *E. coli* isolates were ESBL-producers in Jordan. Shrestha *et al.* (2019) in Nepal documented that 50.9 of *E. coli* isolates were found to be ESBL producers. Moghanni *et al.* (2018) reported that (51.6%) of UPEC isolates producing ESBL. Pootong *et al.* (2018) in Thailand reported that 38.7% of UPEC isolates were ESBL-producers.

4.3. Detection of biofilm formation by Congo-red agar (CRA).

The ability of bacteria to form a biofilm is one of the most important indicators of its pathogenicity and resistance to antibiotics. The biofilm formation was detected by CRA (a qualitative method used to differentiate between biofilm producer and non-producer. Biofilm producer appears as a dry or shiny black colony, while non-producers appear to be pink colony (Poonam *et al*., 2022). A total of 100 UPEC isolates, 71% (71/100 isolates) were found to be biofilm producers while 29% (29/100 isolates) were non-biofilm producers (Figure 4-3).

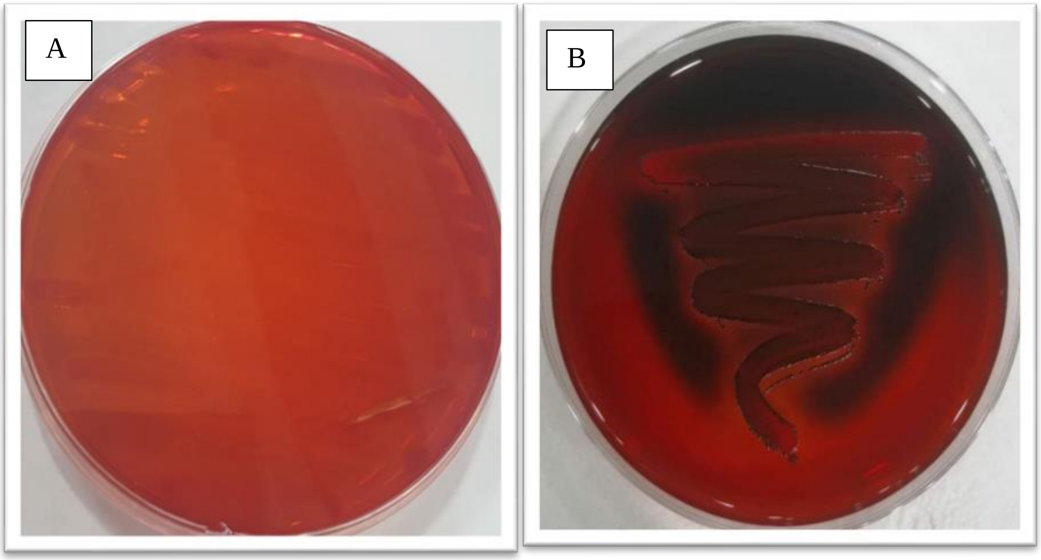

Figure 4-3 Congo red agar plates (A) pink colonies of non-biofilm formation (B) black colonies of biofilm formation isolates

In parallel with current study, Jabir (2022) in Al-Diwanyiah city who noted that 64% of UPEC isolates were biofilm-producers. Jaber & Aal Owaif (2020) in Baghdad city who showed that 93% of the *E. coli* isolates had the ability to produce biofilms. Another studies articulated by Muhammad & Ghareb (2019) in Erbil city and Al-Taai *et al.*(2018) in Diyala city reported that 100% of UPEC isolates were biofilm-producers.

In Pakistan Javed *et al.* (2021) showed a UPEC isolates were assessed for their biofilm-forming ability on CRA (68%) were found to be biofilm producers. Mohsenzadeh *et al.* (2021) in Iran noted that from 100 of UPEC isolates (38%) were biofilm-producers. Katongole *et al.* (2020) noted that 62.5% of UPEC isolates were biofilm producers in Uganda. Umamageswari & Priya, (2019) and Gawad *et al.*, (2018) in Egypt, reported that the biofilm producing isolates were 96% and 76.5%, respectively Dash *et al* (2018) in India who showed the (54.4%) of *E. coli* isolates to produce biofilms.

4.4. Phylogenetic groups and subgroups of *Escherichia coli* isolates:

Polymerase chain reaction was performed to detect *E. coli* diversity using the *chuA* gene, *yjaA* gene and TspE4.C2 DNA fragment. PCR is quickly screening of *E. coli* based on the presence or absence of these particular markers developed by Clermont *et al*. (2000). Figure (4-4) showed 1.5% agarose gel electrophoresis for *chuA* amplicon (288bp), *yjaA* amplicon (211 bp), & TspE4C2 amplicon (152 bp) respectively.

The results of 100 UPEC isolates showed that the majority of isolates were in phylogenetic group B2 (91/100)91%, and subgroup $B2_3$ (75/100) 75%, followed by group D (6/100) 6 %, and subgroup D_1 (4/100).4%, In table (4-3), the least prevalence isolates was in phylogenetic group A (1/100) 1%, and subgroup A_1 (1/100)1%, , followed by B1 (2/100) 2%.

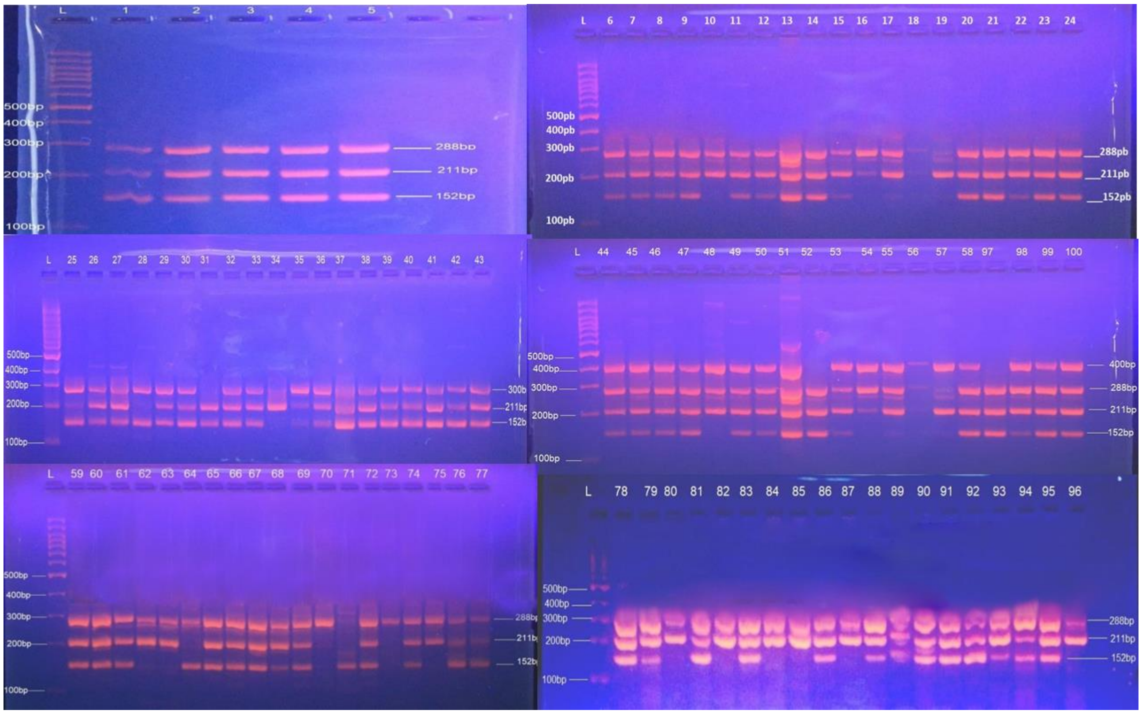

Figure 4-4: Agarose gel electrophoresis of *E. coli* genes used to classify *E. coli* into various phylogenetic groups (Lane M is 1kb DNA ladder, and lanes 1 to 100 are amplified PCR products with the following *E. coli* phylogenetic grouping genes; *chu A* (288 bp), *yja* A (211 bp) and TspE4C2 (152 bp). specimens were amplified on 1.5% agarose gel for 1 hour

Table 4-3 Distribution of UPEC isolates. according to Phylogenetic groups (A, B1, B2, and D) and Subgroups (A0/A1, B1, B2$_2$/B2$_3$, and D1/D2).

	Phylogentic group	**No. (%)**	**Subgroup**	**No. (%)**
	A	1 (1%)	**A_0**	0 (0%)
	B1	2 (2%)	**A_1**	1 (1%)
	B2	91 (91%)	**B_1**	2 (2%)
	D	6 (6%)	**$B2_2$**	16 (16%)
			$B2_3$	75 (75%)
			D_1	4 (4%)
			D_2	2 (2%)
Total		100 (100%)		100 (100%)

In parallel with current finding, earlier study in Wasit city, Gatya Al-Mayahie *et al.* (2022) recorded the highest frequency of UPEC isolates was in group B2 (33.9%), followed by group A (24.1%), group D (17.8%), and group B1 (8.0%). In Al-Hillah city reported that 76.77% of UPEC isolates were belonged to group B2 (Al-Zuhairy & Al-Dahmoshi, 2020), followed by group D, while in Baghdad city, 58% of UPEC isolates were found to belong to group B2 (Mohammed *et al.*, 2022).

Furthermore, in Thiqar city, Hussain and Saleh (2019) showed that 74% of UPEC isolates were belonged to phylogenetic group B2, followed by group A (13%), and group D (13%).in previous study conducted by Ahmed *et al.* (2019), UPEC isolates in phylogenetic groups B2 was the most common (36%) and groups B1 and D in same rates were the prevalent isolates in comparison with group A (8%) in Baghdad city.

Also, studies from different countries indicated that phylogenetic group B2 was the most prevalent among UPEC isolates. Haghighatpanah *et al.* (2022) revealed that the most common phylogenetic group among UPEC isolates in Iran was the B2 group (64.6%), followed by the D group (22.7%). In contrast, subgroup analyses showed that $B2_3$ (50.7%) and D_2 (16.4%) were the most common subgroups (Haghighatpanah *et al*. 2022).

Lin *et al.* (2022) documented that *E. coli* isolates belonged to phylogenetic group B2 (59.6%) were the most prevalent followed, by group D (20.7%), and group A (3.2%) in Taiwan. Javed *et al.* (2021) reported that phylogenetic group B2 (48%) was the most prevalent, followed by group A

(30%), group D(20%) and group B1 (2%) in Pakistan. Mahmoud *et al*. (2020) in Egypt recorded that the majority of UPEC isolates was belonged to phylogenetic group B2 (43%), followed by group D (36%), group A (19%) and group B1 (2%).

Lindblom (2020) in Sweden showed that 53% of UPEC isolates were belonged to phylogroup B2. Khairy *et al*. (2019) in Egypt reported different findings, UPEC isolates belonged to phylogenetic group A1 (31.1%) was the most prevalent, followed by B2 (23.3%), A0 (23.3%), and lastly D1 (12.6%), these results disagreement with our results.

4.5 Molecular detection of ESBL genes of UPEC isolates

The current study was established to detect the prevalence of ESBL production among *E. coli* isolates recovered from inpatients and outpatients with UTIs in Al-Hillah city/Babylon province/Iraq. The results displayed that 84% (126/150) of UPEC isolates were ESBL-producers Table (4-4). The findings achieved in the present study revealed that $bla_{\text{CTX-M}}$ genotype was the high prevalent ESBL-encoding gene. It was found in almost 76% (76/100) of the ESBL-producing *E. coli* figure (4-5), while bla_{TEM} figure(4-6) and bla_{SHV} figure(4-7) were found in 75% (75/100) and 73% (73/100) of isolates respectively.

Table 4-4 Distribution of UPEC isolates. according to ESBL genes

ESBL genes	No. (%)
bla$_{\text{CTX-M}}$	(76/100) 76%
bla$_{\text{TEM}}$	(75/100) 75%
bla$_{\text{SHV}}$	(73/100) 73%

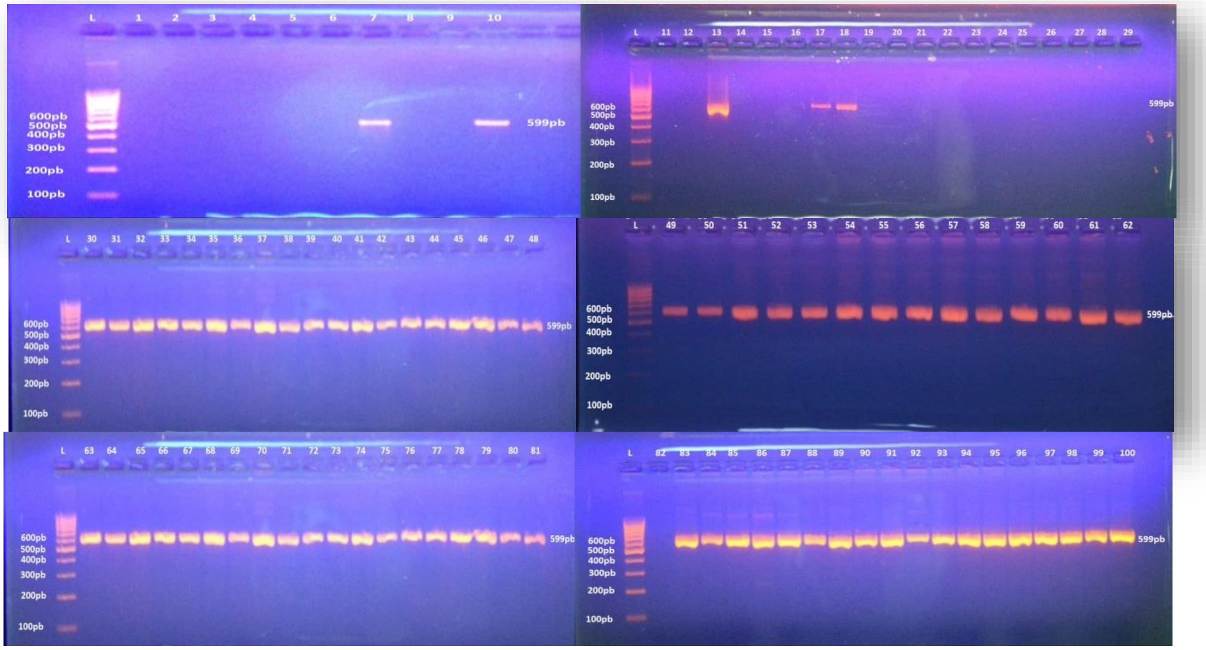

Figure4-5 Agarose gel electrophoresis of *bla*$_{\text{CTX-M}}$(599 bp) carrying *E. coli* isolates isolated from urinary tract infection patients. (Lane L is a 1kb DNA ladder, and lanes 1to 100are amplified *E. coli* PCR products. Samples were amplified on 1.5% agarose gel for 1 hour

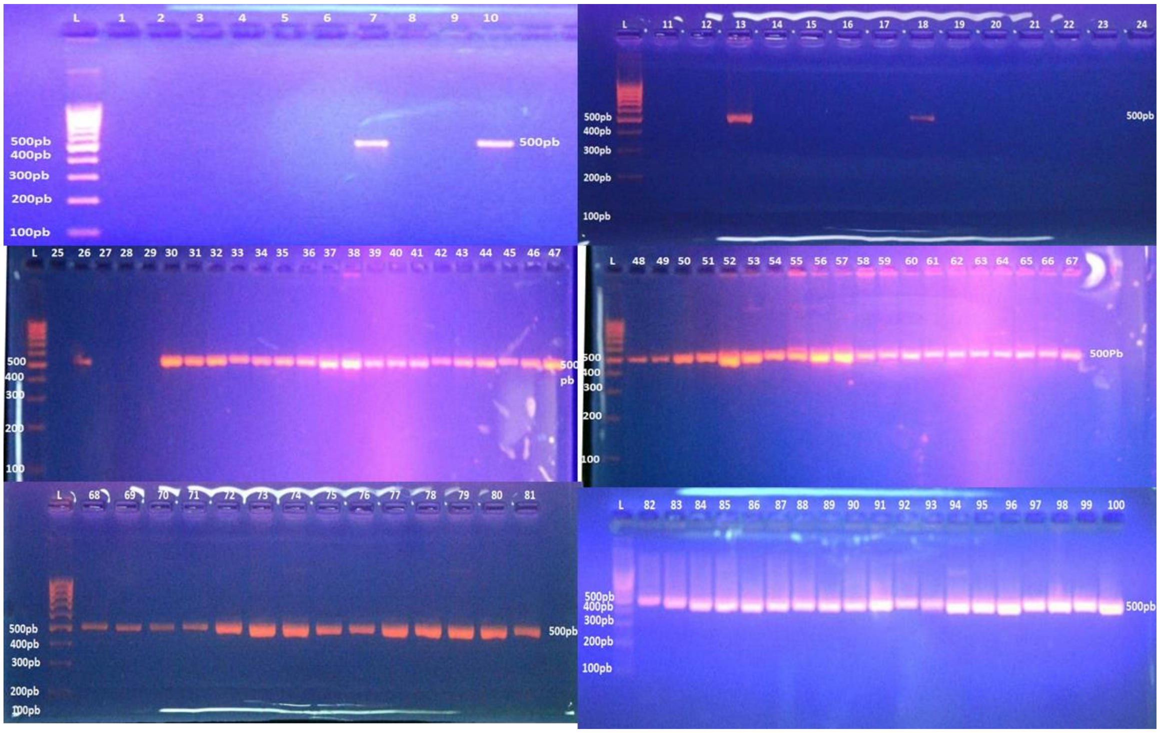

Figure 4-6 Agarose gel electrophoresis of *bla*TEM (500 bp) carrying *E. coli* isolates isolated from urinary tract infection patients. (Lane L is a 1kb DNA ladder, and lanes 1 to 100 are amplified *E. coli* PCR products. Samples were amplified on 1.5% agarose gel for 1 hour.

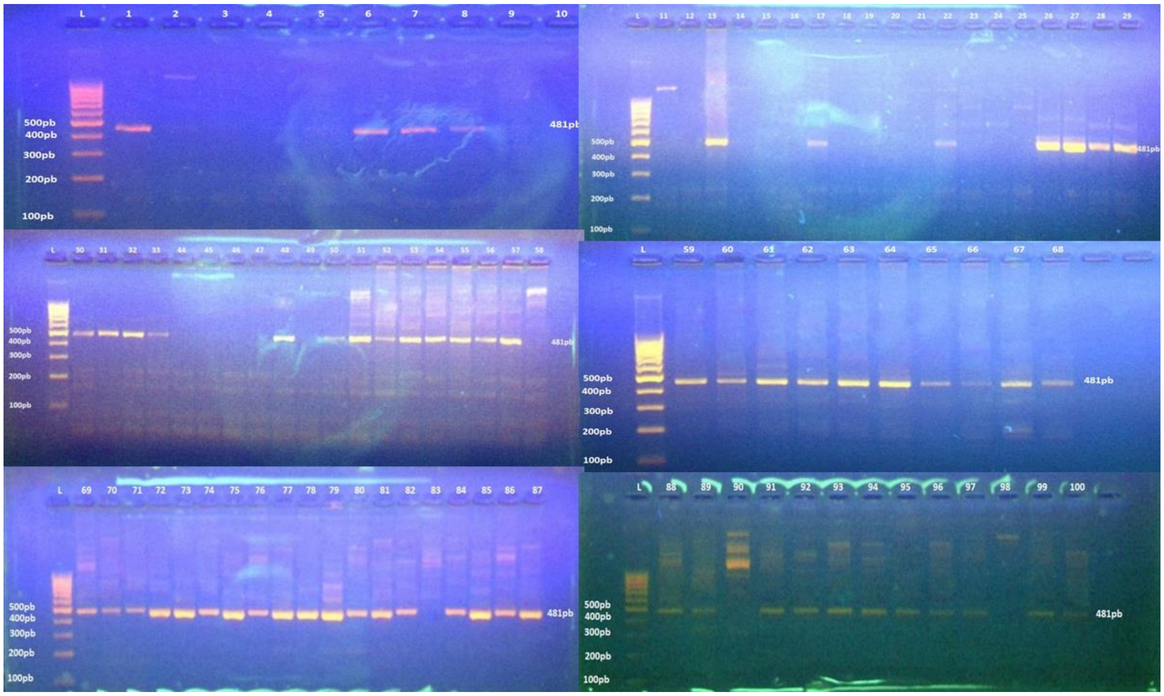

Figure 4-7 Agarose gel electrophoresis of *bla*$_{\text{SHV}}$ (481 bp) carrying *E. coli* isolates isolated from urinary tract infection patients. (Lane L is a 1kb DNA ladder, and lanes 1 to 100 are amplified *E. coli* PCR products. Samples were amplified on 1.5% agarose gel for 1hour.

In parallel with a current finding, earlier study in Kirkuk Kareem & Atiyea (2022) reported that 85% of UPEC isolates were ESBL-producers and the dissemination of *bla*$_{\text{CTX-M}}$ gene was (82.5%) among *E. coli* isolates. In Erbil city reported that 77% of UPEC isolates were positive for ESBL genes and the TEM gene was the most prevalent (81%) followed by CTX-M and SHV respectively (32%) and (16%) this results disagreement with our findings (Pishtiwan & Khadija., 2019).

Ali (2018) in Erbil city reported the CTX-M gene was the most prevalent ESBL gene in a percentage (73.4%). In Al-kut city, Al-Mayahie & Al Kuriashy (2016) recorded the highest frequency of UPEC isolates was the most prevalent to CTX-M gene (70%) followed by SHV gene (11%).

In Zakho city Polse *et al* (2016) reported that CTX-M gene was the most dominant; type (87.2%) while TEM and SHV genes were less dominant types (54.5%) and (21.8%) respectively. Furthermore, the high prevalence of ESBL-producing *E. coli* is associated with the misuse and overuse of antibiotics in Iraq, where many antibiotics are still purchased over the counter.

Our findings were a concordant with the results of other studies carried out in Egypt and Europe. In Egypt Hassuna *et al* (2020) reported that 59.7% of UPEC isolates were ESBL- producers. Muriuki *et al* (2022) in

Kenya recoded that 24% UPEC isolates were ESBL producers with *bla*$_{\text{CTX}}$-M (95.6%), *bla*$_{\text{TEM}}$ (95.6%), and *bla*$_{\text{SHV}}$ (21.7%).

Moghanni *et al* (2018) in Iran displayed (94.5%) of UPEC isolates were positive for CTX-M type, while (48.9%),(8.9%) were positive for TEM, and SHV, respectively. Kanfani *et al* (2016) in Lebanon showed that UPEC isolates were ESBL- producing (87%) the CTX-M gene was the most predominant (80%) followed by TEM gene (39%).

In India Thangavelu *et al* (2022) recorded that CTX-M was the most common ESBL (64 %), TEM (56 %) and SHV (40%). Alqasim *et al* (2018) in Saudi Arabia recorded that 33 % *E. coli* isolates were positive for ESBLs genotypic methods. Among all ESBL-positive *E. coli* isolates, the CTX-M was the most prevalent ESBL type (31/33 isolates; 93.94%).

Conclusions

&

Recommendations

Conclusions and Recommendations

Conclusion

The current study disclosed the high prevalence of ESBL-producing *E. coli* isolates in patients with UTI.

The analysis of the antibiotic resistance of *E. coli* isolates assayed in this study showed a high level of resistance to Aztreonam, Ceftriaxone, Cefpodoxime, Ceftazidime, Ceftazidime-Clavulantae, Cefotaxime, and Cefotaxime-Clavulantae.

The majority of *E. coli* isolates among patients with UTI in the Al-Hillah hospitals belonged to phylogenetic group B2 followed by D, B1 and A.

Escheichia coli in the phylogenetic group B2/ subtyping $B2_3$ also showed the highest prevalence of ESBL genes (TEM, SHV, and CTX-M) and biofilm production.

findings indicated that the members of phylogenetic group B2 isolates may serve as reservoirs for the dissemination of ESBL genes in health care setting.

Recommendation

findings suggested that systems for antibiotic susceptibility surveillance and the detection of molecular analysis of ESBL-producing bacteria in Iraqi hospitals must be established.

Also strict hospital infection control policies with restriction of the consumption of expanded-spectrum cephalosporins are necessary.

Caution is urgently needed to prevent inappropriate and indiscriminate consumption of antibiotics as medications, especially in developing countries such as Iraq, where the drugs can be obtained without prescription due to lack of drug regulation.

Formation a relationship between phylogentic group(A,B1,B2 and D) with biofilm

Study a new ESBL genes such as CMY, G1C.

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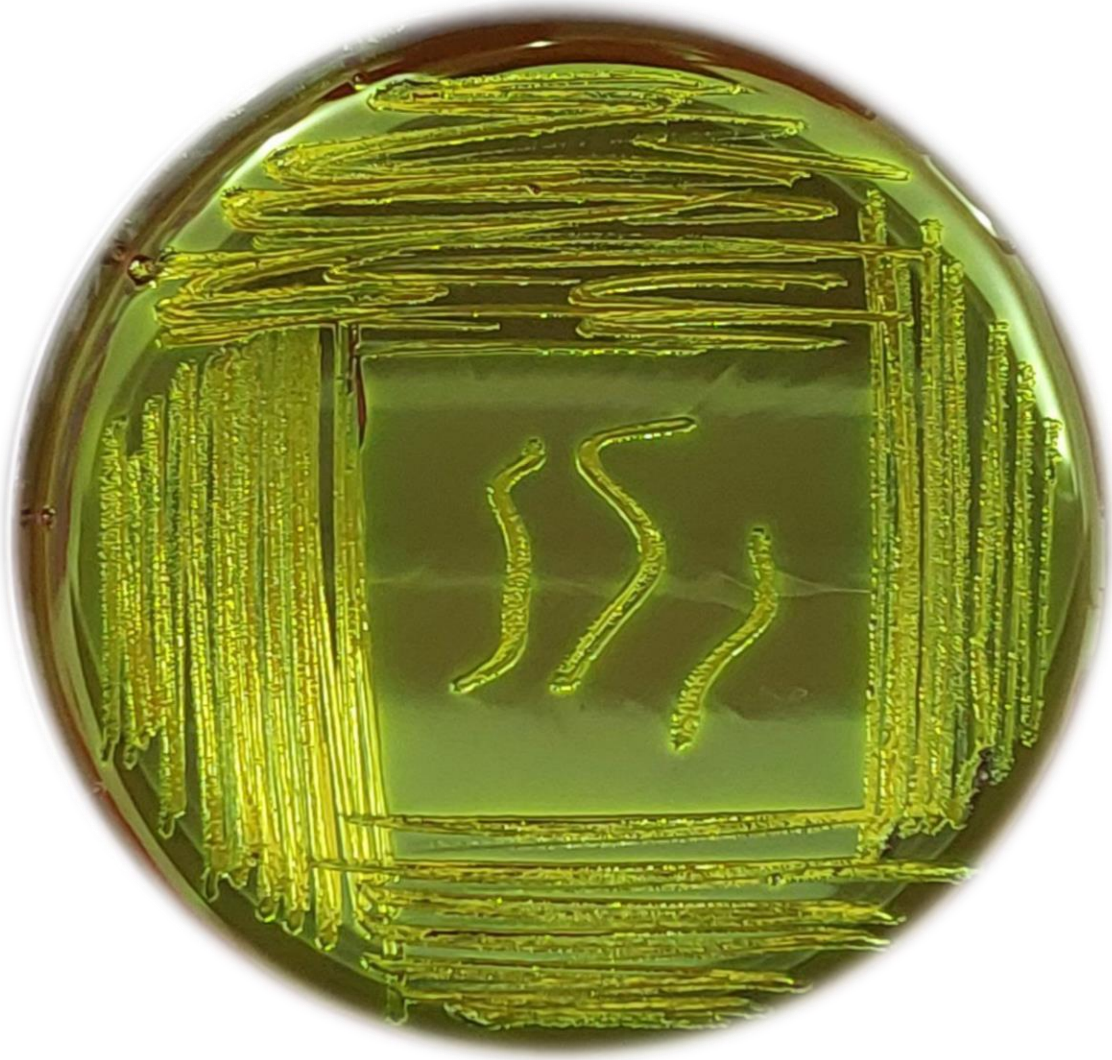
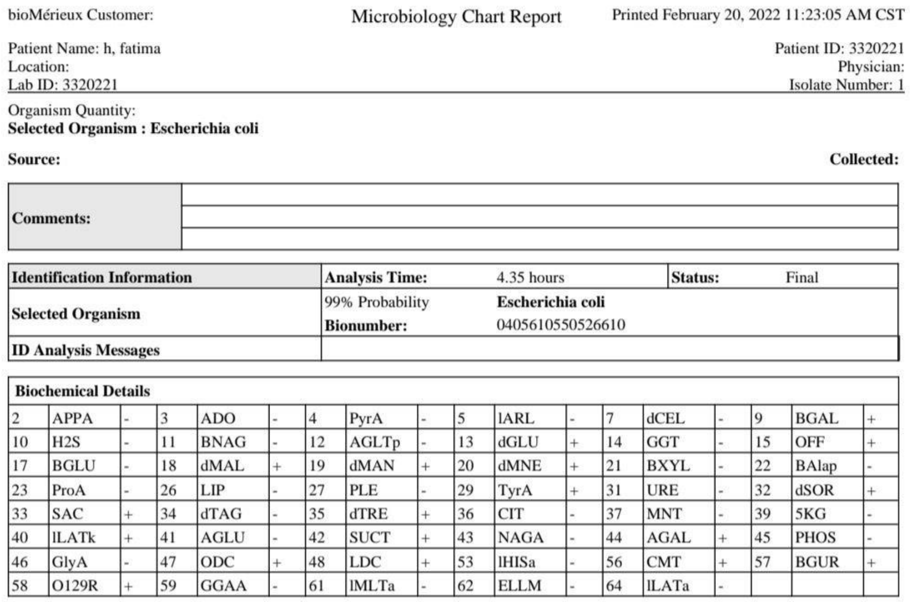
Appendix

Appendix (1): *E. coli* isolates on EMB agar



Appendix (2): Examination report of *E.coli* isolates with VITEK 2 system



bioMérieux Customer: Microbiology Chart Report Printed February 20, 2022 11:23:05 AM CST

Patient Name: h, fatima
Location:
Lab ID: 3320221

Patient ID: 3320221
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

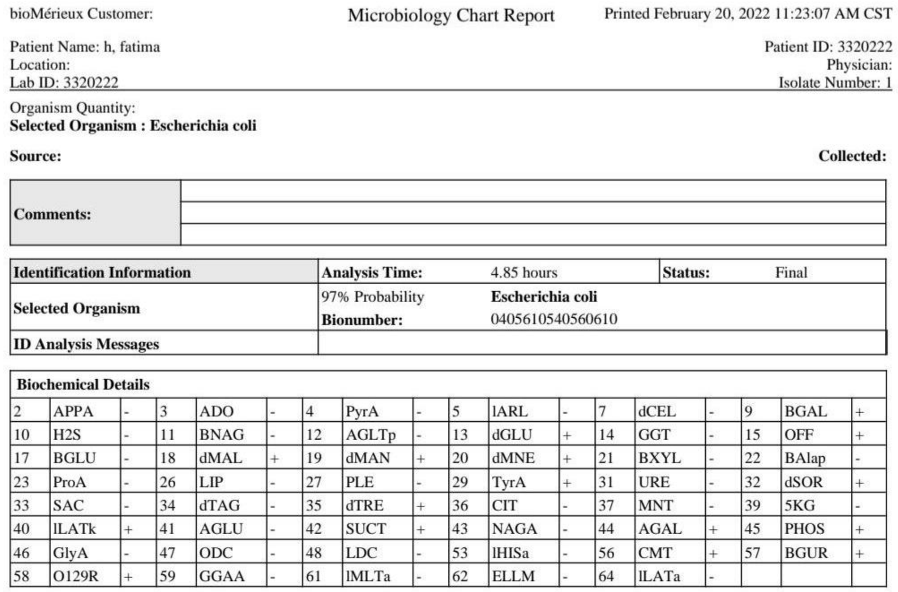
Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.35 hours	**Status:**	Final
Selected Organism	99% Probability	**Escherichia coli**		
	Bionumber:	0405610550526610		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			



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Patient Name: h, fatima
Location:
Lab ID: 3320222

Patient ID: 3320222
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

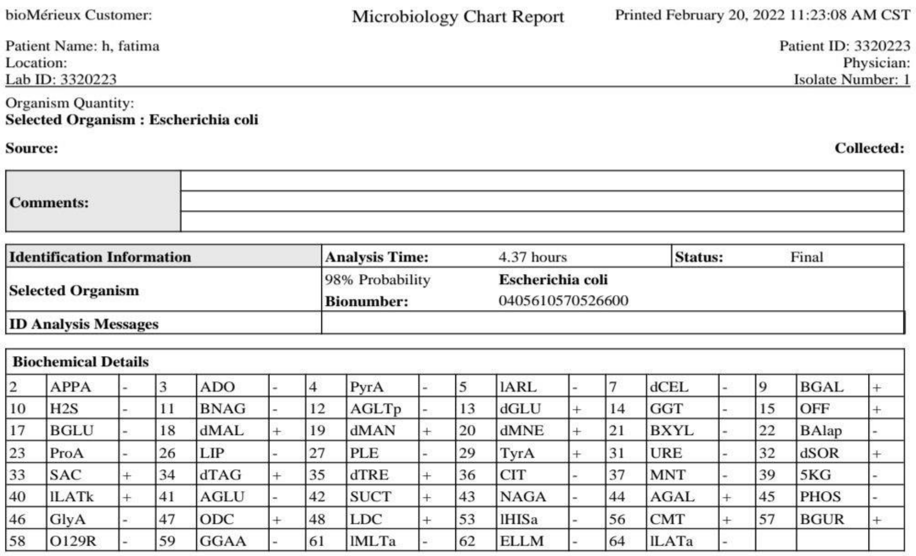
Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.85 hours	**Status:**	Final
Selected Organism	97% Probability	**Escherichia coli**		
	Bionumber:	0405610540560610		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			



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Patient Name: h, fatima
Location:
Lab ID: 3320223

Patient ID: 3320223
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

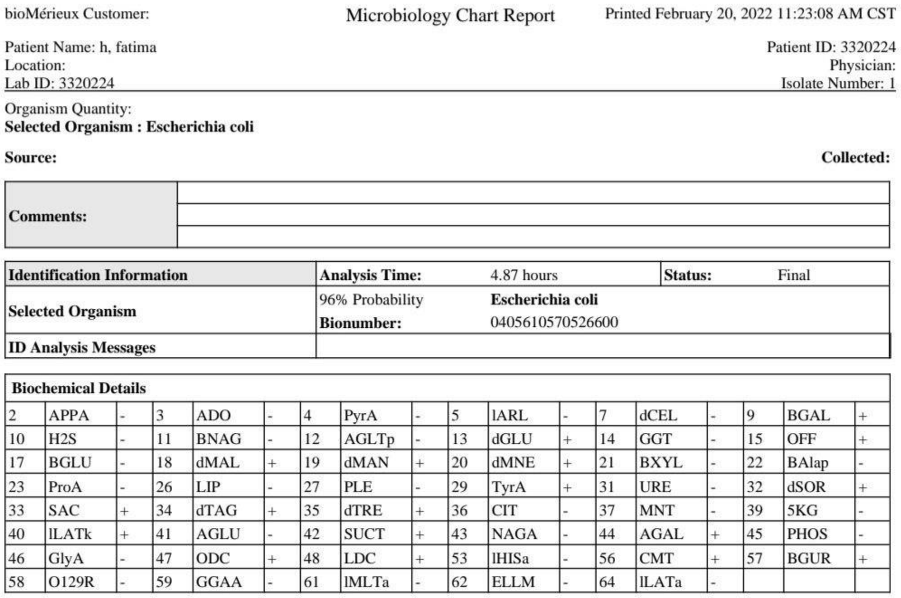
Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.37 hours	**Status:**	Final
Selected Organism	98% Probability **Bionumber:**	**Escherichia coli** 0405610570526600		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			



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Location:
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Patient ID: 3320224
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

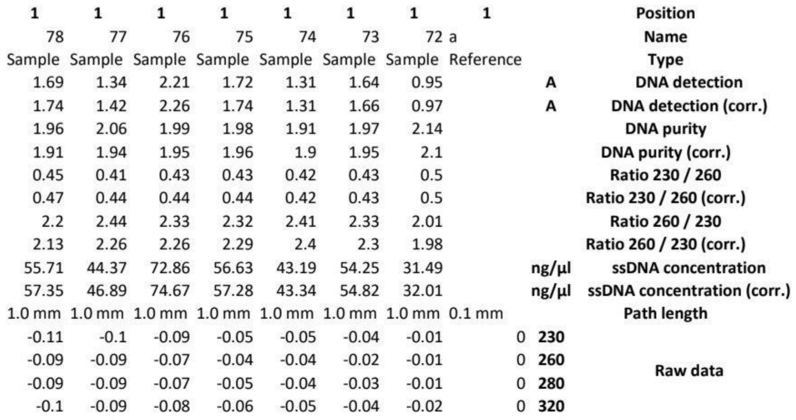
Comments:	

Identification Information	**Analysis Time:**	4.87 hours	**Status:**	Final
Selected Organism	96% Probability **Bionumber:**	**Escherichia coli** 0405610570526600		
ID Analysis Messages				

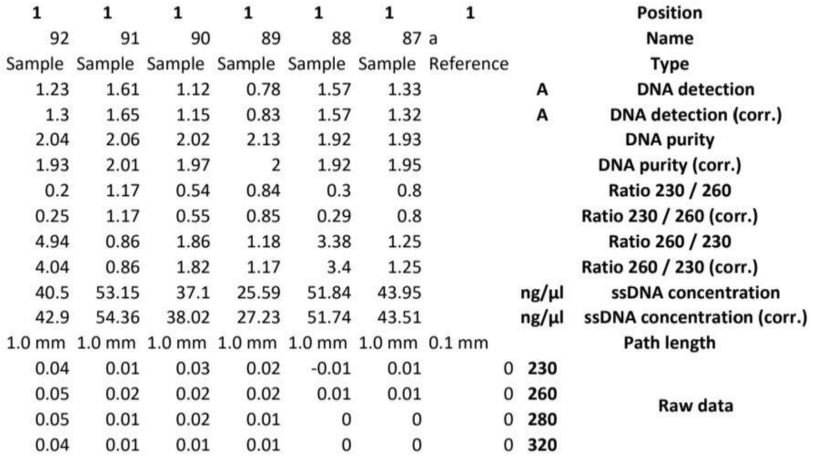
Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Appendix (3): Examination report of *E.coli* isolates with nano drop system to detect DNA purification and concenteration.

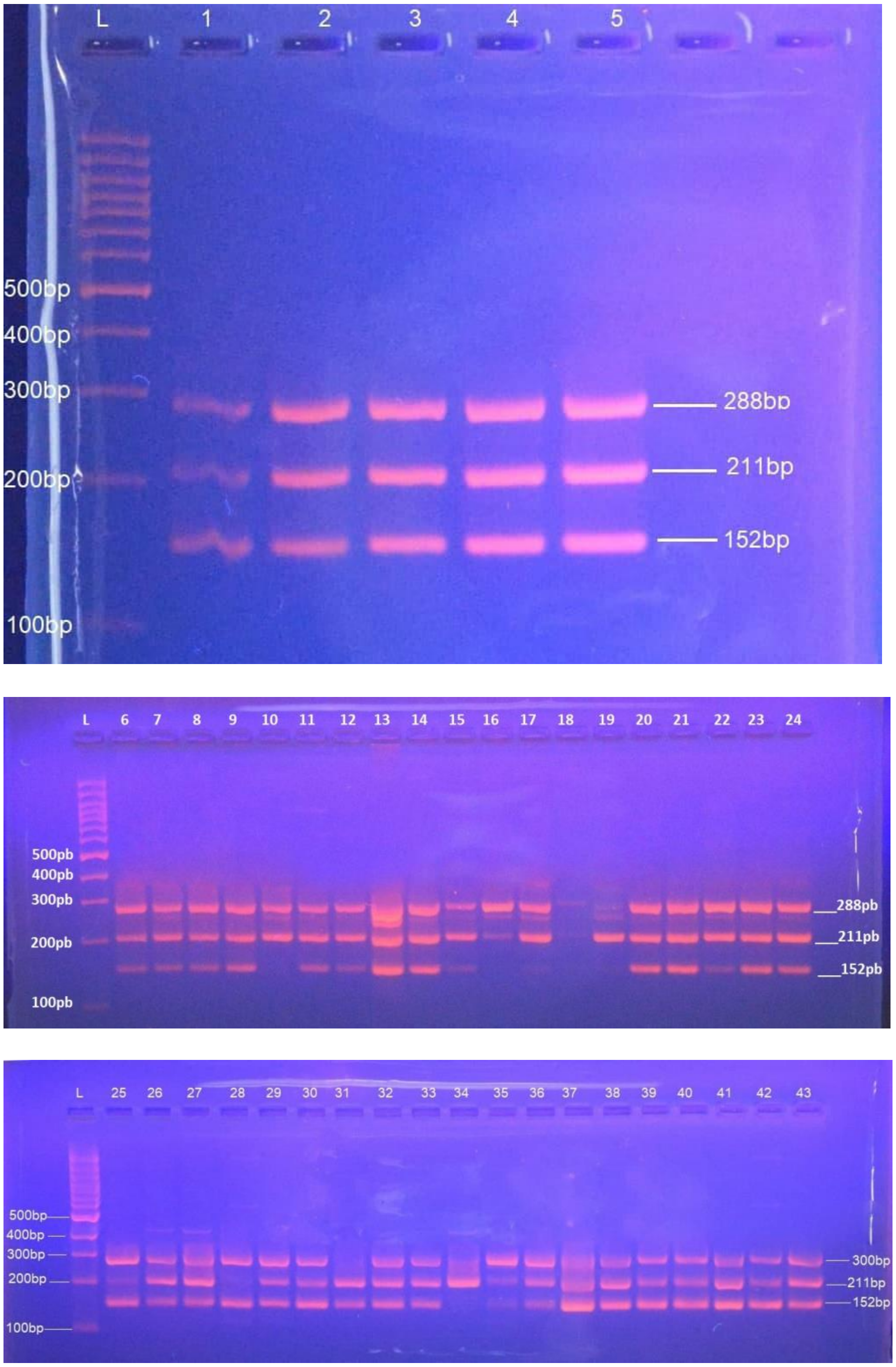


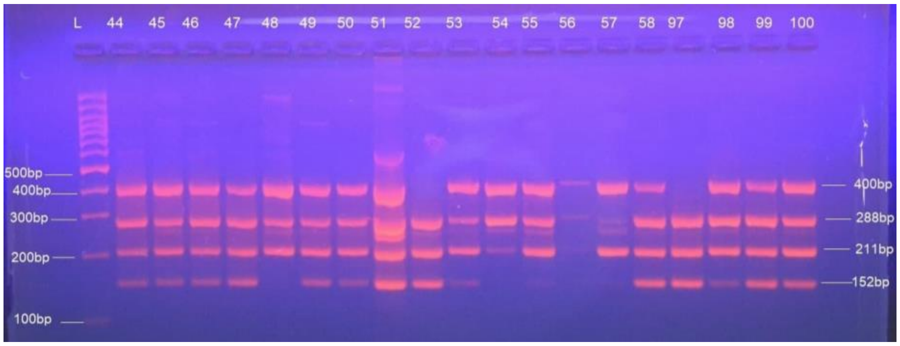
1	1	1	1	1	1	1	1		**Position**
78	77	76	75	74	73	72	a		**Name**
Sample	Sample	Sample	Sample	Sample	Sample	Sample	Reference		**Type**
1.69	1.34	2.21	1.72	1.31	1.64	0.95		**A**	**DNA detection**
1.74	1.42	2.26	1.74	1.31	1.66	0.97		**A**	**DNA detection (corr.)**
1.96	2.06	1.99	1.98	1.91	1.97	2.14			**DNA purity**
1.91	1.94	1.95	1.96	1.9	1.95	2.1			**DNA purity (corr.)**
0.45	0.41	0.43	0.43	0.42	0.43	0.5			**Ratio 230 / 260**
0.47	0.44	0.44	0.44	0.42	0.43	0.5			**Ratio 230 / 260 (corr.)**
2.2	2.44	2.33	2.32	2.41	2.33	2.01			**Ratio 260 / 230**
2.13	2.26	2.26	2.29	2.4	2.3	1.98			**Ratio 260 / 230 (corr.)**
55.71	44.37	72.86	56.63	43.19	54.25	31.49		**ng/µl**	**ssDNA concentration**
57.35	46.89	74.67	57.28	43.34	54.82	32.01		**ng/µl**	**ssDNA concentration (corr.)**
1.0 mm	1.0 mm	1.0 mm	1.0 mm	1.0 mm	1.0 mm	1.0 mm	0.1 mm		**Path length**
-0.11	-0.1	-0.09	-0.05	-0.05	-0.04	-0.01	0	**230**	**Raw data**
-0.09	-0.09	-0.07	-0.04	-0.04	-0.02	-0.01	0	**260**	
-0.09	-0.09	-0.07	-0.05	-0.04	-0.03	-0.01	0	**280**	
-0.1	-0.09	-0.08	-0.06	-0.05	-0.04	-0.02	0	**320**	



1	1	1	1	1	1	1		**Position**
92	91	90	89	88	87	a		**Name**
Sample	Sample	Sample	Sample	Sample	Sample	Reference		**Type**
1.23	1.61	1.12	0.78	1.57	1.33		**A**	**DNA detection**
1.3	1.65	1.15	0.83	1.57	1.32		**A**	**DNA detection (corr.)**
2.04	2.06	2.02	2.13	1.92	1.93			**DNA purity**
1.93	2.01	1.97	2	1.92	1.95			**DNA purity (corr.)**
0.2	1.17	0.54	0.84	0.3	0.8			**Ratio 230 / 260**
0.25	1.17	0.55	0.85	0.29	0.8			**Ratio 230 / 260 (corr.)**
4.94	0.86	1.86	1.18	3.38	1.25			**Ratio 260 / 230**
4.04	0.86	1.82	1.17	3.4	1.25			**Ratio 260 / 230 (corr.)**
40.5	53.15	37.1	25.59	51.84	43.95		**ng/µl**	**ssDNA concentration**
42.9	54.36	38.02	27.23	51.74	43.51		**ng/µl**	**ssDNA concentration (corr.)**
1.0 mm	1.0 mm	1.0 mm	1.0 mm	1.0 mm	1.0 mm	0.1 mm		**Path length**
0.04	0.01	0.03	0.02	-0.01	0.01	0	**230**	**Raw data**
0.05	0.02	0.02	0.02	0.01	0.01	0	**260**	
0.05	0.01	0.02	0.01	0	0	0	**280**	
0.04	0.01	0.01	0.01	0	0	0	**320**	

Appendix (3): Representative agarose gel electrophoresis of *E. coli* genes used to classify *E. coli* into various phylogenetic groups (Lane M is 1kb DNA ladder, and lanes 1 to 100 are amplified PCR products with the following *Escherichia coli* phylogenetic grouping genes; *chu A* (288 bp), *yja A* (211 bp) and TspE4C2 (152 bp). Samples were amplified on 1.5% agarose gel.

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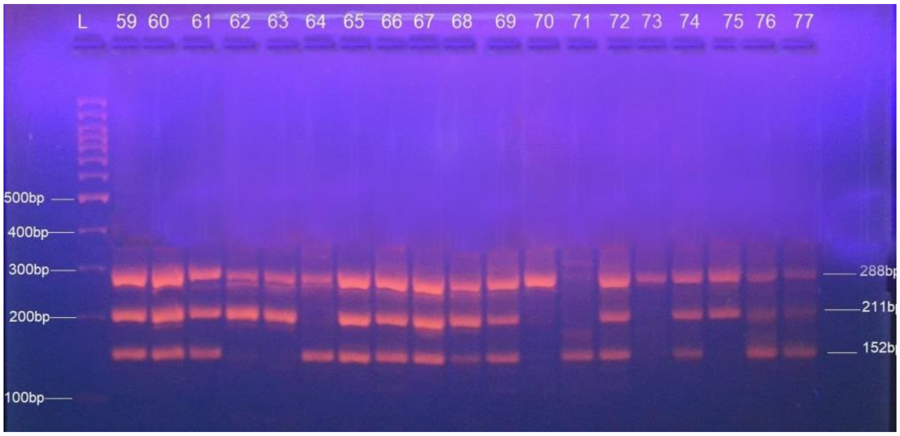
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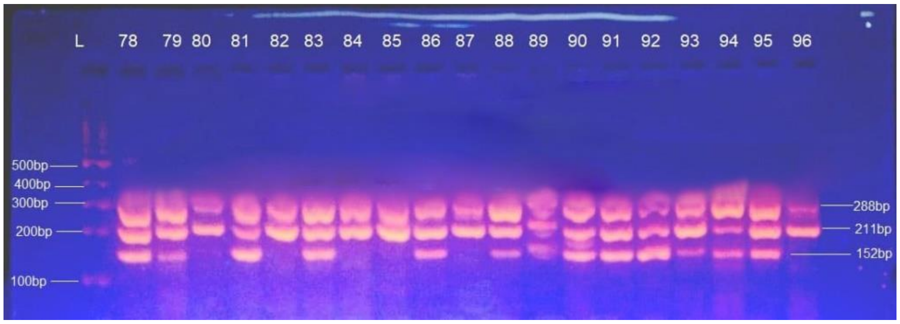
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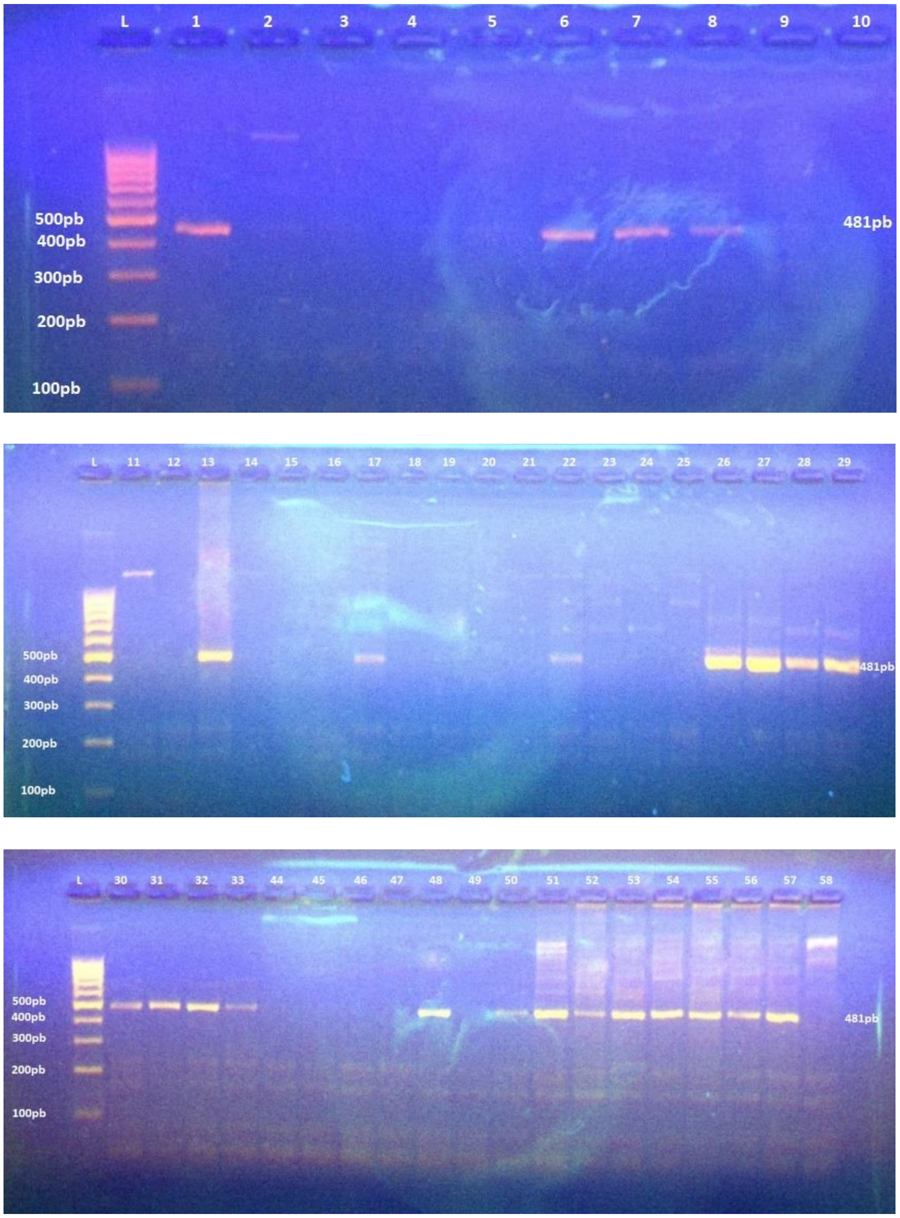
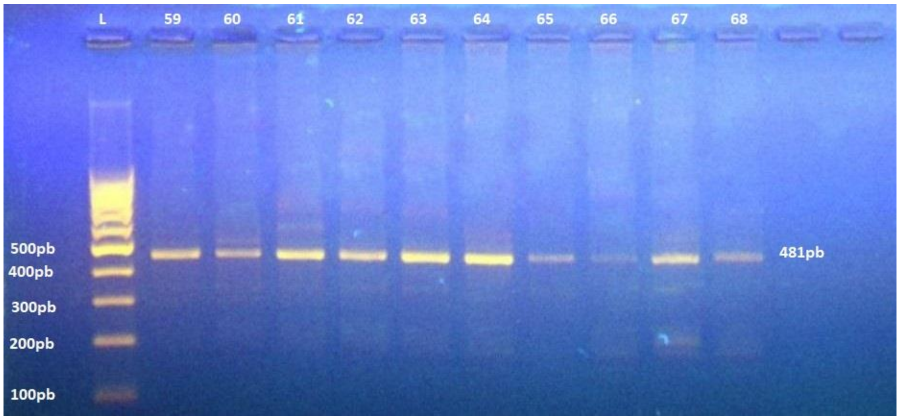
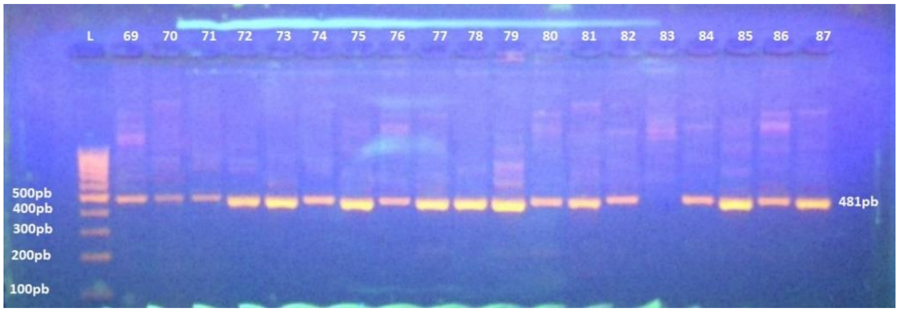
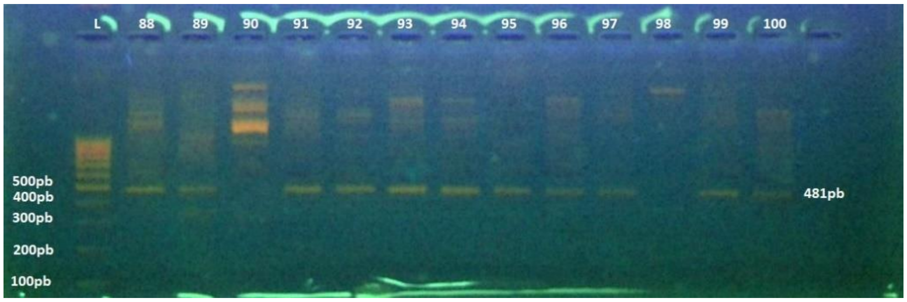
211bp
152bp

Appendix (4): Representative-agarose gel electrophoresis of bla_{SHV} (481 bp) carrying *E. coli* isolates isolated from urinary tract infection patients. (Lane L is a 1kb DNA ladder, and lanes 1 to 100 are amplified *E. coli* PCR products. Samples were amplified on 1.5% agarose gel.

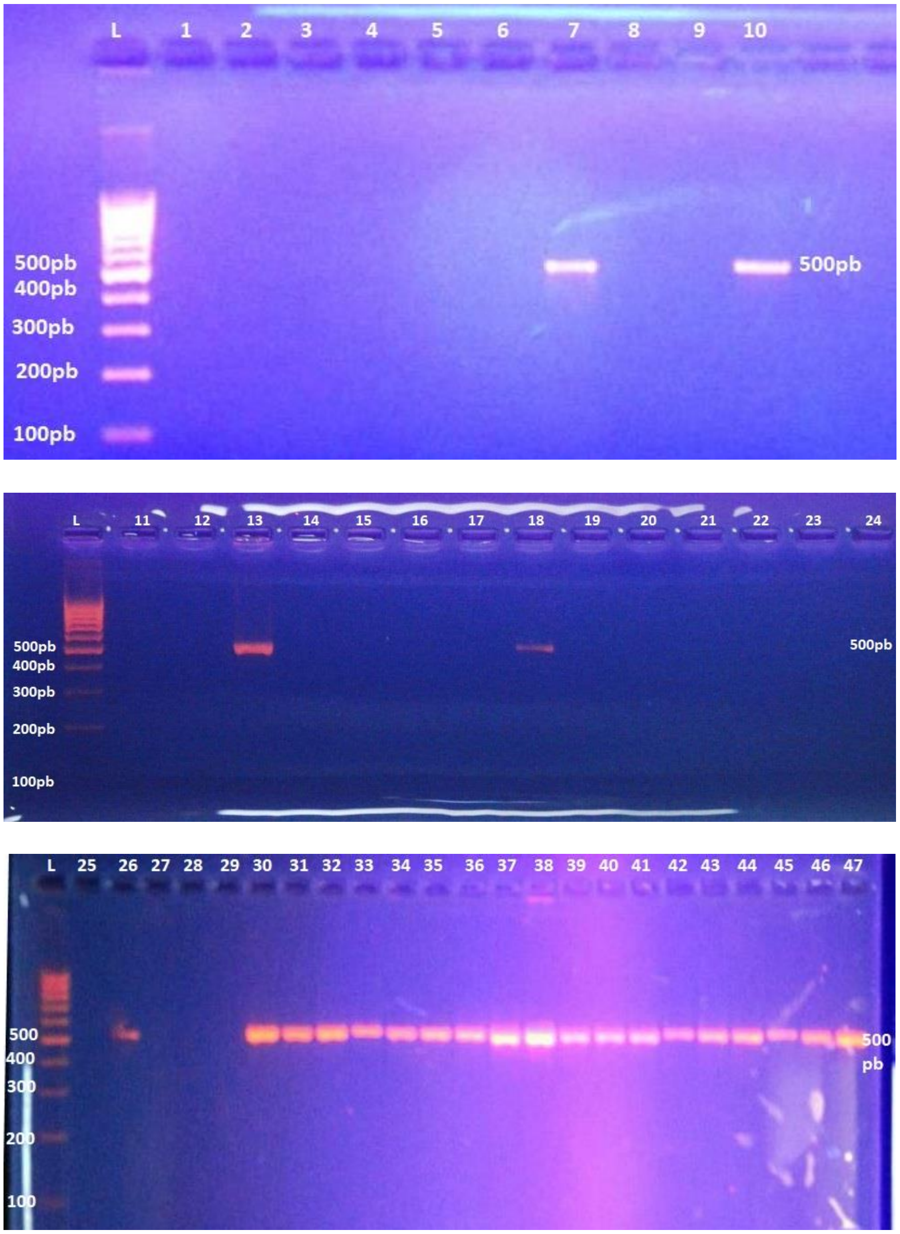


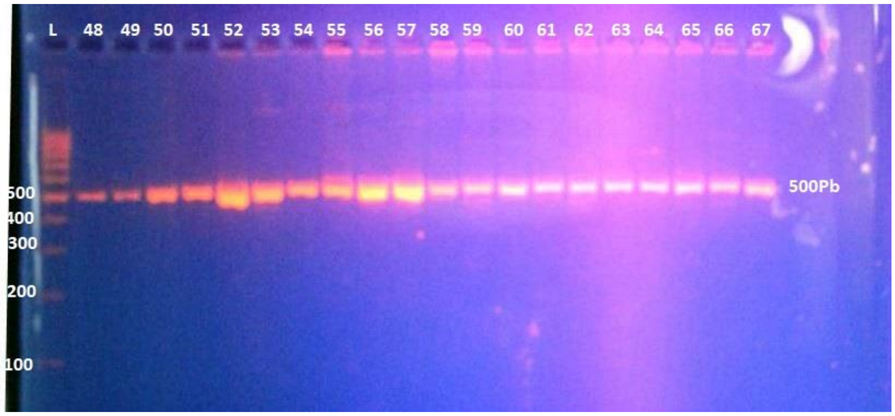

L
59
60
61
62
63
64
65
66
67
68
500pb
400pb
300pb
200pb
100pb
481pb

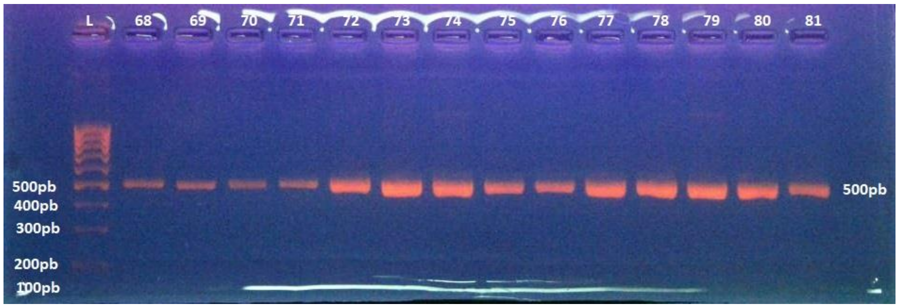

L
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
500pb
400pb
300pb
200pb
100pb
481pb

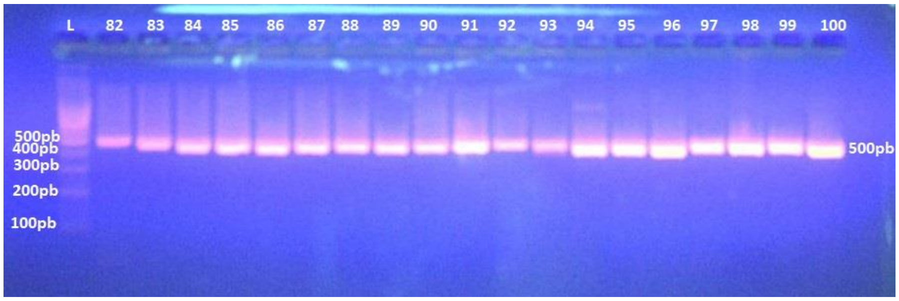

L
88
89
90
91
92
93
94
95
96
97
98
99
100
500pb
400pb
300pb
200pb
100pb
481pb

Appendix(5): Representative–agarose gel electrophoresis of *bla*$_{\text{TEM}}$ (500 bp) carrying *E. coli* isolates isolated from urinary tract infection patients. (Lane L is a 1kb DNA ladder, and lanes 1 to 100 are amplified *E. coli* PCR products. Samples were amplified on 1.5% agarose gel.

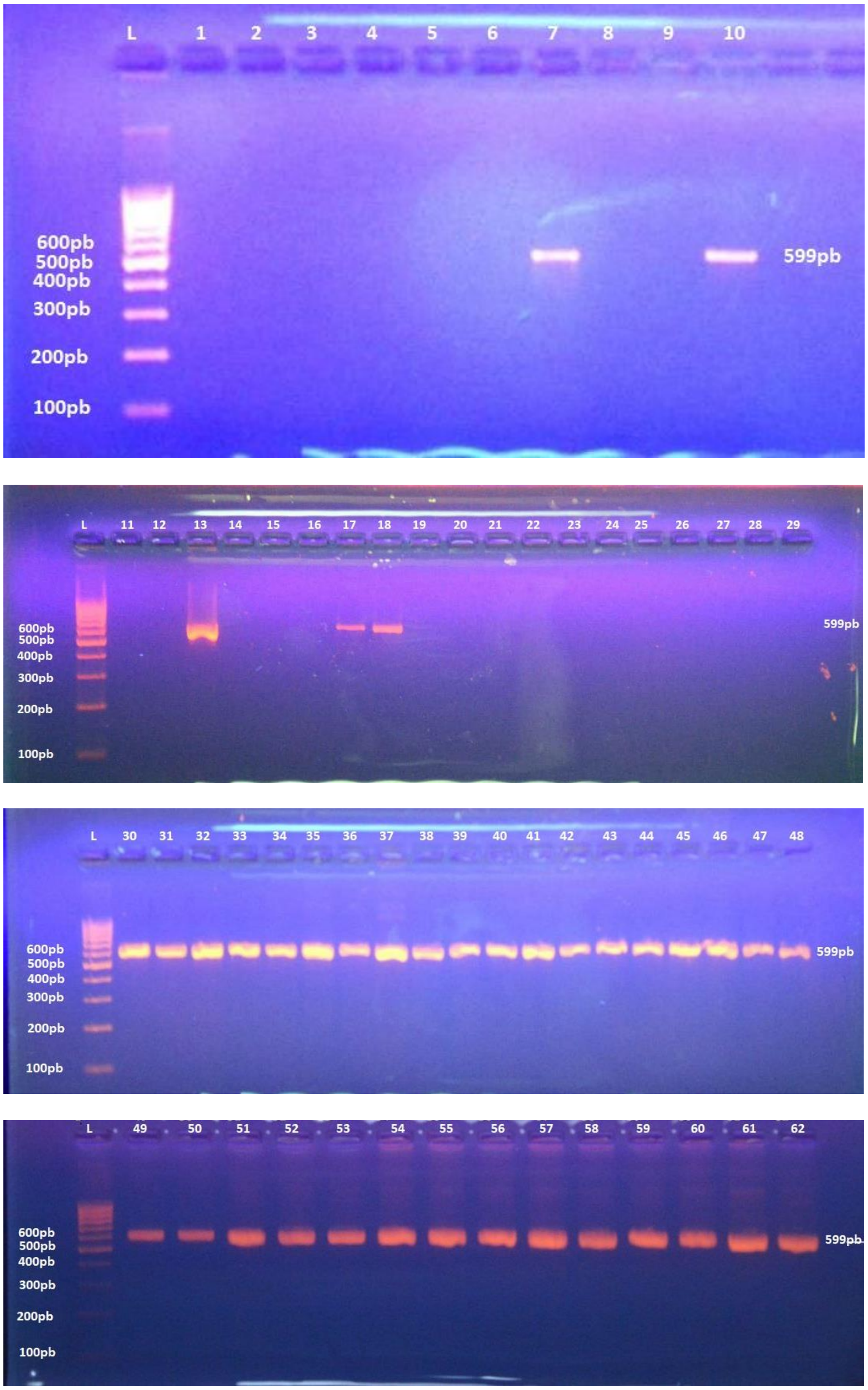


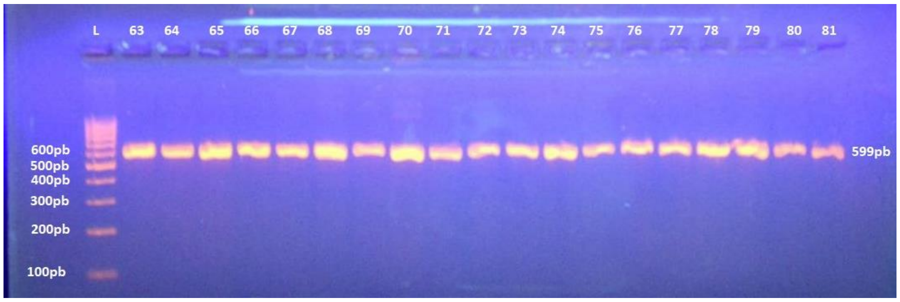

L 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67
500
400
300
200
100
500Pb

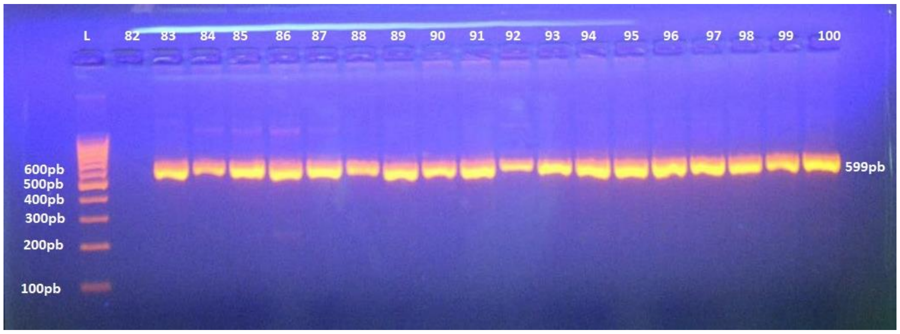

L 68 69 70 71 72 73 74 75 76 77 78 79 80 81
500pb
400pb
300pb
200pb
100pb
500pb


L 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
500pb
400pb
300pb
200pb
100pb
500pb

Appendix(6): Representative agarose gel electrophoresis of blaCTX-M (599 bp) carrying *E. coli* isolates isolated from urinary tract infection patients. (Lane L is a 1kb DNA ladder, and lanes 1 to 100 are amplified *E. coli* PCR products. Samples were amplified on 1.5% agarose gel.




L
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
600pb
500pb
400pb
300pb
200pb
100pb
599pb


L
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
600pb
500pb
400pb
300pb
200pb
100pb
599pb

الخلاصه

إن إنزيمات البيتا لاكتاميز ذات الطيف الواسع (ESBLs) هي إنزيمات تنتجها العائلة المعويه Enterobacteriaceae التي تتوسط مقاومة البنسلين والجيل الثالث من السيفالوسبورينات والمونوباكتام. الإشريكية القولونية هي أكثر مسببات الأمراض شيوعًا وتعتبر كمستودع لانزيمات البيتا لاكتاميز . يزداد انتشار ESBL بشكل واسع ويسبب صعوبه في علاج الأمراض المعدية. وكذلك تشكل تهديدًا صحيًا عالميًا خطيرًا وأصبح تحديًا لمقدمي الرعاية الصحية.

تم جمع 204 عينة ادرار من مرضى تم تشخيص إصابتهم بعدوى السبيل البولي من مستشفيات الحلة والمختبرات الخاصه. خلال الفترة ممتدة من تشرين الاول 2021 إلى نيسان 2022. واوضحت النتائج ان (204/150) 73.5 ٪ ، من هذه العينات هي بكتريا اشريكيا القولونيه وأخرى هي الكلبسيلا الرئويه بنسبة (204/54) 26.5٪ تم الكشف عن عزلات الاشريكيا القولونيه بواسطة الزرع على اوساط Eosin methylene blue و MacConkey's agar للتشخيص. تم تحديد المزيد من العزلات على أنها الايشيريكيا القولونيه باستخدام نظام الفايتك .

تم اجراء فحص حساسيه المضادات الحياتيه لانزيمات البيتا لاكتاميز تجاه سبعة انواع من المضادات و أظهرت النتائج أن (150/109) 72.7٪ من عزلات الإشريكيا القولونية هي نتائج إيجابية لإنتاج ESBL ، بينما أظهرت(150/41) 27.3٪ عزلات الإشريكيا القولونية نتائج سلبية. لإنتاج ESBL. إجمالي 109 عزلة ، كانت المقاومة عالية ل Ceftazidime/Clavulanta (CZC) و (CTC)Cefotaxime/Clavulantae بنسبة 100% ؛ كانت المقاومة بنسبة 86.66٪ للمضاد Cefotaxime (CTX) و المضاد Ceftazidime (CAZ) بنسبة 78.66٪ و المضاد Aztreonam (ATM) بنسبة 84٪ والمضاد Ceftriaxone (CRO) بنسبة 87.33٪ ؛ اما المضاد Cefpodoxime (CPD) بنسبة 83.33٪.

تم الكشف عن تكوين الأغشية الحيوية بواسطة اكار الكونغو الاحمر(CRA) وأظهرت النتائج أن من اصل 100 عزلة من الإشريكيا القولونيه المسببة لالتهاب السبيل البولي(100/71) 71 % عزلة كانت منتجة للغشاء الحيوي بينما(100/29) 29٪ عزلة كانت غير منتجه للاغشية الحية.

استخدم في هذة الدراسة تفاعل البلمره المتسلسل Multiplex PCR لتحديد جينات ال Phylogenetic group والمجاميع الفرعية Subgroup لجينات yjaA(211pb) ؛ chuA(288pb) ؛ TspE4.C2(152pb) ل 100 عزلة بكتيرية وأظهرت النتائج ان الغالبية من العزلات تنتمي لمجموعة B2 بنسبة (100/91) 91% والمجموعة الفرعية $B2_3$ بنسبة (100/75) 75% ؛ تليها المجموعة D (100/6) 6% والمجموعة الفرعية D1 (100/4) 4% وان العزلات الاقل انتشارا تنتمي للمجموعة A (100/1) 1% والمجموعة الفرعيه A1 (100/1) 1% تليها المجموعةB1 (100/2) بنسبة 2% .

تم إجراء الكشف الوراثي عن جينات انزيمات البيتا لاكتاميز ذات الطيف الواسع ESBL (bla_{SHV} bla_{TEM} $bla_{CTX\text{-}M}$) باستخدام تقنية تفاعل البلمرة (PCR). كشفت الدراسة الحالية عن وجود نسبة عالية (150/126) 84٪ من عزلات UPEC المنتجة للـ ESBL بين مرضى مدينة الحلة. وأظهرت النتائج أن النمط الجيني $bla_{CTX\text{-}M}$ هو السائد. بنسبة 76٪ (100/76) من الاشيريكيا القولونيه المنتجة لانزيمات البيتا لاكتاميز ، بينما كانت نسبه bla_{TEM} وbla_{SHV} هي 75٪ (100/75) و 73٪ (100/73) من العزلات على التوالي. تحليل الاكتشاف الوراثي لعزلات الإشريكيا القولونية المنتجة لـ ESBL CTX-M التابعة لمجموعة ExPEC Phylogenetic group B2.

تشير نتائج الدراسة الحالية إلى أن عينات عدوى السبيل البولي التي تم تحليلها قد تكون بمثابة مصدر أساسي لانتشار الإشريكيا القولونية المنتجة لـ ESBL والممرضة، وهو أمر مهم لفهم الظروف الحالية للأشخاص المقيمين في الحلة ، مثل أظهرت البيانات انتشارًا عاليًا لـ ESBL بين عزلات الإشريكيا القولونية.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل، كلية العلوم
قسم علوم الحياة

الكشف الجزيئي عن جينات ذات الطيف الواسع لأنزيمات البيتا لاكتاميز في عزلات الايشريكيا القولونية المسببة لخمج السبيل البولي

رسالة مقدمة الى
مجلس كلية العلوم جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير
في العلوم/ علوم حياة

من قبـل

(فاطمه عباس ناصر حسين)
(بكالوريوس علوم حياة/ جامعة بابل / 2019)

بإشراف
أ.د.مروج سعدي علوش

1444 هـ   2023م