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Phenotypic and Molecular Study of Some Extended Spectrum Beta-lactamases Genes in *Escherichia coli* Isolated from Clinical Cases in Misan Province /Iraq

A Thesis

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيماً﴾

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Supervisor Certification

This is to certify that this thesis entitled (**Phenotypic and Molecular Study of Some Extended Spectrum Beta-lactamases Genes in *Escherichia coli* Isolated from Clinical Cases in Misan Province /Iraq)** was prepared under my supervision at the Department of Biology, College of Science, University of Misan, as partial requirement of degree of Master in Biology .

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Dedication

To the soul that left me…My great father

To the mercy of God on earth…My mother

To the supporter in all my life…My husband

To my happiness and my wealth…My children

To my brothers and sister

To our martyrs heroes

Rabab

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Summary

This study is conducted to detect Extended Spectrum β- Lactamase (ESBLs) phenotypically and genotypically in *Escherichia. coli* isolating from different clinical cases (urine, seminal fluid, wound swab and blood) from main hospitals in Maysan province, completed in a period from October 2018 till end of December 2018.

Two hundred and ninety one (n= 291) samples were collected from clinical cases and cultured on Blood and MacConkey agar media for primary isolation of bacteria, further cultured on selective and differential media such as (EMB and chrome agar). Microscopic and Morphological examination, conventional biochemical tests, API 20E system and Vitek 2 system were used to confirm identification of *E. coli*. Antimicrobial susceptibility test is performed using Modified Kirby-Bauer method. Furthermore, screening test and Double Disc Synergy Test (DDST) were used to determine ESBLs *E. coli* producers. The results revealed that positive bacterial growth appeared in 235 isolate [138 urine, 28 seminal fluid, 30 wound swab and 39 blood]. The number of *E. coli* among positive growth were 105 isolates and the most common were isolated from urine 91 isolates, followed by seminal fluid 6, wound swab 4, and blood 4 isolates. Among 105 *E. coli*, ESBLs producers were detected in 87(82.9%) in screening test, while in confirmatory test the results were 19(21.8%).

The results of antibiotic susceptibility test showed that all *E. coli* were fully sensitive to imipenem(100%), followed by amikacin (90.5%), while ampicillin, piperacillin, augmentin, oxacillin, were the lowest effective antibiotics against *E. coli* where their resistances were 98.1%, 96.2%, 90.5% and 93.3% respectively, in addition to the resistance to cephalosporin's; cefotaxime, ceftazidime, cefpodoxime, ceftriaxone, cefipeme and cefoxitin were 94.3%, 93.3%, 93.3%, 87.6%, 92.4% and 86.7% respectively.

Other antibiotics showed a variable degree of resistance where it were recorded as following 81.9%, 68.6%, 53.3%, 46.7%, 35.2%, and 34.3% to azetreonam,

trimethoprim/sulphamethoxazole, gentamicin, norfloxacin, nitrofuranition and ciprofloxacin respectively. All identified *E. coli* (n=105) were submitted to molecular detection of ESBLs genes on chromosome and plasmid (bla_{TEM}, bla_{SHV}, bla_{OXA} and bla_{CTX-M}) using monoplex PCR technique. The frequency of the detected genes among *E. coli* were as following; 100% of isolates carried bla_{CTX-M}, bla_{OXA} *and* bla_{TEM} genes on chromosome, while bla_{SHV} was detected in 45.7%. On Plasmid was 100% for bla_{TEM} *and* bla_{CTX-M} genes whereas 99.05%, 41% for bla_{OXA} and bla_{SHV} genes respectively.

The present study revealed that there are high prevalence of antibiotic resistance especially to Beta-lactam antibiotics mediated by ESBLs as indicated by screening test, furthermore the molecular study showed high levels of occurrence of bla_{CTX-M}, bla_{OXA}, bla_{TEM} genes irrespective of the origin.

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List of Abbreviations

Abbreviations	Meaning
A/E	**Attaching and Effacing**
Api-20E	**Analytical profile index for Enterobacteriaceae**
BCI	**blood Culture Infection**
Bla	**β-Lactamase encoding gene**
CLSI	**Clinical and Laboratory Standards Institute**
CPS	**Capsule polysaccharide**
CRE	**Carbapenem-resistant Enterobacteriaceae**
DAEC	**Diffusely Adherent *E. coli***
DDST	**Double disk Synergy Test**
DEC	**Diarrheagenic *E. coli***
DFIs	**Diabetic foot infections**
DHFR	**Dihydrofolate reductase**
EAEC	**Enteroaggregative *E. coli***
EHEC	**Enterohemorrhagic *E. coli***
EIEC	**Enteroinvasive *E. coli***
EMB	**Eosin Methyl Blue**
EPEC	**Enteropathogenic *E. coli***
ESBL	**Extended-Spectrum β-Lactamase**
ETEC	**Enterotoxigenic *E. coli***
ExPEC	**Extraintestinal Pathogenic *E. coli***
GNB	**Gram Negative Bacteria**
GI	**Gastrointestinal tract**
HU	**Hemorrhagic colitis**
HUS	**Hemolitic- uremic syndrome**
IL-8	**Interleukin 8**
IMP	**Imipenemase**

KPC	***Klebsiella pneumoniae* carbapenemases**
LPS	**lipopolysaccharides**
MBLs	**metallo-b-lactamases**
MDR	**Multidrug resistance**
NMEC	**Neonatal meningitis *E. coli***
NDM-1	**NEW Delhi-metallo β-lactamase**
PAI	**Pathogenicity islands**
PAmpC	**plasmid-mediated AmpC**
PBPs	**Penicillin binding Proteins**
PCR	**Polymerase chain reaction**
Psi	**Per square inch**
STEC	**shiga toxin producing *E. coli***
Stx	**Shiga toxin**
TBE	**Tri Borate EDTA**
TD	**Travelers' diarrhea**
TLR5	**Toll-like receptor 5**
UPEC	**Uropathogenic *E. coli***
UTI	**Urinary tract infection**
VFs	**Virulence facters**
VIM	**Verona integron encoded metallo β-lactamase**
Vitek	**Vitality index of traditional environmental knowledge**

Introduction

Introduction

Escherichia coli (*E. coli*) is one of the most widespread microbes in the world, capable to cause intestinal or extra intestinal disease and lead to a wide range of diseases (Saleh and Hussain, 2019). It is a group of genetically heterogeneous bacteria, usually colonizes the digestive system of human infants through a few hours after birth and remain mutual benefit for decades, wherever found it indicates a fecal contamination of water or food (Barer and Irving, 2018). In addition to its presence in the human intestines its also present in the intestine of warm-blooded animals (George and Prasad, 2015).

E. coli is considered one of the most important opportunistic and nosocomial infection (Aswani *et al*., 2014; Al-Hasnawy *et al.*, 2018). It can be divided into pathogenic and non-pathogenic strains, pathogens strains divided according to site of intestinal infection into: extraintestinal pathogenic which causes several infection and named based on site of infection such as: uropathogen *E. coli* (UPEC) that causes urinary tract infection, and intestinal pathogens strains which causes diarrhea which in turn divided in six classes based on type of diarrhea and virulence factors (Bavaro, 2012 ; Kwak *et al*., 2016).

E. coli is the main pathogen causing urinary tract infections and important pathogens causing blood stream infections, otitis media, wounds infections, and other complications in humans , also its the common cause of food and water-borne infections (Saravanan and Raveendaran, 2013). It can cause these infections due to its ability to the acquisition of mobile genetic factors which carrying many virulence genes (Bok *et al*., 2015; Martin, 2017).

The most common virulence factors in *E. coli* are adhesions, iron uptake, toxins and capsules responsible for attachment, adherence and invasion in the host and then lead to infection (Yun *et al*., 2014). Also it has surface structures such as flagella, capsule, and lipopolysaccharides (LPS), which give bacteria antigenic properties and also possesses fimbreae or pilli, which help them to

hold of the host tissue, which give them the ability to form biofilm (Zowawi *et al.*, 2015; Terlizz *et al.*, 2017).

Since *E. coli* is becoming resistance to current available antibiotics, it is therefore considered major global public health concern (Subash *et al.,* 2014). This may exacerbated by the highly flexible genome of *E. coli*, and tendency to spread resistance through horizontal gene transfer and clonal spread. (Bajaj *et al*., 2016) . *E. coli* has the ability to evolve constantly, moreover, their strains enables to survive under different environmental conditions and selective pressures (Laehnemann *et al*., 2014).

β-lactam antibiotics are one of the most widely used therapeutic classes of antibacterial prescribed in human and veterinary practices and other domains of life as agriculture due to their safety profile and broad antimicrobial spectrum (Zaniani *et al*., 2012; Rahman *et al*., 2018). Despite the importance of these antibiotic for treating many bacterial infections, but their extensive and heightened use in different fields has become a selective force for driving resistance globally (Shaikh *et al*., 2015). Where the continuous and random exposure of the bacterial cell to this antibiotic that leads to the production of beta lactamase enzymes continuously and thus to the occurrence of genetic mutations in the encoded genes, which resulted in the emergence of resistance encoded genes for the extended-Spectrum Beta-lactamase(ESBLs) enzymes that give resistance to bacteria towards many groups of Beta-lactam antibiotic such as penicillins and cephalosporins (Rezai *et al*., 2015). However, bacterial resistance has originated fast due to the production of these enzyme (Abdi *et al*., 2014). *E. coli* is produced ESBLs in high percentage, these enzyme represent a major group of β-Lactamases enzymes (Al-Hamadani *et al*., 2013).

ESBLs are encoded by some genes such as bla_{TEM} ,bla_{SHV} and $bla_{CTX\text{-}M}$ for the narrower spectrum ß-lactamases by mutations that alter the amino acid configuration around the enzyme active site (Jena *et al*., 2018). These enzyme are typically encoded by plasmids that can be exchanged between bacterial species (Topaloglu *et al*., 2010). Also bla_{OXA} gene was small group that was

distinguishable by its ability to hydrolyze oxacillin (Evans and Amyes, 2014). ESBLs may be defined as a group of enzymes that are capable of conferring resistance to beta lactam with substantial potential to hydrolyse beta-lactam rings are able to hydrolyse penicillins, cephalosporins and monobactam antibiotics but not cephamycins, and carbapenems, Moreover, ESBL production is often accompanied by resistance to fluoroquinolones, co-trimoxazole or aminoglycosides (Flores-Mireles *et al*., 2015). So, the production of ESBLs which produce by *E. coli* is very troubling, greatly limiting treatment options, so, *E. coli* infections are becoming difficult treated (Hano *et al*., 2018).

Since the production of these enzymes leads to promote resistance to many antibiotics groups and are associated with the difficult of treatments and high morbidity and mortality rates so the detection of these enzymes are very important for optimal patients care (Juma *et al*., 2016; Aljanaby and Alfaham, 2017).

According to the previously mentioned informations and due to lack of studies in Maysan province, the present study was aimed to**:**

1- Isolation and identification of *E. coli* from different clinical sources

2- Determination of the susceptibility patterns of the identified isolates toward the commonly used antibiotics.

3- Phenotypic detection of ESBLs producing *E. coli.*

4- Detection of plasmid and chromosomal mediated ESBLs represented by bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$ and bla_{OXA} genes by monoplex PCR.

Chapter One

Literature Review

1. Literature Review

1.1. *Escherichia coli*

E. coli was first described by Theodor Escherich in 1884 and since that time, it has become a critical model organism that has been used to understand the fundamentals of molecular biology (Escherich, 1988; Blount, 2015). Certain strains of bacteria were responsible for diarrhea in infants and gastroenteritis, an important discovery of public health , *E. coli* bacteria were initially called Bacterium coli, the name later changed to *E. coli* to honor its discoverer (Tamerat *et al*., 2016).

There are several species of *Escherichia* but *E. coli* and *E. vulneris* are the major causes of infections in human (Olowe *et al.*, 2017).

E. coli is a coliform bacterium that occurs as a commensal in the gastrointestinal tract of humans and other warm blooded mammals (Conway and Cohen, 2015). It is a gram negative bacteria belongs to the family Enterbacteriaceae, (0.5-0.7) µm in diameter and (1.0-3.0) µm in length, arranged singly or in pairs, non spore forming rod, with some having prominent polysaccharide capsule which provides resistance against many host defense mechanisms (Bardiau *et al*., 2010). Also, it is aerobic or facultative anaerobic (Oliveira *et al.*, 2017). It is ferment a variety of carbohydrates which lead to produce acid and gas from the fermentation to give the one of the characteristics of diagnosis, these bacteria are motile by peritrichous flagella (Brooks *et al*., 2013).

Cell wall of *E. coli* is composed of a thin peptidoglycan layer and an outer membrane, the outer membrane is composed of lipopolysaccharides that includes lipid A, core oligosaccharides and a unique polysaccharide , referred to as the O-antigen, the outer membrane surrounds the cell wall provides a barrier to certain antibiotics such that *E. coli* is not damaged by penicillin, between the outer membrane and the cytoplasmic membrane there is a space filled with a concentrated gel-like substance called periplasm (Brenner *et al.*, 2005).

Therefore, the cell wall of the Gram-negative bacteria, including *E. coli*, is an important factor in its virulence that protects the microbes from antibiotics and analytical enzymes (Melnyk *et al.*, 2015).

It is Growing in pH between (4.4 - 9) and the optimal temperature for growth (36 - 37) C° (Jawetz *et al*., 2016; Wanger *et al*., 2017). It is positive for the catalase test and negative for the oxidase and urease test, whilst positive for the indole test, which is the best test are recommended that to distinguish it from other intestinal family members, as well as non-consuming citrate as the sole source of carbon, and it is positive for the Methyl red test and negative for the Vogase-Proskauer test (Hemraj *et al*., 2013).

E. coli has many virulence genes which located on mobile genetic elements, this indicates that horizontal gene transfer plays a mainly role in the development of various bacterial pathotypes (Subash *et al*., 2014).

These bacterium is persists in the environment and contamination water or food; until its next host consumes viable bacteria, the following ingestion, a stressor facing by *E. coli* is acidity in the stomach, which it survives because stationary phase bacteria induce protective acid resistance systems (Van Elsas *et al.*, 2011). Upon reaching the colon, *E. coli* must get to the nutrients it needs to way out lag phase and grow from minimal to high numbers, failure to transmission from lag phase to logarithmic phase will lead to elimination of the invading *E. coli* bacteria, successful colonization in the colon by *E. coli* depends on competition for nutrients with intensive and diverse microflora (Bonnet *et al.*, 2014).

It penetrates the mucus layer and has the capability to avoid host defenses and grow rapidly, exceeding the turnover rate of the mucus layer resides in mucus until being escaping into the lumen of the intestine, some cells are discarded in the host feces and the cycle begins again. This cycle of colonization and extraintestinal survival is the fact for commensal and pathogenic *E. coli* (Bergstrom *et al.*, 2012).

1.2. Classification of *E. coli*: (Tchaptchet and Hansen, 2011).

Kingdom	Eubacteria
Phylum	Proteobacteria
Class	Gamma proteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genes	*Escherichia*
Species	*coli*

1.3 -Epidemiology

E. coli is the main part of the intestinal family that invades the intestines after birth and coexists naturally, while some strains have opportunistic susceptibility to host disease after optimum conditions for growth (Gharajalar and Sofiani, 2017). It has evolved truly since its discovery, and pathogenic *E. coli* have been reported to cause a spectrum of diseases (Baker, 2015).

E. coli is a source of hospital infections, particularly for children with low immunity and under nourishment, which leads to the use of antibiotics, this causes the emergence of antibiotic resistance bacteria (Korzeniewsk *et al*., 2013). This resistance leads to the failure of treatment, especially resistant to antibiotics which are the first choice in the treatment of infections such as the third generation where it leads to recurrence of infection and increase diseases and raise mortality (Barber *et al*., 2013). The acquisition of strains bacteria to determinants of resistance and expansion in the acquisition of resistance already present in the bacteria *E. coli* considered one of the most important reasons for bacterial resistance to antibiotics (Costa *et al*., 2013).

It is remained as one of the most important bacteria causing infections in pediatrics due to producing ESBLs which making them resistant to beta-lactam antibiotics(Rezai *et al*., 2015). Also it is one of the most common causes of

diseases and deaths of diarrheal children worldwide (Bettelheim and Goldwater, 2014). Moreover, it is also one of the most common bacterial species responsible for Urinary Tract Infection (UTIs) especially in pregnant women (Brooks *et al*., 2016; Lhwak and Abbas, 2018).

1.4. Types of *E. coli*

1.4.1. Normal flora *E. coli*

The most common basic habitation of normal flora *E. coli* is the part of the normal flora bacteria in the gastrointestinal tract, also called commensal *E. coli* or nonpathogenic *E. coli* (Gillespie and Hawkey, 2006).

The exits of intestinal microflora can prevent the growth of pathogenic bacteria strains, which have survived the gastric juice of the upper gastrointestinal tract and have reached the large intestine, through the competitive inhibition of pathogenic strains; (Katouli, 2010). The metabolic functions of this flora comprise the synthesis of essential vitamins and fermentation of indigestible dietary residues (Gerritsen *et al*., 2011). High and irregular use of antibiotics may lead to change in the intestinal microflora which causes the disease (Distefano, 2015).

1.4.2. Intestinal pathogenic *E. coli*

The *E. coli* have ability to cause gastrointestinal infection, intestinal pathogenic *E. coli* which have evolved many mechanisms that can cause disease through: colonize of the mucosa, evade host defense, multiply and damage the host. It can be divided into six types depending on their specific characteristics and specific virulence factors; enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), diffusely adherent (DAEC) and (EIEC) enteroinvasive (Kaber *et al*. 2004; Rivas *et al*., 2015; Malema *et al*., 2018).

1.4.2.1. Enteropathogenic *E. coli* (EPEC)

EPEC is a major cause of infantile diarrhea and mortality in low-income countries (Ale *et al*., 2017). The central mechanism of EPEC pathogenesis is a lesion called 'Attaching and Effacing' (A/E), which is characterized by intimate adherence of bacteria to the intestinal epithelium. The adherence of bacteria to the enterocytes is mediated by intimin, an outer membrane protein encoded by the *eae* gene (Magdy *et al*., 2015).

1.4.2.2. Enterohemorrhagic *E. coli* (EHEC)

EHEC are major food-borne pathogens whose survival and virulence in the human digestive tract remain unclear owing to paucity of relevant models. EHEC interact with the follicle-associated epithelium of Peyer's patches of the distal ileum and translocate across the intestinal epithelium via M-cells, but the underlying molecular mechanisms are still unknown(Cordonnier *et al*., 2017).

Ruminants, especially cattle, are a natural reservoir of shiga toxin producing *E. coli* (STEC) and human infection is linked with the consumption of contaminated food, STEC belong to a wide range of serotypes; however, only a limited number has been associated with human disease, among which EHEC O157:H7 is the most prevalent serotype associated with outbreaks and sporadic cases worldwide (Rangel *et al*., 2005). *E. coli* O157:H7 is considered a main food-borne organisms in cattle and other ruminants which considered the major reservoirs for this organism (Shabana, 2014). Also infections of this bacteria can be acquired by direct contact with animals and by person-to-person spread (Khosravi *et al*., 2016).

1.4.2.3. Enterotoxigenic *E. coli* (ETEC)

The ETEC is the most common group, particularly in the developing countries, the diarrhea caused by ETEC is characterized by a rapid onset of watery, non-bloody diarrhea with remarkable volume, accompanied by mild or

no fever, other common manifestations of this diarrheal infection are malaise, abdominal cramping pain, vomiting and nausea (Abbasi *et al*., 2014). It is the mainly cause of travelers' diarrhea in south Asia (Sanders *et al*., 2019). Where it colonizes the gastrointestinal tract (GI) by fimbrial adhesion, they are non-invasive. However, they produce two types of toxins which allow rise to intestinal secretion causing the watery diarrhea (Dubreuil *et al.*, 2016).

1.4.2.4. Enteroinvasive *E. coli* (EIEC)

EIEC are a group of intracellular pathogens able to enter epithelial cells of colon, multiplicate within them, and move between adjacent cells with a mechanism similar to *Shigella*, the etiological agent of bacillary dysentery (shigellosis) in humans, which is characterized by invasion and inflammatory destruction of the human colonic epithelium (Pasqua *et al*., 2017; Belotserkovsky and Sansonetti , 2018) .The transmission is achieved by the faecal–oral route, contaminated food and water being the major sources of infection, able person-to-person transmission has been also respected (Gomes *et al*., 2016). The EIEC produce syndrome through infection can be indistinguishable from infection of *Shigella* (DuPont, 2014).

1.4.2.5. Enteroaggregative *E. coli* (EAEC)

The classical definition of EAEC pathogenesis indicates that the microorganism has the ability to adhere to epithelial cells in a very characteristic "stacked-brick" pattern and is capable of forming biofilms (Arenas-Hernández *et al*., 2012). EAEC strains are important causes of diarrhea worldwide and are the second most important bacterial cause of travelers' diarrhea (TD) (Bamidele *et al*., 2019). In addition, it has been identified as the causative agent of AIDS-associated diarrhea in both developing and industrialized countries also known to distribute widely in water and food, and occasionally with animal feces, diagnosis of EAEC has long been problematic (Zhang *et al*., 2016).

1.4.2.6. Diffusely Adhering *E. coli* (DAEC)

Adherent *E. coli* (DAEC) were the last diarrheagenic *E. coli* pathogroup (DEC) to be recognized. It is a bacterial pathogen that induces unique alterations on epithelial cells, resulting in diarrhea illness and epithelia damage that may also contribute to the development of other intestinal diseases (Meza-Segura and Estrada-Garcia, 2016). DAEC remains controversial, Previously, that motile DAEC strains isolated from diarrheal patients induced high levels of interleukin 8 (IL-8) secretion via Toll-like receptor 5 (TLR5), none the less, DAEC strains from healthy carriers hardly induced IL-8 secretion, regardless of their possessing flagella (Tanimoto *et al*., 2019).

1.4.3. Extraintestinal pathogenic *E. coli* (EXPEC)

EXPEC include: Uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) according to Pitout (2012a). UPEC can produce of specific virulence factors which enable the bacteria to adhere to uroepithelial cells and to cause UTI (Kaira and Pai 2017). Also neonatal meningitis caused by neonatal meningitis *E. coli* (NMEC) in infants (Brooks *et al.*, 2013).

Other most major *E. coli* infections are cholecystitis, appendicitis, peritonitis, postoperative wound infections, and sepsis (Mahon *et al.*, 2014). These Infections which caused by the EXPEC strains have been widely reported in the community settings as well as hospitals and long-term care facilities thus, causing a pronounced burden on the medical and economic resources across the globe (Poolman and Wacker, 2016).

1.5. Virulence factors of *E. coli*

E. coli strains have a number of virulence factors, which enable them to colonize and causes disease also persist in face of highly effective host defenses, some of these fall within the cellular structure and others are excreted outside the body (Brooks *et al*., 2010). Among these virulence Biofilm, which is defined

as a layer of polysaccharide and proteins, many bacteria of the same species forming it to protect themselves when they feel threatened by adhesion to the surface (Vuotto et al., 2014). Also, hemolysin; an enzyme which has a great importance in increasing the ferocity of the bacteria, as it works on exiting of hemoglobin from the red blood cells, and thus lossing iron (KuKanur *et al*., 2015). There are several types of hemolysin according to the form of decomposition which include Alpha, Beta and Gama (Jaweetz *et al*., 2016). The most common type of hemolysin of *E. coli* was α-Hemolysis, which is specifically non-linked to the cell of bacteria and it is extracellular type that excreted in the logarithmic phase (Brooks *et al* ., 2016).

Bacteriocin which called colicins when produced by *E. coli*, are antimicrobial proteins, can target other *E. coli* cells by binding to cell surface receptor proteins and activating their import, resulting in cell death , it is encoded on plasmid (Soltani *et al.*, 2018; Sharp *et al.*, 2019). Additionally, endotoxin is one of the most important components of the bacterial cell wall of the Gram negative, which consists of lipopolysaccharide (LPS) containing the somatic antigen O that gives a bacteria the ability to colonialize on the host cells, flagellum are structures also contain the antigen H (Flagellar antigen H) these antigens give the bacteria the ability to overcome the host's immune system and siderophores are an important and specialized iron transport system of bacteria and play a significant role in virulence of *E. coli*, which allows them to use hemoglobin as an iron source that stimulates the growth of bacteria (Terlizzi *et al*., 2017).

E. coli also have another virulence factor like capsule is called K-antigens, where it is the major surface antigens of *E. coli* and it is the first line of defense in the bacteria, which consists of polysaccharide surrounding the bacterial cell (Yang *et al*., 2018). Other virulence factor called cytotoxic necrotizing Factor (CNF1) is a major virulence factor of pathogenic *E. coli* strains (Reppin *et al* ., 2017). It is obtained their name from necrosis effect

on rabbit skin when it was isolated and tested (Markus, 2017). Moreover it had adhesins and fimbriae or pilli which was of the most important factors that help bacteria adhere to host tissue, give bacteria the ability to form the biofilm and then increase their resistant to antibiotic (Neamati *et al*., 2015; Spaulding *et al*., 2017).

Therefore, the knowledge of virulence factors of *E. coli* will help in better understanding of the organism pathogenicity and guided empirical therapy which can result in a better treatment outcome (Kaira and Pai, 2017).

1.6. Antibiotics and Resistance

Antimicrobials can be defined as any natural, synthetic or semisynthetic origin substance which kill or inhibit the growth of microorganisms, on the other hand, antibiotic refers to a low molecular weight substance produced by microorganisms which act against another microorganism at low concentrations (Giguère, 2013).

Indeed, each new antibacterial drug development has been followed by the detection of resistance to it, antibiotic resistance is defined as the ability of microbes to resist the effects of drugs, as a result the drugs become ineffective to neither kill nor inhibit the microbes (CDC, 2015).

The emergence and rapid spread of antimicrobial resistance is now a global concern (Ventola, 2015). Where the antimicrobial resistance in bacteria may be the result from selection pressures applied by antimicrobial use (WHO, 2014). Also bacteria can acquire antimicrobial resistance either by chromosomal mutation and acquisition of resistance genes by horizontal transfer (Ruppé *et al*., 2015).

1.6.1. Chromosome-mediated Antibiotic resistance

E. coli have a circular chromosome, is double stranded DNA, range from 4.500 to 5.520 million base pairs, *E. coli*, chromosome is roughly 1000 times longer than the cell itself (Donnenberg, 2002). Pathogenicity of *E. coli* is, due to the presence of many virulence genes, located on chromosomes or plasmids or both that encodes important virulence factors, if present on the chromosome, these genes are typically found in specific regions called pathogenicity islands (PAI) (Koga *et al*., 2014). Many of the Gram negative bacteria have naturally occurred chromosomally mediated β-lactamase, which may be help the bacteria in finding a niche when faced with competition from other bacteria that naturally produce β-lactams (Tham, 2012). Also bacteria have the ability to acquire different types of ESBLs enzymes with both plasmid transmissible and chromosome encoded (Adeyankinnu *et al*., 2014). In addition to, the mutations in chromosomal genes resulting in an increase in the expression of intrinsic resistance mechanisms (either antibiotic-inactivating enzymes or efflux pumps), permeability alterations by loss of outer membrane porins, or target modifications (Ruppe *et al*., 2015).

1.6.2. Plasmids-Mediated Antibiotic Resistance

The term plasmid was first introduced by the American molecular biologist Joshua Lederberg in 1952 (Black, 2012). Bacterial plasmids are double-stranded, circular, self-replicating and DNA extrachromosomal genetic elements, which are not essential for bacterial growth. They may not be exist in all species or all strains in a species. A strain can have more than one type of plasmid that vary in size and the genetic code it carries, in addition to, it can be transferred from one cell to another cell spontaneously or during manipulation (Ray and Bhunia, 2014). Plasmids are main mechanism for the spread of antibiotic resistant genes and confer traits of antibiotic resistance in bacterial populations (Sandhu, 2010).

The transfer of gene plasmid-mediated can occur either between closely related strains or between widely related strains from diverse species or genera and can play a significant role in the mobility of resistance genes (Ray and Bhunia, 2014; Cruz and Hedreyda, 2017).

Resistance to β-lactams and other antibiotics in the Enterobacteriaceae is frequently associated with plasmid resistance, β-Lactamase-mediated resistance is increasingly associated with plasmid-encoded ESBLs and carbapenemases, specifically the CTX-M family of ESBLs, the KPC family of serine carbapenemases, and the VIM, IMP, and NDM-1 metallo-β-lactamases. These enzymes are now appear in multiple combinations of ESBLs and carbapenemases, thereby there confer resistance to virtually all β-lactam antibiotics (Bush, 2010; Reinthaler *et al.*, 2010). Additionally, Bacterial plasmids mainly transfer via conjugation mechanism (Huddleston, 2014). Whereas this process does not transfer the chromosome mediated antibiotic resistance genes (Tabassum *et al*., 2018). So, *E. coli* repeatedly caused urinary tract, wound and blood infection resulting in significant morbidity and mortality due to having plasmid encoded ESBLs which in turn lead to treatment failure (Ali , 2018). Therefore, Plasmids have an important role behind the success of the ESBL genes as they both mediate transfer and immobolize maintenance of these genes in new hosts (Søraas, 2014).

1.7. Antibiotics Group

1.7.1. Beta-Lactams Antibiotics Group

These antibiotics are of great importance among other antibiotics. They are broad-spectrum against Gram-negative and Gram-positive bacteria which cause many infections (Ehmann and Lahiri, 2014). They have a deadly effect of Gram-positive bacteria than Gram-negative, because Gram-negative contain within the composition the external membrane which obstructs the arrival of the antibiotic to the target (Brook *et al*., 2007).

These antibiotics contain the beta lactam ring, which makes it more important and effective against bacteria. They are inhibit cell wall formation of bacteria through the binding antibiotic with special proteins within the cell membrane structure called Penicillin binding Proteins (PBPs) , where inhibit the action of Transpeptidase enzyme, which creates peptide chains linking the layers of peptidoglycan, which forms a component of the cellular wall (Carroll *et al*., 2016). This binding causes decomposition the cell wall of bacteria and then its death (Brook *et al*., 2007). There are seven types of these proteins in *E. coli* (la, lb, 2, 3, 4, 5, 6) which are arranged by molecular weight (Davey *et al*., 2015).

Resistance to β-lactams may be a result of several mechanisms including: altering the permeability of the cell membrane which results in the loss of some channel proteins (porins) making it difficult for the antibiotic to reach the cell (Fernando and Kumar, 2011). Additionally, other mechanisms such as modification of penicillin-binding proteins, production of β-lactamases and overexpression of efflux pumps (Talbot, 2013). None the less, in Gram negative β-lactams resistance is mainly due to the hydrolytic action of β-lactamases, these enzymes inactivate beta-lactam antibiotics by hydrolyzing the peptide bond of the four-membered beta-lactam ring the encoded genes of these enzymes called bla gens which are carried either on the chromosome or plasmid(Al-Jubori *et al*., 2012).

These antibiotics make up approximately 50% of all prescribed antimicrobials and are categorized on the basis of the β-lactam ring in their chemical structure into four major groups; penicillins, cephalosporins, carbapenams, and monobactams (Rahman *et al*., 2018).

1.7.2. Aminoglycoside Antibiotics Group

It is an important group of antibiotics and used to treat the infections caused by Gram positive and Gram negative bacteria such as gentamicin, amikacin,

tobramycin, streptomycin, kanamycin , these antibiotic are similar in chemical, toxic and pharmacological properties（Dagil *et al*., 2013). These antibiotics have Bactericidal effectively (Wang *et al*., 2012). Where they inhibit protein synthesis through irreversibly binding to ribosomal Subunit 30S , thus stopping the peptide chain elongation phase and then cell death (Brook *et al*., 2007). In most Gram negative bacteria the resistance to these antibiotic attributed to produce protein encoded enzyme from mutant plasmid which inhibit the building of the protein by irreversibly associating with the (30S) ribosomal unit, thus stopping the phase of the peptide chain elongation and then the death of the cell (Wassef *et al*., 2010; Cirit *et al*., 2019).

Additionally, the resistance to amikacin and other aminoclaysides also depends on the lack of permeability of the antibiotic due to the change in permeability of the membrane which weakens the active transport in the cell (Carroll *et al*., 2016). These antibiotics have harmful side effects when used in long-term treatment, where they influence on the eighth nerve and lead to deafness（Ototoxicity), as well as toxicity (Quiros *et al*., 2010).

1.7.3. Quinolones Antibiotics Group

Quinolones and fluoroquinolones inhibit nucleic acid synthesis by binding to DNA gyrase and DNA topoisomerase IV. These enzymes are responsible for relaxing and supercoiling the DNA within a cell, binding with these enzymes can interfere with the DNA replication process (Džidić *et al*., 2008). fluoroquinolones, which can be inhibited by target alteration of target enzyme (Blair *et al*., 2015). That occurs through acquisition of mutations, in one or more genes, that encode the targets of these antibiotics and change it (Paterson, 2006; Redgrave *et al*., 2014). Also resistance attributed to accumulation of these antibiotics (Paterson, 2006)

This group of antibiotics is divided due to structure and efficacy against microbes to four generations antibiotic nalidixic acid is one of the first

generation quinolons (Hall *et al*., 2011). Other generations called fluoroquinolones including the second generation such as ciprofloxacin, third generation like Levofloxacin and fourth generation such as trovafloxacin (Oliphant *et al.,* 2002).

1.7.4.Trimethoprim Antibiotic

Trimethoprim is an antibiotic that inhibits the synthesis of bacterial DNA. (Tortora *et al*., 2016). Where it works to inhibits the enzyme Dihydrofolate reductase (DHFR) through interferes with folic acid metabolism (Murray *et al*., 2013). This antibiotic is used in the treatment of urinary tract infection, especially the complex infections (Davey *et al*., 2015; Carroll *et al*., 2016). The resistance to this antibiotic by modification of target enzyme (DHFR) which encoded by DRF genes that are either located on the plasmid or chromosome (Kumar *et al*., 2012).

1.7.5. Nitrofurantoin Antibiotic

This antibiotic is used in the treatment of urinary and intestinal infection, affecting the process of protein building through the damage caused directly by the DNA (Brooks *et al*., 2007). It is effective against Gram negative and Gram positive bactreia, also it is more effective at PH (5.5) (Katzung, 2001). The using of nitrofurantoin has increased exponentially since recent guidelines respositioned it as first line therapy for uncomplicated lower urinary tract infection (UTI), where when given short term for lower UTI, it has good clinical and microbiological efficacy and their toxicity is mild. (Huttner *et al*., 2015).

1.8. β-lactamases enzyme

They are enzymes that give the bacteria resistance to β-lactam antibiotics group, genes encoding β-lactamase enzymes which can be found either chromosomally or on mobile genetic elements such as plasmids, (Drawz and Bonomo, 2010).

They are a broad group of enzymes which have been classified using two schemes - (a) Ambler molecular classification and (b) Bush-Jacoby-Medieros functional classification. In the Ambler classification system, β-lactamases are grouped according to protein sequence similarity. There are four classes (A-D) in the Ambler scheme where class A, C and D are serine β-lactamases and class B are metallo β-lactamases (Paterson and Bonomo, 2005).

Conversely, functional similarities (substrate and inhibitor profile) of the enzymes are the basis of Bush-Jacoby-Medieros classification (Bush and Jacoby, 2010). This classification scheme is of more relevance to physicians or microbiologists in diagnostic laboratory because it considers β-lactamase inhibitor and β-lactam substrates that are clinically relevant (Dhillon and Clark., 2012). The emerging β-lactamase enzymes in Enterobacteriaceae which are increasingly problematic are Extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases and carbapenemases (Pitout, 2012b).

Table (1-1): Various classification schemes and representatives of Beta-lactamase enzymes (Bush and Jacoby, 2010; Rahman *et a*., 2018).

Ambler (molecular) Class	Bush and Jacoby group(2009)	Substrate/ target	Inhibition profile Clavulanic acid	Tazobactam	Member example
A	2a	pencillins	Yes	No	Pc-1
	2b	Pencillins,some of the1st-generation cephalosporin	Yes	No	TEM-1,TEM-2 SHV-1
	2be	Extended spectrum Cephalosporin, monobactam	Yes	No	TEM-3,SHV-2 CTX-M-15, PET-1, VEB-1
	2br	Pencillins	No	No	TEM-30, SHV-10
	2ber	Extended spectrum Cephalosporin, Monobactam	No	No	TEM-50
	2c	Carbenicillin	Yes	No	PSE-1, CARB-3
	2ce	Carbenicillin Cefepime	Yes	No	RTG-4
	2e	Extended spectrum beta-lactams	Yes	No	CepA
	2f		Changeable	No	KPC-2,IMI-1 SME-1
B	3a	Carbapenems	No	Yes	IMP-1, VIM-1CcrA IND-1,NDM-1
	3b	Carbapenemes	No	Yes	CphA, Sfh-1
C	1	Cephalosporins	No	No	AmpC, P99, ACT-1,CMY-2, FOX-1,MIR-1
	1e	Cephalosporins	No	No	GCI,GMI-37
D	2d	Cloxacillion	Changeable	No	OXA-1, OXA-10
	2de	Extended spectrum Cephalosporin	Changeable	No	OXA-11, OXA-15
	2df	Carbapenemes	Changeable	No	OXA-23, OXA-48

1.8.1. Extended Spectrum β-lactamases(ESBLs)

The designation ESBLs were used to define β-lactamases with a broad spectrum of hydrolysis, resulting from the occurrence of amino acid substitutions in the structure of the enzymes (Livermore, 2008). ESBLs belong to class A according to Ambler's classification, and group 2be functional group of Bush's classification (Bush and Jacoby, 2010).

They are a predominant cause of β-lactam antibiotics resistance in Gram-negative bacilli (GNB) (Ruppe *et al*., 2015). These enzymes confer resistance to an important class of drugs such as penicillin, amoxicillin, third generation cephalosporin (Kuenzli, 2016). In addition to their resistant to monobactam (azetronam) (Ghafourian *et al*., 2014). Also these enzyme can hydrolyze fourth generation cephalosporins and compromise the efficacy of all β-lactams, except cephamycins and carbapenems, in the worldwide these group of enzymes are found widely and causes a severe infections on human health which leads to various diseases (Saravanan *et al*., 2018).

The incidences of infections which are caused by ESBLs producing GNB are increasing in prevalence worldwide, both in the healthcare as well as community settings, posing significant therapeutic challenges (Kassakian and Mermel, 2014). These enzymes are diverse, complex and rapidly developing ESBLs producing strains can increase morbidity and mortality rates, in part as a result of linked resistance to other antibiotic families, which restrict therapeutic options and raises healthcare costs (Inwezerua *et al*., 2014; Shaikh *et al*., 2015).

The prevalence of these enzyme increasing day by day in nearly every center of different countries and necessary steps to prevent the spread and emergence of resistance should be taken (Kumar *et al*., 2014).

ESBLs are break down the amide bond, which present in the beta-lactam antibiotic ring making them ineffective molecules (Al-Jubori *et al*., 2012). So, the rate of multiple drug-resistant strain was highly in ESBLs-producing *E. coli* than in non ESBLs-producing *E. coli* (Alyamani *et al.*, 2017).

ESBLs encoding genes are located on chromosome, plasmids or transposons that can transfer easily between two or more bacteria such as *E. coli*, *K. pneumoniae*, *Enterobacter cloacae* and others. (Moghaddam *et al*., 2014; Niumsup *et al*., 2018). Bellow some genes that encoding for ESBLs:

1.8.1.1. CTX-M Type ESBLs

Although a bit recently discovered, they are the most increasingly reported types of enzymes associated with resistance, they are plasmid based encoded cefotaximases , constitute the fast growing family of ESBLs (Zhao and Hu, 2013). Where Cefotaximases are enzyme rapidly growing cluster that have disseminated geographically, they are transferred to species other than Enterobacteriaceae and might be responsible for the presence of $bla_{\text{CTX-M,}}$ genes are mostly encoded by transferable plasmids (Jamali *et al* ., 2017) . The CTX-M enzymes have been recognized as the most prevalent among Enterobacteriaceae ESBLs producers (Day *et al*., 2016; Zeynudin *et al*., 2018). These this enzymes were reported at first time in *E. coli* species in 1990 (Smet *et al*., 2010). Since these enzymes are most encoded on plasmid so they are more easily horizontally transmissible, and they hydrolyze β-lactam antibiotics that resulting in resistance to penicillins, cephalosporins, and aztreonam (Reinthaler *et al.,* 2010).

CTX-Ms are named after their extended activity against cefotaxime compared to ceftazidime and the origin of its first isolation (Munich, Germany) (Birbrair, and Frenette, 2016). The CTX-M type enzymes belong to a group of class A ESBLs according Ambler classification that in general exhibit much

higher levels of activity against cefotaxime and ceftriaxone than ceftazidime (Ghafourian *et al*., 2014; Shaikh *et al*., 2015).

Among other ESBLs, CTX-M enzymes have been proven to be the most efficacious in terms of promiscuity and its predominance abundance in diverse epidemiological settings, where they have largely replaced and outnumbered other ESBLs types such as TEM (D'Andrea *et al*, 2013). The presence of CTX-M type ESBLs is often associated with co-resistance phenotypes in particular to fluoroquinolones and aminoglycosides, in addition to tetracycline, and trimethoprim/sulfamethoxazole co-resistance, which is commonly observed among TEM and SHV type ESBLs (Lahlaoui *et al*., 2014). Thus far, 172 variants of CTX-M were identified worldwide (http://www.lahey.org.2018).

1.8.1.2. TEM Type ESBLs

TEM are mostly encoded by Gram-negative bacteria, almost 90% of the resistance against ampicillin in Gram-negative bacteria are due to bla_{TEM} encoded genes (Livermore, 1995). The TEM-type ESBLs are often plasmid mediated derived from mutations in the classic TEM (TEM-1 and TEM 2) genes by single or multiple amino acid substitution around the active site. *E. coli*, isolated from a patient named Temoneira (hence, named TEM) in Athens, Greece, harboring resistance encoded by bla_{TEM-1}gene was the first ever report in 1965 (Steward *et al*., 2000). TEM-1 is able to hydrolyze penicillin and 1st-generation cephalosporin such as cephaloridine, TEM-2 derived from the original TEM-1 enzymes as a result of single or multiple amino acid sequence mutations (Zhao and Hu, 2013).

These enzymes become the most commonly encountered β-lactamase among gram negative bacteria (Sharma *et al*., 2010). The most disseminated TEM-type ESBLs among Enterobacteriaceae in Europe in the clinical setting are TEM-24, TEM-4 and TEM-52, while in isolates from animals TEM-52, TEM-106 and TEM-116 are the most common (Coque *et al*., 2008). These enzymes composed

of more than 223 variants according to the database of the Lahey Clinic (http://www.lahey.org 2018).

1.8.1.3. SHV Type ESBLs

SHV types of enzymes are mostly found in *Klebsiella* species (especially *K. pneumoniae*) most often encoded on plasmid, nevertheless, a number of species have been shown to carry $bla_{SHV\text{-}1}$ gene within the chromosome (Zhao and Hu, 2013). SHV indicates sulfhydryl variable (Randall *et al*., 2010). Extended-spectrum SHV β-lactamases belong to functional group 2be, while very recently they were assigned to subclass A1 of serine β-lactamases, clustering with TEM and CTX-M enzymes among other clinically relevant β-lactamases(Bush, 2013; Philippon *et al*., 2016). The hydrolytic spectrum of SHV-type ESBLs includes activity against oxyimino-β- lactams such as cefotaxime, ceftazidime, ceftriaxone and aztreonam (Bush and Jacoby, 2010).

SHV-type ESBLs are point mutants of both narrow-spectrum β-lactamases SHV-1 or SHV-11 that had origin in the *Klebsiella*. *pneumoniae* chromosome (Poirel *et al*., 2012). SHV-2 was the 1st SHV-ESBL type detected in *Klebsiella ozaenae* isolated from Germany, in 1983, this enzyme originated from point mutation in SHV-1 which resulted in substitution of glycine by serine at the 238 positions and extension of its hydrolytic substrate profile to include cefotaxime and to a minor degree ceftazidime (Perilli *et al*., 2011).

Since these enzymes are ordinarily encoded by self-transmissible plasmids therefore that frequently carry resistance genes to other drug classes and have become widespread through the world in several Enterobacteriaceae, this confirm their clinical significance (Liakopoulos *et al* ., 2016). The family of these enzymes comprises 193 variants according to the internet site of the Lahey Clinic (http://www.lahey.org 2018).

1.8.1.4. OXA Type ESBLs

OXA-type ESBLs are mainly reported in *Pseudomonas aeruginosa*, and all the β-lactamase variants so far discussed belong to molecular class A, except OXA-type β-lactamases which belonging to molecular class D and functional group 2d, due to their oxacillin hydrolyzing capabilities (Koroska *et al*., 2017; Rahman *et al*., 2018).

The OXA- β-lactamases are so named because of their oxacillin hydrolyzing abilities, these β-lactamases are characterized by their ability to hydrolyze cloxacillin and oxacillin 50% more than benzyl penicillin, they predominantly occur in *Pseudomonas spp*, but have been detected in many other gram negative bacteria (Cuzon *et al*., 2008).

Oxacillinases (OXA) with carbapenemase activity categorized as class D enzymes according to Ambler classification , have been extensively reported throughout the world (Nordmann *et al*., 2011). Also, there are poorly inhibited by clavulanic acid (Bhattacharjee *et al*., 2007). Altogether, OXA type *β*-lactamases is explosively increasing based on the amino acid sequence variations and so far 498 variants have been reported and arranged in the database (http://www.lahey.org 2018).

1.8.2. AmpC β-lactamases

The AmpC β-lactamases comprise other important group of beta-lactamases from *E. coli* which exhibit a hydrolytic profile similar to the ESBLs, whereas having an additionally hydrolytic activity toward cephamycins like cefoxitin and cefotetan, the inhibition of AmpCs is usually caused by cloxacillin and boronic acid, but not by the common b-lactamase inhibitors by clavulanic acid and tazobactam (Helmy and Wasfi, 2014).

This group of enzymes is class C based on Ambler classification, and group 1 according to the Bush-Jacoby-Medieros system (Jacoby, 2009). AmpC β-

lactamase was first detected enzyme from *E. coli* which were resistant to penicillin while plasmid-mediated AmpC (pAmpC) genes were reported from *Enterobacter cloacae* in 1980s (Philippon *et al*., 2002). There are some examples of AmpC β-lactamases such as; CMY, ACT, FOX and DHA types which derived from chromosomally encoded AmpC cephalosporinases of bacteria such as *Enterobacter. spp*., *Citrobacter freundii, Morganella morganii*, *Aeromonas* spp and *Hafnia alvei* (Rubin and Pitout, 2014). The Lahey database is a curated list of β-lactamase genes, a total 136 variants of CMY have been described (http://www.lahey.org, 2017).

1.8.3. Carbapenemases

Carbapenemases are a group of enzymes which are capable of hydrolyzing all classes of β-lactam drugs including carbapenems which are considered to be the last alternative for effective treatment of life threatening infections (Poirel *et al*., 2016). There are three classes of Carbapenemases (A, B, D) which are the most widespread and clinically significant representatives of each class namely *Klebsiella pneumoniae* carbapenemases (KPC, class A); metallo-b-lactamases (MBLs, class B) and oxacillin- hydrolyzing metallo-b-lactamases (OXA, class D) they have frequently been reported in different *E. coli* strains (Beyrouthy *et al*., 2013). In spite of this, it is also worthy to note that in most instances the production of KPC and OXA carbapenemases as well as MBLs of the IMP and VIM type has been associated with strains of *K. pneumoniae* and *Acinitobacter baumannii* (Bartolini *et al*., 2014).

Chapter Tow

Materials

and

Methods

2. Materials and Methods

2.1. Materials

2.1.1. Equipments and Instruments

The equipments and instruments used in the present study are listed below:

Table (2-1): Equipments and instruments with their manufacturers

Equipments and instruments	Company/Country of origin
Autoclavable tube	AFCO/Jordan
Autoclave	Hirayama/Japan
Biosafety cabinet	Human Lab / Korea
Burner	Amel / Turkey
Cooling Centrifuge	Eppendorf/Germany
Densitometer	Biomerieux/Farance
Digital camera	Sony/Japan
Disposable petri dish	Al-Hani company/Lebenon
Distillator	Lab Teach/Korea
Electrophoresis apparatus	Bioneer/Korea
Eppendrof tubes	BDH/UK
Flask (500ml,1000ml)	Lab glass/Japan
Frezon	AUCMA/China
Gel Documentation System	Biometra/ Germany
Incubator	Binder/Germany
Light Microscope	Olympus/Japan
Micropipettes (0.5-10), (5-50), (100-1000)	Watson/Japan
Microwave	Shonic/China
Petri dishs	Plastilab/ Lebanon

Refrigetor	Concord/Lebanon
Sensitive electronic balance	Denver instrument/ Germany
Slide	Super star/ India
Standard wire loop	John Bolten/England
Sterilized cotton swabs	AFCO / Jordan
Thermal cycler apparatus	Bioneer/Korea
Tips	Sterellin Ltd./UK
U.V- Transilluminator	ELETTROFOR/ Italy
Vitek 2 compact system	Biomerieux/ France
Vortex	CYAN/Belgium
Water bath	Memmert/Germany

2.1.2 Chemical and Reagents

The chemical and reagents used in the present study are listed below:

Table (2-2): Chemical and reagents and their manufacturers

Materials	**Manufacturers/Country of Origin**
50X TBE (Tris- Boric acid-EDTA)	Biobasic/Canada
Absolute ethanol	Scharlau /Spin
Agarose gel	Promega /USA
DNA ladder (100- 2000bp)	Bioneer /South Korea
Ethidium bromide	Promega /USA
Free nuclease water	Bioneer/ South Korea
Glycerol	Sigma / USA

Kovacs reagent	Himedia /India
Loading Dye	Biobasic /Canada
Methyl red	BDH/UK
Sodium chloride (NaCl)	BDH/UK
α- naphthol (C10H8O)	BDH/UK

2.1.3. Culture Media

The media which are used in the present study to isolate and identify *E. coli* are listed below:

Table (2-3): Media and their manufactures

Media	Manufactures/ Country of Origin
Blood agar	Himedia/India
Brain heart infusion agar	Oxoid /UK
Brain heart infusion broth	Himedia/India
Chrome agar	Liofilchem/Italy
Eosin Methylene Blue(EMB)	Himedia/India
Kligler agar	Himedia/India
MacConkey agar	Himedia/India
Methyl red-Vogous proskaur broth	Oxoid/UK
Mueller-Hinton agar	Himedia/India
Peptone water	Himedia/India
Simmons citrate agar	Mast-diagnostic/UK

2.1.4 .Types of Antibiotics Discs and their Concentrations

The antibiotics disc used for detection susceptibility pattern to *E. coli* in the present study are listed in table (2-4) below:

Table (2-4): The Antibiotics discs used in the present study

NO	Name of Antibiotic	Symbol	Concentration (µg)
1	Ampicillin	AM	10
2	Piperacillin	PIP	100
3	Oxacillin	OX	1
4	Amoxicillin/clavulanic acid	AMC	20/10
5	Cefoxitin	FX	30
6	Ceftazidime	CAZ	30
7	Cefotaxime	CTX	30
8	Cefpodoxime	CPD	10
9	Ceftriaxone	CRO	30
10	Cefepime	CEF	30
11	Aztreonam	AZT	30
12	Imipenem	IPM	10
13	Amikacin	AK	30
14	Gentamicin	CN	10
15	Norfolxacin	NOR	10
16	Ciprofloxacin	CIP	5
17	Nitrofurantin	FU	300
18	Triomethoprim/ Sulfamethoxazole	STX	(1.25/23.75)

2.1.5. The Kits

The kits used in the present study are listed in table (2-5) and their components in appendices (1) and (2).

Table (2-5): Types of kits used in the present study and their Manufacturers

Name of Kit	Purpose	Manufacturers /Origin
AccuPower® PCR PreMix	For amplified primer by PCR	Bioneer／South Korea
AccuPrep® Plasmid Mini extraction kit	Extraction bacterial DNA Plasmid	Bioneer／South Korea
Api 20E	Identification of Enterobacteraceae Family	BioMerieux (France)
Gram' stain	Differentiation of Microorganisms	SyrBio (Syria)
Presto™ Mini g DNA Bacteria	Extraction bacterial genomic DNA	Geneaid／Taiwan
Vitek 2- GN Kit	Identification of Gram-Negative bacteria	BioMerieux (France)

2.1.6. Primers used in present study

The specific primers for bla_{CTX-M}, bla_{SHV}, bla_{OXA} and bla_{TEM} genes which have been chosen in table (2-6) were prepared by the company of Bioneer/ South Korea.

Table (2-6): Primers sequences used for genes amplification

Name	Primers sequences (5′-3′)		Size bp	References
bla_{TEM}	**F**	ACTGCGGCCAACTTACTTCTG	374	(Kaye *et al*., 2004)
	R	CGGGAGGGCTTACCATCTG		
bla_{CTX-M}	**F**	CGCTTTGCGATGTGCAG	550	(Paterson *at el*., 2003; Hujer *at el*., 2006)
	R	ACCGCGATATCGTTGGT		
bla_{OXA}	**F**	ATATCTCTACTGTTGCATCTCC	619	(Karami *et al*., 2008)
	R	AAACCCTTCAAACCATCC		
bla_{SHV}	**F**	GGGTTATTCTTATTTGTCGC	615	(Feizabadi *et al*., 2010)
	R	TTAGCGTTGCCAGTGCTC		

2.2.Methods

The general steps for this research are shown below in figure(2-1)

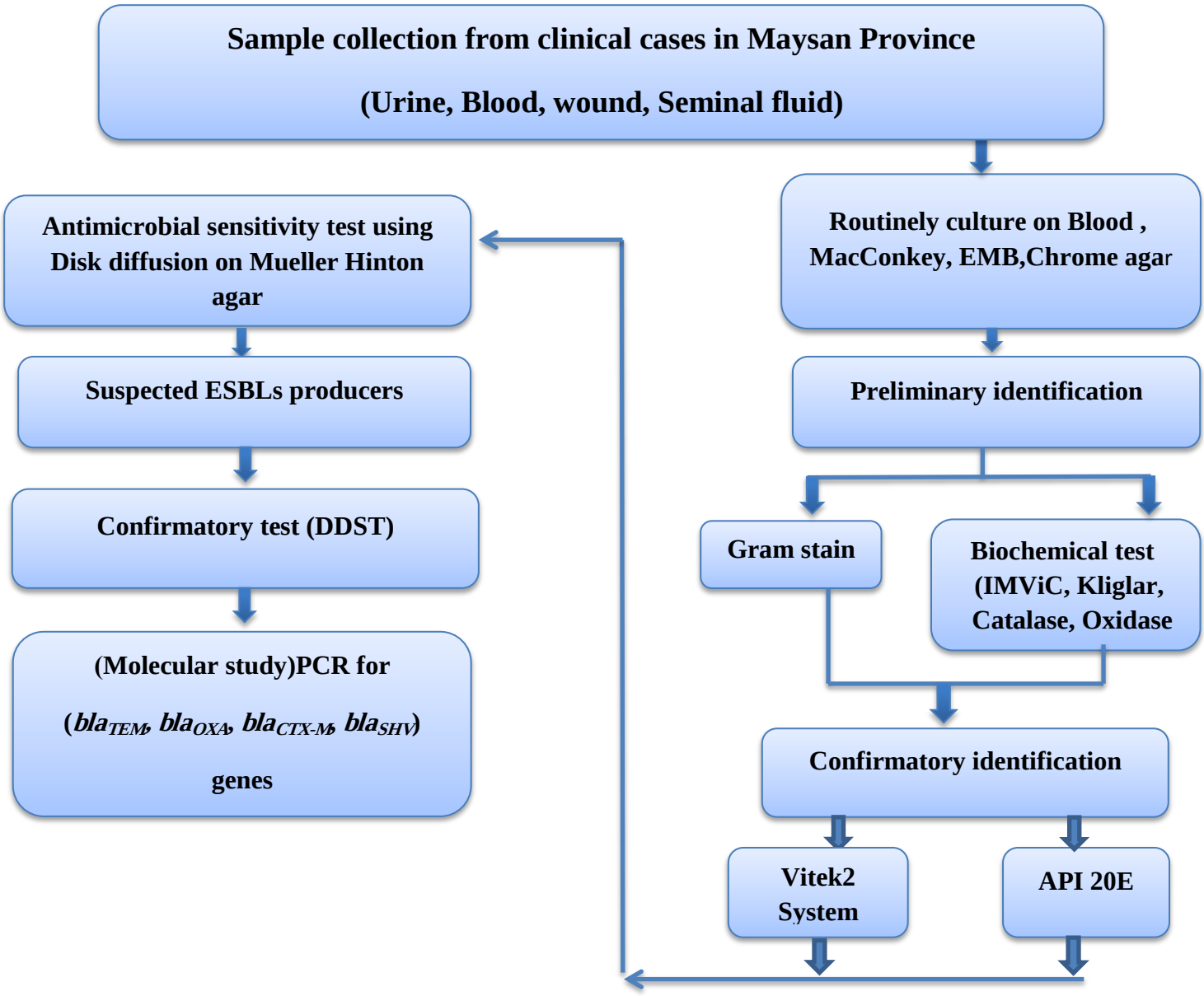


Figure (2-1): The most important steps in current the study**.**

2.2.1. Sterilization Methods

2.2.1.1. Sterilization by Autoclaving

The cultural media were sterilized by autoclaving at 121C˚ for 15 minutes under pressure 15 psi.

2.2.1.2. Sterilization by Dry Heat

The glassware was sterilized by oven at 180 C˚ for 2 hours.

2.2.2. Preparation of Reagents and Solutions

The following reagents were used in the present study, and prepared as described by (MacFaddin, 2000).

2.2.2.1. Catalase Reagent (3%)

The catalase reagent which is hydrogen peroxide (3%) was prepared from the stock solution, in a black bottle and used for detection of the ability of the bacteria to produce catalase enzyme.

2.2.2.2. Oxidase Reagent

This reagent was prepared, freshly in a black bottle via dissolving. 0.1gm of material(Tetramethyl p-phenyl diamine- dihydrochloride), in 10 ml of (D.W).

2.2.2.3. Methyl Red Reagent

The reagents were prepared through dissolving 0.1 gm of methyl red in 300 ml of 96% ethanol, and then completed to 500 ml with distilled water (D.W). This reagent was used as indicator in methyl red test.

2.2.2.4. Voges-Proskauer Reagents

were prepared as follows:

Reagent A: 5gm of α-naphthol were dissolved in 100ml of 96% ethanol.

Reagent B: 40gm of KOH were dissolved in 100ml D.W.

This reagent was used as indicator in Voges-Proskauer test.

2.2.2.5. Normal saline (0.85%)

This solution was prepared by dissolving 0.85 gm of sodium chloride in 90 ml of distilled water, and then the volume was completed up to 100 ml by distilled water.

2.2.2.6.Tri Borate EDTA (TBE) buffer

Preparation of 1X TBE buffer: The 1X TBE buffer was prepared from 50X TBE buffer (as stock solution) by adding 20 ml of this stock solution to 980 ml of distilled water (Sambrook and Rusell, 2001).

2. 2.3. Preparation of culture media

All the culture media listed in table (3-3) were prepared according to the instructions of the manufacturer.

2.2.4. Isolation of *E. coli*

2.2.4.1. Samples collection

Two hundred and ninety one (291) samples were collected from different clinical cases since the beginning of October 2018 till the end of December 2018 from Al-Sader Teaching, Birth and Child and Al-Zhrawai Hospitals of Maysan Province.

2.2.4.2. Culturing of Samples

Selected samples (urine, blood, wound swab and seminal fluid). About 5 ml from the middle of the urine stream in sterile containers were labeled and directly transported to laboratory and cultivated on blood and MacConkey agar and incubated overnight at 37 Cº under aerobic conditions. While blood sample were collected , about 5 ml of blood were inoculated in brain heart infusion broth and incubated overnight at 37 Cº under aerobic conditions, in next day one drop has been taken by syringe and put it on blood and MacConkey agar and incubated overnight at 37 Cº under aerobic conditions and we repeated that

for a week, whereas sample of wound and seminal fluid were cultured on blood and MacConkey agar directly. All suspected bacterial isolates were streaked on Eosin Methylene Blue (EMB) and chrome agar.

2.2.5. Diagnosis of *E. coli*

2.2.5.1. Conventional Tests

The growing colonies of *E. coli* isolates were initially identified depending on:

2.2.5.1.1. Morphological Characteristics

The morphological characteristics of the growing colonies of *E. coli* include size, color, shape, edge and height of the colonies on enrichment, selective and differential media (Blood agar, MacConkey agar, (EMB) and Chrome agar (Goldman and Lorrence, 2009).

2.2.5.1.2. Gram Staining

Gram stain solutions were prepared and used according to the method recommended by Bailey and Scott (2002).

2.2.5.1.3. Biochemical Tests

The following biochemical tests were described according to MacFaddin (2000).

2.2.5.1.3.1. Indole Test

The peptone water was prepared according to the instructions of the manufacturer in autoclaveable test tubes then sterilized by autoclaving. The test tubes were inoculated with fresh colony and incubated at 37 C^{o} for 24hours; after that one drop of Kovac's reagent was added for each tube. The formations of red ring in less than 1 minute indicate a positive result.

2.2.5.1.3.2. Methyl Red Test

Methyl red-Voges Proskauer broth was inoculated with a fresh bacterial culture and incubated at 37°C for 24 hrs. when, a few drops of methyl red solution were added and mixed. The result was read immediately, positive test was bright red.

2.2.5.1.3.3.Voges-Proskauer Test

Methyl red-Voges-Proskauer broth was prepared according to the instructions of manufacture and after that inoculated with a fresh bacterial culture and incubated at 37°C for 24 hrs. Ten drop of 40% KOH solution and more 10 drop of 5% solution of α-naphthol to each tube. A positive reaction was indicated by the development of cherry red color in 2 or 5 minutes.

2.2.5.1.3.4. Citrate Utilization Test

Simmon's citrate agar was prepared according to the instructions of manufacture and poured into test tubes (5 ml into each tube) then sterilized by autoclaving. The test tubes were let in a slant position to harden, and inoculated with fresh colony then incubated at 37Cº for 24-48 hours. Changing the color of agar from green to blue color indicates a positive result.

2.2.5.1.3.5. Kliglers Iron Agar Test

Using a straight inoculation needle an inoculums was stabbed into bottom of tube and streaked over surface of the agar slant and then incubated at 37Cº for 24h .

2.2.5.1.3.6. Catalase Test

A single isolated colony was transferred from the cultured petri dish by a sterile loop and placed on a glass slide then 1-2 drops of 3% H2O2 were mixed with the colony on the slide. The appearance of gas bubbles indicates a positive test.

2.2.5.1.3.7. Oxidase Test

A piece of filter paper was placed in a Petri dish and 3 drops of a freshly prepared oxidase reagent were added. By sterile loop, a colony was removed from a cultured plate and smeared on the filter paper. The oxidase positive organisms give blue color within 5-10 seconds; in oxidase negative organisms, color does not change.

2.2.5.2. Confirmatory Diagnosis

2.2.5.2.1. Analytical profile index for Enterobacteriaceae test API 20E Test

This test has been used clinically for the rapid recognition of the bacterial isolates. This test, strip contains 20 small tubes with upper orifice (Cupule) and lower orifice (tube) which contain dried material represent the biochemical test, color changes happened in the tubes either during incubation or after addition of the reagents. These colors reveal the presence or absence of chemical active and thus, a positive or negative result; The instructions for use are:

1. Preparation the strip: To fashioning humid condition, 5 ml of D.W was added to the strip container.
2. Preparation bacterial suspension: Transferred pure single colonies from culture with suspected *E. coli* Isolates was transferred by a loop into a buffer suspension medium (NaCl 0.85 suspension) and mixed well to form a suspension .
3. Inoculation of strip: The tube of each strip has been filled with a bacterial suspension by a sterile tubes that include CIT, VP and GEL must be filled the capule of them. Pasture pipette except some
4. To formation anaerobic condition in some tube the upper part of tubes LDC, ADH, ODC, H2S and URE were covered with a sterile mineral oil.
5. The strip container was closed and incubated at 37ºC for 18-24 hrs .

6. Reading the result:

After incubation, the reagents were added as: a drop of Kova's reagent into IND tube and a drop of TDA reagent into TDA tube reading the result immediately, also drop of VP1 reagent then drop of VP2 directly into VP tube and waited for 10 minutes then reading the result. Then after that, all results were recorded into numerical profile and compared with the company index. Each strip was divided into seven parts and each part has three tests numbered as 1, 2 and 4 and each positive test has been given a specific number, but the negative test has been given the number zero. Final output there are seven numbers which compare with the index to give the genus and species of the bacterial isolates. (Blazevic *et al*., 1979).

2.2.5.2.2. VITEK 2 Diagnostic System

The Vitek 2 system was used in this present study in order to confirm the diagnosis *E. coli* isolates. Where in this system, a bacterial suspension is required from the suspected *E. coli*, and this suspension is placed in the inoculated tube. This suspension is transferred to the card, which is incubated in thermally controlled conditions, the color changes in the card, which are produced by the metabolic activity of the bacteria, are measured in interrupted form (every 15 minutes) by light intensity, after that the information's were analyzed, stored and printed automatically (Pincus , 2006).

The steps are described in more detail as follows:

I- Preparation of bacterial suspension

A sterile swab was used to transfer a sufficient number of bacterial colonies of a pure culture and suspended in 3 ml of sterile saline in a clear plastic test tube. The turbidity is adjusted by density check measurement at the range (0.5-0.7).

II- Inoculation of identification card

An identification card was inoculated with bacterial suspension using an integrated vacuum apparatus. A test tube containing the bacterial suspension was placed into a special rack (cassette) and the identification card was placed in the neighboring slot, while inserting the transfer tube into the corresponding suspension tube. The cassette can accommodate up to 10 tests or up to 15 tests. The filled cassette was placed either manually or transported automatically into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the bacterial suspension was forced through the transfer tube into micro-channels that filled all the test wells.

III- Card sealing and incubation

An inoculated card was passed by a mechanism ,which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types were incubated on-line at 35.5 + 1.0 ºC. Each card is transferred from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next reading time. Data were collected at 15 minute intervals during the entire incubation period.

2.2.6. Methods of preservation

The bacterial isolates were preserved on brain heart infusion agar slant at temperature 4 ºC , the bacterial isolates were maintained every month during the study by culturing on new culture media. For long preservation, brain heart infusion broth supplemented with 15% glycerol had been used to frozen at temperature(-20 ºC) for long term (several months or year) (MacFaddin, 2000).

2.2.7.Testing for Antibiotic Susceptibility

All *E. coli* isolates were subjected to antibiotic susceptibility depended on disc diffusion method as described by Bauer *et al*.(1966). The selection of antibiotic disc was done according to the guidelines recommended by the Clinical and Laboratory Standard (CLSI, 2017). Three or Five pure isolated colonies from fresh culture bacteria has been suspended in 5 ml of brain heart broth and incubated at 37C˚, for 24hr, the density of the suspension produces from growth culture was adjusted depending on density check measurement by adding sterile normal saline. Inoculated Mueller-Hinton agar plates by dipping a sterile swab into the inoculums. The swab was streaking over the surface of the medium several times with the plate was rotated through an angle of 60°,to ensure diffusion after each application finally, also the swab was pressed around the edge of.

Antibiotic discs , as shown in table (2- 4) was placed to each plate by a pair of sterile forceps. approximately 15 mm far of the edge of the plate. Each disc gently pressed down to ensure even attached with the medium. The plates were put in incubator at 37°C. After 18 or 24 hours incubation, the diameter present in each zone (including the diameter of the disc) were measured and recorded in mm. The results then were interpreted according to CLSI (2017).

2.2.8. Phenotypic Detection of ESBLs producing *E. coli*

2.2.8.1. Screen Test (primary test)

ESBLs production by β-lactam resistant isolates was initially screened by using diffusion disks of cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), aztreonam (30 μg) and cefopodoxime (10 μg), placed on inoculated plates containing Muller-Hinton agar according to the CLSI recommendations (CLSI, 2017). After 18 hrs incubation at 37C˚, the diameters of the inhibition zones around the antibiotics were measured by caliper. The isolates which showed

inhibition zones < 27 mm for cefotaxime, <22mm for ceftazidime, <25 mm for ceftriaxone, < 27 mm for aztreonam and <17mm for cefopodoxime were suspected for ESBL production.

2.2.8.2. Double Disk Synergy Test (DDST) (confirmatory method)

This method was carried out as modified by Bedenić *et al.* (2010) as follows: This test was done by placing a disc of augmentin (20μg amoxicillin + 10μg clavulanic acid) on to Mueller–Hinton agar on which test inoculum was spread and discs were placed around augmentin, containing 30 μg for cefotaxime, 30 μg for ceftazidime, 30μg for ceftriaxone, and 30 μg for azetreonam with keeping the distance(15-20) mm (edge to edge) from a disk of augmentin (30 μg). After that the petri dish was incubated for 16-20 hours at 35C˚(±)2C˚. Any enhancement of the zone of inhibition between a β-lactam disk and augmentin disk was indication of *E. coli* ESBL producer.

2.2.9. Molecular study

Polymerase Chain Reaction (PCR) assay was performed for detection of *E. coli* ESBLs gene markers (***bla*$_{TEM}$, *bla*$_{CTX\text{-}M}$, *bla*$_{OXA}$** and ***bla*$_{SHV}$**). This method was carried out as in the following steps:

2.2.9.1.Genomic DNA extraction

Genomic DNA was extracted from *E. coli* by using Genomic DNA Mini Bacteria Kit according to company's instructions; the bacterial culture has been inoculated in 10 ml of medium brain heart infusion broth and incubated at 37ºC at 24 hours in the incubator as follows**:**

Step 1: Cell Harvesting(pre-lysis)

- Fresh culture (1ml) was Added to a 1.5ml. tube microcentrifuge.
- Culture was Centrifuged for 1 minute at 13,000 rpm. then discard the supernatant.

- Added 180µl of GT buffer by micropipette, then re-suspend the cell pellet by vortex.
- Added 20µl of proteinase K (make sure distilled water added) and incubate at 60C˚ for 10 minutes, during incubation the tubes were inverted every 3 minutes.

Step 2: Lysis Step

- Added 200µl of GB buffer to the sample and mix by vortex for 10 seconds.
- The tubes were incubated at 60C˚ for at less 10 minutes to ensure the sample lysate is clear, during incubation the tubes were inverted every 3 minutes. At this time the Elution buffer was pre-heated (200µl per sample) to 70C˚ (for step DNA Elution).

Step 3: DNA Binding

- Added 200µl of absolute ethanol to the sample lysate and mix immediately by shaking vigorously.
- GD column were placed in a 2 ml collection tube.
- Mixture (including any insoluble precipitate) was transfer to GD column then centrifuge at 13,000 rpm for 2 minutes.
- The 2m1 collection tubes containing the flow-through were discard .
- The GD column were placed in a new 2ml collection tube.

Step 4: Washing Step

- Added 400µl of W1 Buffer to the GD column and then centrifuge at 13,000 rpm for 30 seconds then discard the flow-through, place the GD column back in the 2ml collection tube.
- Added 600µl of Wash buffer (make sure ethanol was added) to the GD column, centrifuge at 13,000 rpm for 30 second then discard the flow-through, place the GD column back in 2ml collection tube .
- The columns matrix were centrifuge at 13,000 rpm for 3 minutes to dry.

- The dried GD column were transferred to a clean 1.5 micro centrifuge tube.

Step 5:Elution

- pre-heated Elution Buffer was added into center of the column matrix.
- We waited for at least 3 minutes to allow Elution buffer to be completely absorbed.
- The tubes were placed in Centrifuge at 13,000 rpm for 30 seconds to elute the purified DNA.
- At last, the DNA was Stored at 2-8°C.

2.2.9.2. Plasmid DNA Extraction

1. cultured *E .coli* (1-5ml) were harvesting by centrifugation for 5 min at 3,000 rpm and the media completely were removed by pipetting.
2. Added 250 µl of Buffer (1) to the collected cells and completely responded by vortex or pipetting.
3. Added 250 µl of Buffer (2) and mixed by inverting the tube 3-4 times, gently.
4. Added 350 µl of Buffer (3) and immediately mixed by inverting the tube 3-4 times gently.
5. Tube centrifuged for 10 min at 4°C, 13,000 rpm in a micro-centrifuge.
6. The cleared lysate was transferred to the DNA binding column tube and centrifuge for 1min at 13,000 rpm.
7. The flow-through were poured off and re-assembled the DNA binding filter column with the 2.0 ml collection tube.
8. Added 500 µl of Buffer (D) and wait for 5 min, then the tubes were centrifuged for 1 min at 13,000 rpm
9. Added 700 µl of Buffer (4) to the DNA binding column tube and centrifuge for 1min.at 13,000 rpm.

10. The columns were dried by additional centrifugation for 1min.at 13,000 rpm.

11. The DNA binding filter columns were transferred to the new 1.5 ml micro-centrifuge tube .

12. Added 50-100 µl of Buffer (5) to the binding filter column, and wait for at least 1min.for elution.

13. The plasmid DNA were eluted by centrifugation for 1min.at 13,000 rpm.

2.2.9.3. Detection of DNA content by Agarose Gel Electrophoresis

The DNA extraction have been carried out, agarose gel was prepared according to Sambrook and Russell (2006). Agarose gel electrophoresis was adopted to confirm the presence and integrity of extracted DNA of *E. coli* isolates according to the gel electrophoresis protocol which included the following steps:

1- One hundred of 1X TBE buffer were taken in a Flask

2- Agarose powder (1) gm was added to 100 ml of 1X TBE buffer.

3- The solution was heated to boiling by using a water bath until all gel particles were dissolved.

4- Three microliters of ethidium bromide (0.5 µg/ml) were added to the agarose solution, and then the agarose was stirred in order to get mixed.

5- The solution was left to cool down at 50-60C˚.

6- After sealing both edges of the gel tray with a cellophane tape and fixing the comb in 1 cm away from one edge, the agarose solution was poured into the gel tray.

7- The agarose was allowed to solidify at room temperature for 30 minutes. The fixed comb was carefully removed and the gel tray was placed in the gel tank. The tank was filled with 1X TBE buffer, until the buffer reached 3-5 mm over the surface of the gel.

8- DNA sample was transferred 5µl from it to Eppendrof tube, 2µl of loading dye were added to tube and mixing well ,then the mixture was loaded in to the wells in agarose gel. Electric current was allowed at 65 volt for 1hour. Finally, the bands were visualized on a UV transiluminator at wave length 350 nm .

2.2.9.4. Preparation of primers solution

The oligonucleotide primers were suspended by dissolving the lyophilized product after spinning down briefly with De-ionized water depending on manufacturer's instruction as stock suspension. Working primer tube was diluted with De-ionized water, the final picomoles depended on the procedure of each primer.

2.2.9.5. Master Mix

The components of the master mix were prepared by the company of Bioneer, South Korea.

2.2.9.6. Polymerase Chain Reaction protocol

The protocol used according to Bioneer manufacturer's instructions. All PCR components were assembled in PCR tube and mixed by cooling microcentrifuge at (850 rpm) for 10 sec.

- The steps were conducted are as following:

1- Template DNA and primers were dissolved before usage.

2- Template DNA and primers were added into the AccuPower®. Taq premix tubes as in table (2-7) for getting 20ml reaction volume.

Table (2-7): The volume of mixture of PCR

PCR Master mix	Volume (µl)
De-ionized water	6
Master mix	5
Forward primer	2
Reverse primer	2
DNA template	5
Final volume	20

3- The lyophilized blue pellet was completely dissolved and spin down either by using centrifuge 15 sec, vortex or by pipette up and down several times.

4- The Eppendorf PCR tubes were placed in the thermocycler and the right PCR cycling program parameter conditions were showed in table (2-8) and (2-9).

Table (2-8): PCR Program of bla_{OXA}, bla_{CTX-M} and bla_{SHV} genes:

PCR step	Temperature (Cº)	Time(min)	Repeat
Initial Denaturation	94	5	1
Denaturation	94	1	34 cycle
Annealing	55	1	
Extension	72	1	
Final Extension	72	5	1
Hold	4		-

Table (2-9): PCR Program of bla_{TEM} gene:

PCR step	Temperature (Cº)	Time	Repeat
Initial Denaturation	95	5min	1
Denaturation	94	30s	30 cycle
Annealing	62	30s	
Extension	72	30s	
Final Extension	72	10min	1
Hold	4		-

2.2.9.7. Agarose Gel Electrophoresis

TBE 1X buffer was added to the electrophoresis tank, tray with agarose which had previously attended was immersed in electrophoresis tank. Each well is loaded with 5µl of DNA sample. Then standard molecular weight of DNA ladder (5µl) is loaded in a first well. Electrophoreses run at 65 volt for 1hr, the gel was visualized by and then photographed by using a gel Documentation system **(**Mishra *et al*., 2010).

Chapter Three

Results

and

Discussion

3. Results and Discussion

3.1. Samples collection and isolation

A total of 291 samples were collected during the period from October 2018 to the end of December 2018, and cultured according to the standard laboratory technique, the number of positive growth was 235 samples which included 138 samples from urine, 28 samples of seminal fluid, 30 samples of wound swabs and 39 were blood samples. 105 (44.7%) *E. coli* were recovered from all type of positive growth as showed in table (3-1).

These results were strongly similar to the study of Al-Dulaimi *et al*. (2015) where their result was 44.8% in Babylon city, also corresponded with a study reported in India by Singh *et al*. (2016) and Andrew *et al*. (2017) in Southwestern Uganda where identified *E. coli* was 44% for both. While the results were more than those obtained in the study conducted by Al-Jelehawy (2016) in Diwania city which that 22.27% and higher than other study reported in India by Akila *et al*. (2017), and in Ethiopia by Gashe *et al*. (2018) where the results were 21.96% and 25.4% respectively, also in a study reported in Babylon done by Al-Hasnawy *et al*. (2018) that was 15%. The results of the present study were less than that of a studies conducted by Dagi *et al*. (2015) in Turkia and Bora *et al*. (2014) in India where the results were 70% and 73.6% respectively.

Navidinia *et al*. (2014) and Sáez-lópez *et al*. (2018) concluded that *E. coli* which caused most extraintestinal infections was commensal strain in original, but become pathogen by adaptation stratagems or gained of virulence factors. Moreover, there is Physiological study which conducted by Murthy (2006) who noted that *E. coli* have the ability to adapt themselves to their different environments.

Table(3-1): Number and percentage of *E. coli* from positive growth of different clinical cases.

Positive culture	Number of isolate	(%)
E. coli	105	44.7
Other causes	130	55.3
Total positive culture	235	100

Data in current study showed that the high rate of *E. coli* isolated from urine 91(65.9%), followed by seminal fluid, wound swab and blood as indicated in the table (3-2) which show 21.4%, 13.3%, 10.3% respectively.

Table(3-2): Number and percentage of *E. coli* of isolates according to source of infection.

Samples type	Infection type	No. Samples	No. Positive growth	No. *E. coli* isolates	*E. coli* Percentage(%)
Urine	Urinary tract infection	159	138	91	65.9
Seminal fluid	Genitourinary tract infection	39	28	6	21.4
Wound swab	Wound infection	41	30	4	13.3
Blood	Septicemia	52	39	4	10.3
Total	-	291	235	105	44.7

A similar conclusion was reached by Omololu-Aso *et al*. (2017) who concluded that *E. coli* was the most gram negative bacteria found in clinical laboratories samples including the majority of urinary, wound, blood and peritoneal samples. Also similar pattern of results concluded by Lhwak and Abbas (2018) in Babelyon city where the results was 63.3% and with study reported in other country for researchers Ouedraogo *et al*. (2016) who reported in their study on urinary tract infections, *E. coli* was the most frequent isolate (67.5%) among all the identified bacteria. Also, compatible with the result of a study reported by Sohail *et al*. (2015) who studied urinary tract infection and they found that the most predominant isolated bacteria was *E. coli* (62%). While these result was contrary to the findings of Saleh and Hussein (2019) in Thi-Qar city which reported that 30% of *E. coli* recorded from Immunosuppression Patients, also went beyond previous reports conducted in Al-Najaf province by Majeed and Aljanaby (2019) where *E. coli* presented 38.9% of identified bacteria isolated from patients.

The leading causes to the highest rate of isolation from urinary tract infection might attributed to the possession of *E. coli* to pilli (adhesins) which help for attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion resulting in invasive infection and UTIs (Amiri *et al*., 2009). Moreover, Abraham (2011) considered in his study that *E. coli* is responsible for two types of urinary tract infection which are a symptomatic and symptomatic infection, and they are consider major etiological agent for this infection. Contamination also may be cause this infection.

As shown in Table (3-2) which mentioned the percentage of *E. coli* in seminal fluid was 21.4% that conform with study reported by Moretti *et al*. (2009) where the result was 20.3% from sperm culture also they concluded in their study that sperm bacterial contamination is quite frequent and could contribute to the decay of the sperm quality of infertile men. While in a study conducted by Anago *et al*. (2015) the result was 1.2%, whereas, Vilvanathan *et al.* (2016) was

deduced that prevalence of *E. coli* in seminal fluid was 10%. Solomon and Henkal (2017) were considered that *E. coli* is the most common nonsexually transmitted urogenital tract microbes, these pathogens cause epididymitis, epididymo-orchitis, or prostatitis and contribute to increased seminal leukocyte concentrations. Nonetheless Villegas *et al*. (2017) mentioned that the most *E. coli* isolated from semen is uropathogenic (UPEC) and can damage sperm in different ways. Also it has an important role in causing male infertility associated with genital tract infections, the main mechanism postulated for male infertility by *E. coli* is the profound damage to different sperm processes and function, either by direct contact and/or through secreted toxins.

In the current study, the result of wound swab was 13.3%. It was cognate to the percentage of study conducted by Nasser (2017) in Baghdad city which was 7.5%, while another study performed in Nepal by Nepal *et al*. (2017) was 2.9%, whilst a study implemented by Roy *et al*. (2017) in Bangladesh indicated that the percentage of isolated *E. coli* was 23.7% where they considered in their study that *E. coli* is the second most common isolate in wound infection.

Insan *et al*. (2013) defined wound as a breach in the skin and the exposure of subcutaneous tissue following loss of the skin integrity which provides a moist, warm and nutritive environment that is conductive to microbial colonization and proliferation. Additionally, Kumburu *et al*. (2017) and Janssen *et al*. (2018) noted that the wound infections are an emerging medical problem worldwide, the economic burden and morbidity and mortality rates are huge.

In the present study the percentage of *E. coli* in blood cultures was 10.3% which was directly in line with the result reported by Kumburu *et al*. (2017) where the result was 9.1% in Northern Tanzania, also corresponded with a study conducted in Iraq by Aljanaby *et al*. (2017) that was 14%, while other studies conducted by Nasser (2017) and Nepal *et al*. (2017) the results were 5% and 2.2% respectively.

Lamy *et al*. (2016) noted that blood stream infections (BSIs) are a major cause of death in the developing countries and the detection of microorganisms is essential in managing patients, despite major progress has been made to improve identification of microorganisms, Blood culture (BC) remains the gold standard and the first line tool for detecting BSIs. ESBLs producing strains of *E. coli* are a significant cause of BSIs in hospitalized and non-hospitalized patients that was recorded by Tumbarello *et al.* (2008) who mentioned in their previouly study that delaying effective antimicrobial therapy in BSIs caused by ESBL producers significantly increases mortality .

The differences in the ratio of identified bacteria are may be due to the level of health in different communities, also may be due to the heterogeneity in the laboratory methods used to isolate and diagnose bacterial pathogens in the different countries and regions of the developing countries. This may also be attributed to differences in patient environments taking into consideration the hospital environment in addition to antibiotics used in treatment indiscriminate lead to heterogeneity and spread of bacterial species.

3.2. Diagnosis of *E. coli*

3.2.1. Conventional diagnosis

3.2.1.1. Morphological and Microscopic characteristics

Diagnosis of *E. coli* was completed by using documented methods that mentioned in section (2.2.5.1) by the examining the morphological characterization of the growing colonies of *E. coli* on different culture media Include(enrichment , selective and differential media). The samples were cultured on MacConky agar, a blood agar, EMB and Chrome agar as shown in figure (3-1). The colonies of *E. coli* under study was characterized by; its small, smooth and dry, fermented lactose sugar. Pink color on MacConky agar; the morphological characteristics of *E. coli* isolates of present study was similar to that in a study conducted by Barcella *et al*. (2016) and Al-Zubaidy (2016) who showed growth

of circular, pink color, lactose fermenting colonies of *E. coli* on the MacConkey agar media.

While *E. coli* appeared as Gray color colonies and some of them showed their ability to hemolysis on Blood agar that was compatible with researchers Saleh and Hussein (2019). On EMB the colonies appeared green, these results were in line with previous studies obtained by Al-Zubaidy (2016) in Baghdad and Zandi *et al*. (2017) in Iran which exhibited that the *E. coli* displayed green with metallic sheen colonies on eosin methylene blue (EMB) agar.

On Chromogenic medium, the colonies of identified *E. coli* were light pink color where this medium used for the enumeration and identification of micro-organisms from clinical specimens and food, special formula allows also confirming directly the indole tests, this media contain peptone, yeast extract, tryptone, sodium chloride, chromogenic mixture, and agar in addition to final pH was (7.2 ± 0.2). Where Peptone, tryptone and yeast extract are a source of amino acids and vitamins, Sodium chloride maintains the osmotic balance of the environment, Chromogenic mixture allows identification of microorganisms based on colony color and morphology. These results were matched with Verhaegen *et al*. (2015) and Al-Shweely (2018) where *E. coli* form pink colonies.

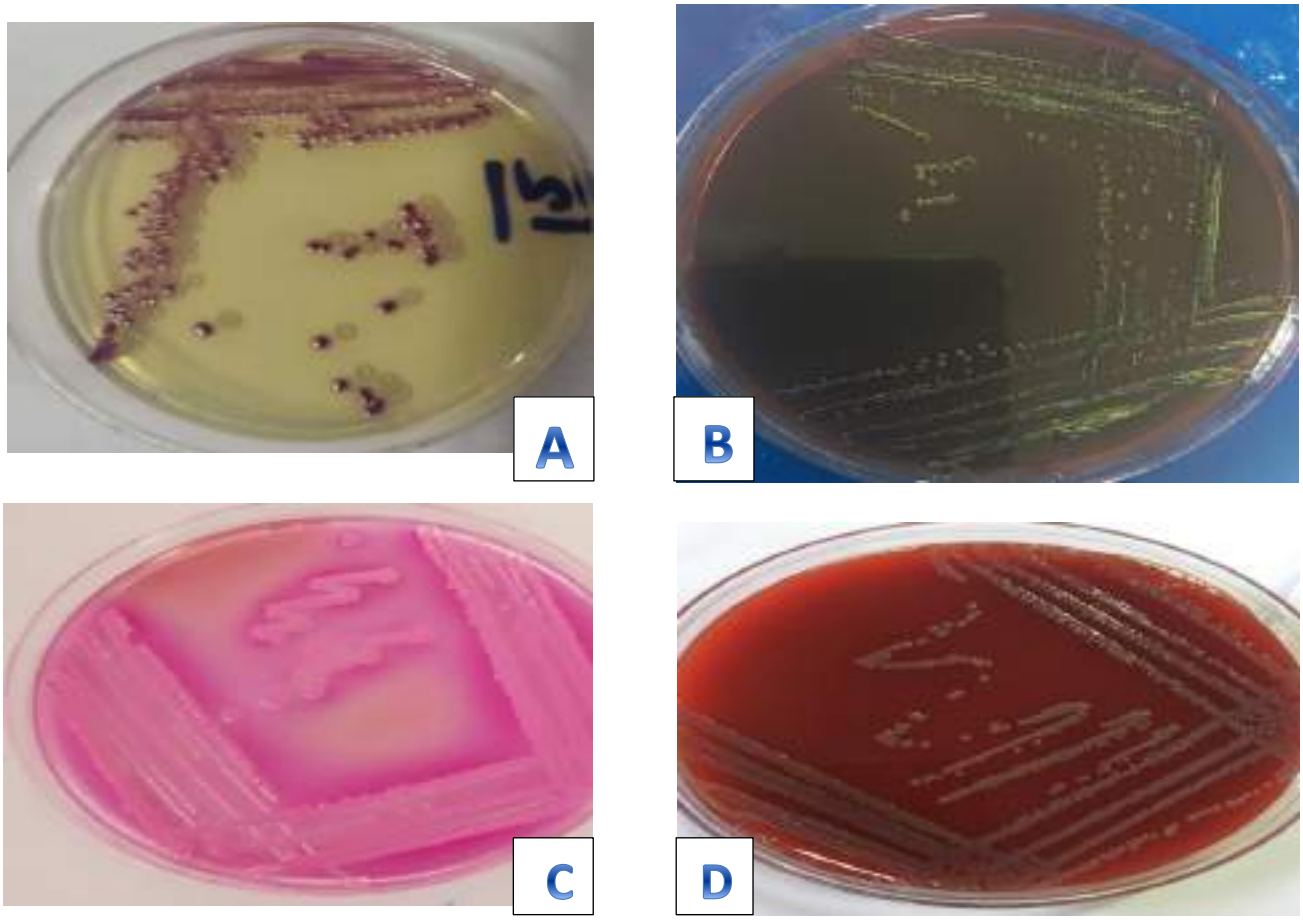


Figure (3-1): Morphological characteristic of *E. coli* on different culture Media (A): *E. coli* colony on Chrome agar, (B): *E. coli* on EMB agar, (C): *E. coli* on MacConkey agar and (D): *E. coli* colony on blood agar.

The result of Gram staining demonstrated that *E. coli* was observed as pink colored, nonspore , cocco bacilli and vary in length as shown in Figure (3-4). The findings were directly in line with previous studies conducted by Akter *et al*. (2016) who reported that *E. coli* was a Gram negative bacteria with rod shaped appearance that arranged in single or in pair and pink color colonies.

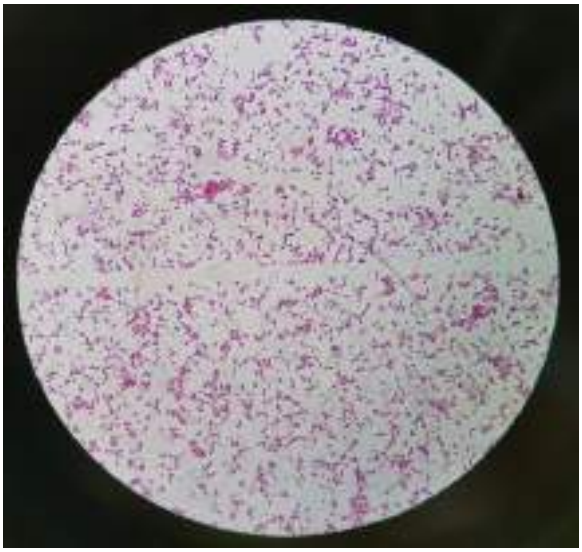


Figure (3- 2): Gram staining of *E. coli* isolated under the light microscope using oil immersion lenses (100X).

3.2.1.2. Biochemical Tests

The biochemical tests were performed on suspected *E. coli* that recovered from different clinical cases , the results of the current study found that 105 *E. coli* were lactose fermenter. Furthermore, *E. coli* was Indole and methyl red tests positive whereas negative for both voges-proskauer , and citrate utilization tests. On kligler agar *E. coli* revealed A/A reaction with gas production and were non H2S producer. Also, it is negative for oxidase and positive for catalase tests as shown table (3-3). Moreover, the findings of biochemical tests were consistent with the findings of Ali *et al*. (2017) in Diyala and Saleh and Hussein (2019) in Thi-Qar city.

Table (3-3): Results of biochemical tests for isolated *E. coli*

Biochemical test	Result	Percentage of *E. coli*
Indole	+	100%
Methyl red	+	100%
Voges-Proskauer	-	100%
Citrate Utilization	-	100%
Kligler agar	A/A with gas, no H2S	100%
Catalaes	+	100%
Oxidase	-	100%

3.2.2. Confirmatory identification of *E. coli*

For further confirmation, *E. coli* isolates were subjected to API 20E and Vitek 2 system as showed in section (2.2.5.2). These tests are considered as confirmatory tests to decrease the time required for the bacterial diagnosis by conventional biochemical test.

3.2.2.1. Analytic Profile Index 20 Enterobacteriaceae (API 20E)

To confirm the preliminary identification of *E. coli*, the (API 20E) system was implemented to got this objective . Sixty three (60%) of isolates were identified by this system as previously mentioned in section (2.2.5.2.1) and shown in Appendix (5) and Figure (3-3) where the Api20E strip is shown after it is inoculated with a bacterial suspension and incubated for 24hrs in 37C˚, which strongly support the conventional tests and this concordance with study conducted by Yousef *et al*. (2013) and Al-Zubaidy (2016) who referred to the use of the API 20E test as a diagnostic golden biochemical tool for identification

of *E coli* isolated from different clinical samples. While the results were 82.7% in study conducted by Al-Oebady (2017).

The major advantage of the API 20E system is that it is more convenient and easier to identify Gram-negative bacteria than the conventional tests (Blazevic *et al*., 1979). The API 20E is one of the original miniaturized systems; still in wide spread use for the identification of members of the Enterobacteriaceae since the 1970s, this system is based on 21 different biochemical tests such as the production of indole, citrate utilization, voges-proskauer reaction, carbohydrate fermentation and other tests (Schmidt and Relman, 1994). Abulreesh (2014) concluded in his study that API 20E was found to produce more accurate identification with isolates of clinical but not environmental origin.

Figures (3-3): The strip of Api-20E system : (+)The test positive; (-)The test negative.

3.2.2.2. Vitek 2 System

Forty two (40%) of suspected *E. coli* were confirmed by Vitek2-automated system. The results strongly support the other conventional results. Appendix (6) shows the result of Vitek 2 system for one of the studied samples.

The Gram negative card was based on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic ac-

tivities, and resistance (Chang *et al*., 2002). There were 47 biochemical tests and one negative- control well. Final identification results were available in approximately 10 hrs. or less.

In the present study all results were excellent which concluded that the VTEK2 Gram-negative identification method is an acceptable automated method for the rapid identification of Gram-negative bacteria as mentioned by Al-Humam (2016). Current results coincides with the study of Ali (2017) who showed the ability of VITEK2 systems to identify more than 150 Gram-negative bacilli. While our result study was higher than the results achieved by Guran *et al*. (2017).

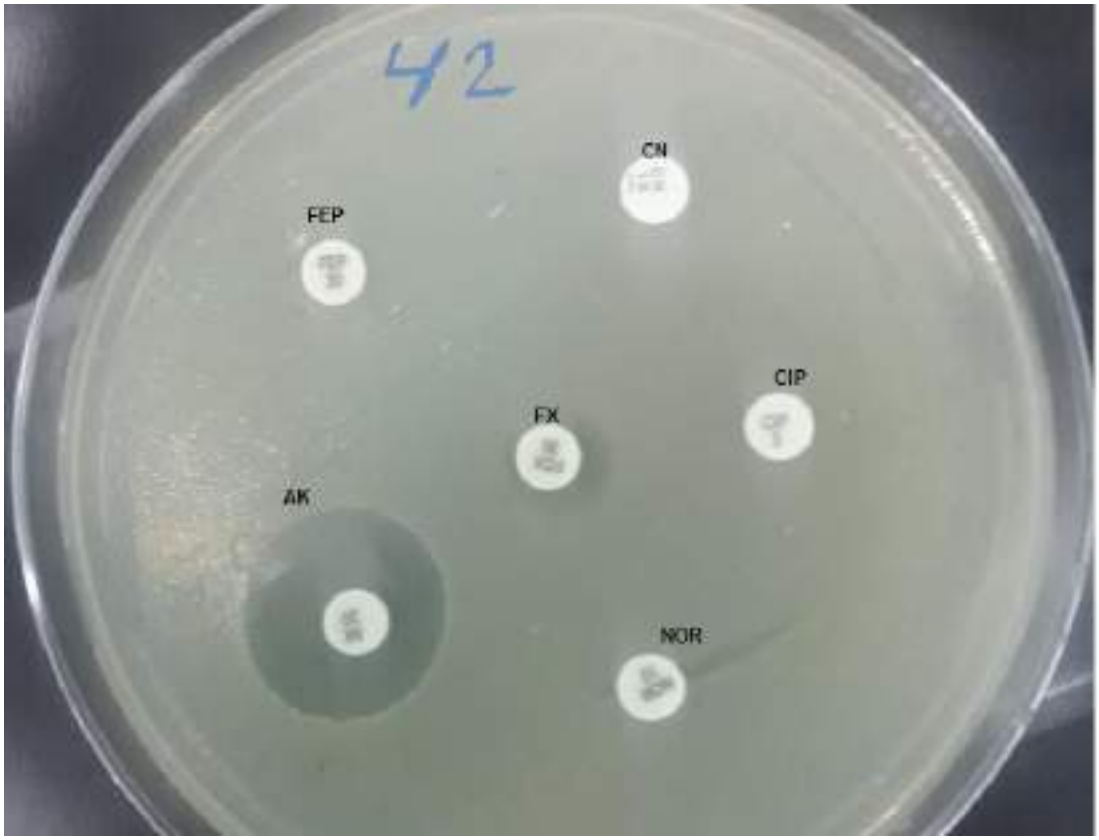
3.3. Antibiotic Susceptibility

The antimicrobial susceptibility test for completely identified (105) *E. coli* isolates. The results demonstrated that *E. coli* varied in their resistance and sensitivity for screened 18 antibiotics belong to the following groups: (penicillins, cephems, monobactam, carbapenems, β- lactamase inhibitor, aminoglycosides, Fluoroquinolones, Nitrofurantoins and folate pathway inhibitors) which used as a traditional treatment for the infections caused by this bacteria as in the table (3-4) and figure (3-4)

Antimicrobial resistance has become a major medical and public health trouble in hospitals and also in the community (Nugent *et al* . 2010). Furthermore, Jena *et al*. (2017) confirmed that knowledge of resistance pattern can help physicians to select suitable empirical antibiotic regimens, so that antibiotics showing high-resistance pattern can be avoided.

Table (3-4): Results of Antibiotics susceptibility test for *E. coli* isolated from different clinical cases(n=105) .

No	Antibiotic	Sensitive No.(100%)	Intermediate No.(100%)	Resistance No.(100%)
1	Ampicillin	2 (1.9)	-	103 (98.1)
2	Piperacillin	4 (3.8)	-	101 (96.2)
3	Oxacillin	5 (4.8)	2 (1.9)	98 (93.3)
4	Amoxicillin/ Clavulanic acid	8 (7.6)	2 (1.9)	95 (90.5)
5	Cefoxitin	8 (7.6)	6 (5.7)	91 (86.7)
6	Ceftazidime	7 (6.7)	-	98 (93.3)
7	Ceftriaxone	11 (10.5)	2 (1.9)	92 (87.6)
8	Cefotaxime	6 (5.7)	-	99 (94.3)
9	Cefpodoxime	4 (3.8)	3 (2.9)	98 (93.3)
10	Cefepime	8 (7.6)	-	97 (92.4)
11	Aztreonam	11 (10.5)	8 (7.6)	86 (81.9)
12	Imipenem	105 (100)	-	-
13	Amikacin	95 (90.5)	4 (3.8)	6 (5.7)
14	Gentamicin	46 (43.8)	3 (2.9)	56 (53.3)
15	Norfloxacin	56 (53.3)	-	49 (46.7)
16	Ciprofloxacin	69 (65.7)	-	36 (34.3)
17	Nitrofurantions	65 (61.9)	3 (2.9)	37 (35.2)
18	Triomethoprim /Sulfamethazole	33 (31.4)	-	72 (68.6)



Figure(3-4): Disk diffusion method show resistance of *E. coli* (42) to the screened antibiotics: CN(Gentamycin), FX(Cefoxitin), NOR(Norfloxacin), AK(Amikacin), FEP(Cefepime), CIP(Ciprofloxacin).

Data in the current study showed high strongly resistance to Ampicillin reached to 98.1% which greatly similar to local studies in Al- Najaf reported by Al-Hilali (2015) and in Al-Diwaniya by Al-Jelehawy (2016) where the results were 99% and 95.91% respectively. Another local study in Tikrit conducted by Alsamarai *et al*. (2016) showed that all isolates are completely (100%) resistant. Also, another study in Saudi Arabia conducted by Yasir *et al*. (2018) where similar pattern of results were obtained that was 99.5%.

Moreover, *E. coli* showed high resistance to piperacillin which was 96.2% that in agreement with local studies for Al-Hilali (2015) in Al-Najaf and Ali *et al*. (2017) in Diyala where the results were 95.2% and 93.3% respectively, also in concordance with a study reported by Cebeci *et al*. (2019) where the result was 98% for *E. coli* isolated from clinical samples in some European country.

The data in table (3-4) show the resistance to oxacillin was 93.3%. This result was broadly in line with a study for Al-Hassnawy *et al*. (2018) in Babylon where all isolates are fully resistance (100%) to oxacillin.

The resistance to amoxicillin/clavulanic was 90.5% which was highly similar to the findings in different places obtained by Jena *et al*. (2017) where the result was 90.9%, which also agreed with many local studies as Aljanaby *et al*. (2017) and Al-Hasnawy *et al*. (2018) where the resistance was 92% and 86% respectively, whereas in the study for Ali *et al*. (2017) in Diyala, the result was 73.3%.

The most reliable interpretation for the resistance of *E. coli* against ampicillin and amoxicillin/clavulanic acid, may be due to predominate gene resistance among *E. coli* isolated in this study and ability *E. coli* to have Beta-lactmase. Sasaki *et al*. (2010) found that 85.1% of the isolates had the ability to produce beta-lactam enzymes. These enzyme such as ESβLs which analyzes the beta-lactam ring (Skurnik *et al.* 2005). The reason of high resistance to penicillins for identified *E. coli* in the current study isolates was may be due to the high production of TEM Extended Spectrum β-lactamase, be genetically localized on the chromosome and the plasmid.

Data in the current study revealed that high resistance to monobactam group which represented by antibiotic aztreonam where the resistance percentage was 81.9%. This to some extent in concordance with the results achieved by Al-Hassnawy *et al*. (2018) that was 92%, while the resistance to this antibiotic in a study of Azekhueme *et al*. (2015) was 41.1%.

The Present study showed that 86.7% of *E. coli* isolates were cefoxitin resistant which coincidently with a study of Hassan *et al*. (2018) in Saudi Arabia where the result was 90%. The frequency of cefoxitin resistance in our study was higher than previous studies recorded in Al-Diwaniya city and Al-Najaf implemented by Al-Jelehawy (2016) and Alfatlawi *et al*. (2019) in Al-Najaf where the result was 34.69% and 59% respectively.

Reduced susceptibility to cefoxitin in the *E. coli* may be an indicator of AmpC activity, but cefoxitin resistance may also be mediated by alterations to outer membrane permeability (Tan *et al*., 2009).

Data in the current study exhibited high resistance to third-generation cephalosporins. These findings were consistent with the study of Al-Hassnawy (2018) in Babylon who concluded that there are a high resistance of bacterial isolates to most antibiotic especially to β –lactam antibiotics.

As shown in table (3-4) resistance to cefotaxime was 94.3% which is similar to the study in Babylon conducted by Al-Hasnawy (2018) where all isolates of *E. coli* fully (100%) resistant to cefotaxime, and also the current study resemble that of Aljanaby and Alfaham. (2017) where the result was 92%.

The resistance ratio to ceftazidime was 93.3%. Similar conclusion was reached by Aljanaby and Alfaham (2017) who obtained the ratio of resistance (96%), while these results went beyond previous report conducted by Gashe (2018) in Ethiopia which showed that the result of resistance to this antibiotic was 65%. Furthermore, the study conducted by Azekhueme *et al*. (2015) who found that resistance to cefotaxime and ceftazidime was 41.7% for both.

In the current study the resistance to ceftriaxone was 87.6%. This result is in agreement with local studies implemented by Aljanaby and Alfaham (2017) and Al-Hassnawy *et al*. (2018) where the ratio was 90% and 87.5% respectively. Moreover, the result of resistance to third generation group was closely related to the result of study in Nepal conducted by Nepal *et al*. (2017) which was 84.8% to ceftriaxone, while incompatible with a study conducted by Polse *et al*. (2016) that was 52% in Zakho.

Resistance to antibiotic cefpodoxime was 93.3% which was on the same line of study reported by Singh *et al*. (2016) in India and in Uganda by Andrew *et al*. (2017) where the results were 88.6% and 96.4%, but incompatible with study of Azekhueme *et al*. (2015) in which the ratio of resistance was 39.6%.

The results in the present study exhibited that 92.4% of resistance to fourth generation of cephalosporins (cefepime) that somewhat convergence with result of Mawlood *et al*. (2018) that was 79.2% in Erbil city while these results were contrary to the findings of Singh *et al*. (2016) which result of resistance to this antibiotic was 36.9%.

In this study, resistance to cephalosporins coincides with a study in Nepal conducted by Wagle *et al*. (2018) who concluded that *E. coli* were the most common pathogen isolated and were highly resistant to common antibiotics for oral use such as cephalosporins. In addition to Moghnieh *et al*. (2018) who deduced that *E. coli* and *Klebsiella* spp have high resistance to third-generation cephalosporins is common in all countries, with prevalence reaching more than 50% in Syria and Egypt.

The present study reported high resistance rates to most antibiotics specially Beta-lactam that may be due to uncontrolled consumption, consequence of easy access to inefficient and cheap antibiotics, also caused mainly by mutations in the common group of class A β-lactamases, which consisting of TEM, SHV and CTX-M β-lactamases that has extended hydrolytic spectrum activity on cephalosprins. Furthermore, Walther-Rasmussen and Hoiby (2004) concluded in their studies that high rate resistance to ceftazidime and cefotaxime may be related with production of enzymes named cefotaximases (CTX-M), where these CTX-Ms showed a much higher degree of activity towards cefotaxime than to ceftazidime .

Also the high resistance against Beta-lactam antibiotic in the present study may be due to the high prevalence of TEM and CTX-M enzymes in *E. coli* isolated in this study that corresponded to a study achieved by Haghighatpana *et al*. (2016) on the other hand, Ruppe *et al*. (2015) deduced in their study that the dramatic increase in the rates of resistance to third-generation cephalosporins in Enterobacteriaceae mainly results from the spread of plasmid-borne ESBLs, especially those belonging to the CTX-M family. So, European

Center for Disease prevention and Control (2014) considered the inclusion; ceftazidime and cefotaxime where these antibiotic can help detect theses enzyme CTX-M whereas cefpodoxime alone can be used to detect other such as TEM type

In the present study, we used two antibiotics from flouroquinolones family belong to the second generation. The result of their resistance was 46.7% to norfloxacin and 34.3% to ciprofloxacin. Two studies in India performed by Singh *et al*. (2016) and Shanthi and Madhumathy (2018) they found that the resistance to norfloxacin were 81.9% and 72.71% respectively.

The result of ciprofloxacin was in agreement with studies for Hamdoon (2011) who found the resistant to this antibiotic was 33.4% and corresponded with a study for Nanakali and Ahmad (2015) that was 32.8% .While this result was in compatible with local study reported by Hussein (2018) in Thi-Qar province where the result was 70% and inverse with other study in different countries as Yasir *et al*. (2018) in Saudi Arabia who explained that the percentage of *E. coli* which resist to ciprofloxacin was 68.2%.

Shanthi and Madhumathy (2018) concluded in their study that high drug concentration in the urine can be obtained when fluoroquinolones are used specifically to treat urinary tract infection so these antibiotics are particularly used to treatment UTI. The effectiveness of these antibiotics starts by inactivate the DNA gyrase, the accumulation of double-stranded bands in the bacterial cell genome, which im pedes the basic movements of DNA and RNA polymerase along the DNA template. In addition to Yadav *et al*. (2015) who observed that ESBL producers are often resistant to other antibiotics in addition to β-lactam, such as fluoroquinolones.

Resistance to gentamicin was 53.3% a similar pattern of findings were reached by Anago *et al*. (2015) and Aljanaby and Alfaham (2017) who found the results were 54.8% and 46% respectively, whereas in a study conducted in Northest Ethiopia by Kibret and Abera (2011) who considered gentamicin was

appropriate for empirical treatment of *E. coli* isolated from different clinical in the study area. The present study went beyond a previous report of Azekhueme *et al*. (2015) showing that resistance to gentamicin was 22.9%. The high resistance to this antibiotic in our current study and its difference from the recent local studies and previous studies may be attributed to the different region and environmental conditions as well as the lack of awareness in most patients and taking medicines without consulting the doctor.

The majority of *E. coli* (90.5%) were sensitive to amikacin. These findings were in accordance with findings reported by Singh *et al*. (2016) where the results was 88.4% while other studies implemented by Al-Hasnawy *et al*. (2018) and Cebeci *et al*. (2019) the result was 73% for both. Most studies have investigated the effectiveness of amikacin against *E. coli* isolates, which may be due to the low use of this type of antibiotic compared to other types due to its Toxicity.

The present study reported that resistance of *E. coli* to trimethoprim/sulfamethoxazole was 68.6%, overall these findings were in accordance with findings reported by Yasir *et al*. (2018) in Saudi Arabia where the result of resistance was 66.4% but it was less than a study for Anago *et al.* (2015) that was 86.9% and more than a local study for Alfatlawi *et al*. (2019) where the rate of resistance was 7%.

In the present study 35.2% of *E. coli* were resistant to nitrofurantion, this result was more than a study for Al-Jelehawy (2016) whose findings was 12.24%, which was also higher than a study by Yasir *et al*. (2018) in Saudi Arabia where the results were 6%, whilst this result was less than a study conducted by Aljanaby and Alfaham (2017) whose result was 80%.

Tulara (2018) explained in his study on ESBLs producing *E. coli* in India that nitrofurantoin was actively against ESBLs *E. coli* and showed that this antibiotic is important for the treatment of many types of infections, including complicated infections in urinary tract and other sites in the body. Also Munoz-Davila (2014) highlighted on this antibiotic and observed that his safety through

using have made it the antimicrobial of choice in the prophylaxis and treatment of lower UTI in adults for many years. Moreover Klesiewicz *et al*. (2018) mentioned that nitrofurantion derivatives displayed higher antimicrobial activity than other antimicrobial agents regardless of bacteria species or resistance mechanism.

Moreover, Haghighatpanah *et al*. (2016) was observed that *E. coli* is an important cause of noscomial infections worldwide, where their antimicrobial resistance leads to treatment failure of hospital infections which caused by ESBLs *E. coli*, so ESBLs is considered one of the major causes of antibiotic resistance in these bacteria.

The most effective β-lactam antibiotics was imipenem where 100% of *E. coli* were sensitive to it, this agree with study for Alfatlawi *et al.* (2019) in Najaf province and Kumar *et al*. (2014) in India where all *E. coli* fully (100%) sensitive to imipenem, another study for Anago *et al*. (2015) in Cotonou city revealed that 96.4% of *E. coli* where sensitive to imipenem.

A Similar conclusion was reached by Shaikh *et al*. (2015) who noted that carbapenems are widely regarded as the drugs of choice for the treatment of severe infections caused by ESBL-producing Enterobacteriaceae. Moreover, in a recent study for Cebeci *et al*. (2019) who considered the antibiotic imipenem and meropenem must be preferred drugs for infection of *E. coli* which isolated from clinical samples. Also, in a study for Reimer (2019) who observed that treatment choices for these ESBLs are limited, and the most effective antibiotics against ESBL-producing Enterobacteriaceae infections are carbapenems.

In the current study, all isolates were multidrug resistance (MDR) because that were resistant to at least three classes of antibiotic, this correlated with the study of Magiorakos *et al.* (2012) who concluded that *E. coli* considered as an MDR if it was resistant to at least three classes of antibiotic. In a large number of previous studies, MDR strains have a high percentage of *E. coli* isolated from clinical specimens such as a study for Al-Hilli (2010) who revealed that all *E.*

coli isolate obtained from Merjan Teaching Hospital in Hilla were considered as MDR. So, the appearance of MDR isolates of *E. coli* has caused many problems in the treatment of *E. coli* (Ahmadikiya *et al*., 2017).

The careless antibiotics usage, without antibiotic sensitivity testing, is the most important factor promoting the emergence of MDR, which causes selection and dissemination of antibiotic resistant pathogens in clinical medicine. Furthermore, easily available of drugs in our province which taken from pharmacy without doctor prescription and instruction, lack of health education in some individuals and the availability of antibiotics is relatively cheap, and high prevalence of ESBLs might be lead to increase of MDR strains, such as what observed by Thiong'o (2014) which concluded that multi-drug resistance among Gram negative bacteria is on the increase due to acquisition and expression of ESBLs.

3.4. Phenotypic Detection of ESβLs

The ESβLs enzymes was screened in all identified *E. coli* (n=105) using the Screening Test according to CLSI (2017). The results revealed that 87 82.9% were positive for screening ESβLs test as showed in table (3-5). These findings were consistent with Kamel *et al*. (2013) who recorded 83.3% of *E. coli* were positive for this test. Also, these results were in accordance with that obtained by Al-Hasnawy *et al*. (2018) where the results was 100% while these results contrary to the findings of Mohammed *et al*. (2016) in which the result was 33.5%. The high incidence of ESBL-producing isolates obtained in this study was probably due to the consumption of large amount of third-generation cephalosporins, trend of self-medication, and the extensive prophylactic misuse of antimicrobials by Iraqi patients and physicians.

All isolates that were positive for the screen test were subjected to a confirmatory test (DDST) where was done according to Bedenić *et al.* (2010) which was explained in section (2.2.8.2), the results appeared that 19 (21.8%)

of isolates were ESβLs producers as in figure (3-5). The enhancement or extending of the inhibition zone around the test antibiotic disk increased towards the (AMC) disc called ghost-zone, this refer to the inhibition of ESβLs. This increase of the inhibition zone occurs because the clavulanic acid present in the amoxicillin-clavulanic acid disk inactivates the ESβLs produced by the tested microorganisms (Chaudhary , 2004). These results were very close to the findings of Mohammed *et al*. (2016) in North Eastern Nigeria that was 23.8%. Also Ashrafian *et al.* (2013) found that 26.5% of *E. coli* were ESBL positive in their study conducted in Iran, while the results of a local study implemented by Ali *et al*. (2017) in Diyala where Phenotypic confirmatory tests showed that 10%, While Zandi *et al*. (2017) showed that 30% of *E. coli* isolated from patients hospitalized in Yazd using combination disk test were ESBLs producers. Therefore the differences among the results of studies may be attributed to the different tests used and type strains of *E. coli*.

Table(3-5): The frequence of ESBLs producing *E. coli* isolates by phenotypic tests.

Screen test No.(105)	**No.(%)**	**Confirmatory test No.(87)**	**No.(%)**
Positive	87 (82.9%)	Positive	19 (21.8%)
Negative	18 (17.1%)	Negative	68 (78.2%)

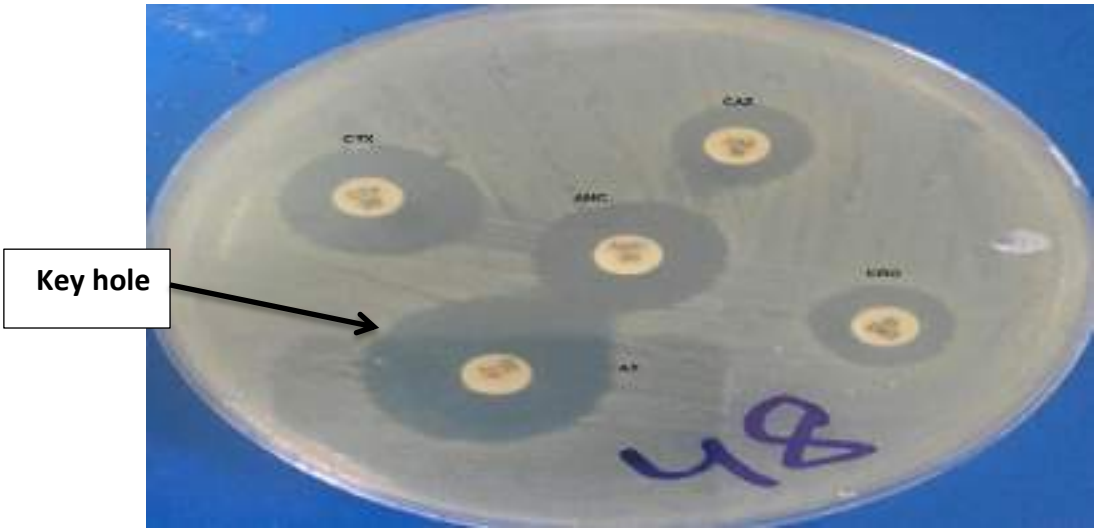


Figure (3-5): The Double Disk of Synergy Test of *E. coli* (n= 48) show the effect of clavulanic acid as inhibitor for the producing of ESBLs and enhancing the zone of inhibition between AT(Aztreonam), CTX (Cefotaxime), CRO (Ceftriaxone), CAZ (Ceftazidime) and AMC (Amoxicillin + Clavulanic acid).

In a study implemented by Kao *et al*. (2010) who found ESBL-AmpC combinations were identified in clinical isolates of Enterobacteriaceae and they concluded such as these isolates might be hinder the accurate detection of ESBLs phenotypes by screening and confirmatory tests according to the recommendations of the Clinical and Laboratory Standards Institute.

Infections caused by ESBLs producing organisms have led to bad results; reduced rates of clinical and microbial response, longer hospital stays and greater hospital expenses (Tschudiu-sutter *et al*., 2010). Also, Karanika *et al*. (2016) noted that the prevalence rate of ESBL producer in healthy people is significant worldwide, this should be taken into consideration in infection control and antibiotic administrative decisions. In addition to a study of Lota and Latorre (2014) were concluded to the prevalence of ESBL *E. coli*-producers ranged from 4%-20.9% in the Philippines from (1999-2013). Moreover, Zandi *et al*. (2017) concluded in their study that frequency of ESBLs and its variants may vary in different geographical settings, as reports indicate their spread in some certain communities and no significant relationship was found between frequency of

ESBLs and age or gender. Additionally to Livermore (2012) who observed that the prevalence of ESBLs genes differs among patient groups .

3.5. Detection of ESBLs genes by PCR technique

Molecular Detection of ESBLs genes (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{OXA}$ genes) among the clinical isolates of *E. coli* on chromosome and plasmid was done by a monoplex PCR as shown in table (3-6).

Table (3-6): Prevalence of *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX}$-M and *bla*$_{OXA}$ genes in *E. coli* (no=105).

Gene	Total isolate(n=105)	Percentage(%)
bla$_{CTX-M}$	Chromosome (105)	100
	Plasmid (105)	100
bla$_{TEM}$	Chromosome (105)	100
	Plasmid (105)	100
bla$_{OXA}$	Chromosome (105)	100
	Plasmid (104)	99.05
bla$_{SHV}$	Chromosome (48)	45.7
	Plasmid (43)	41

3.5.1. Molecular Detection of ESBL Chromosome Encoded Genes

3.5.1.1. *bla*$_{CTX-M}$ gene

Data of the current study showed that *bla*$_{CTX-M}$ gene with 550bp product size was 100% carried on chromosome of subjected *E. coli* as in figure (3-6). This result is very close to the study in different places for Kiratisin *et al*. (2007) in Thailand which reported the highest prevalence of *bla*$_{CTX-M}$ gene among several *bla* genes in all isolates which was 99.6% and Yasir *et al*. (2018) in Saudi Ara-

bia where the result was 95.3%. Also, like to some degree with local studies such as Al-Jelehawy (2016) and Alfatlawi *et al*. (2019) which reported that majority of CTX-M ESBL producers were *E. coli*, their results were 91.83% and 86.3% respectively and another local previous study such as Hawkey (2008) reported that all ESBL producing *E. coli* isolates in his study harbored $bla_{\text{CTX-M}}$ gene on chromosome. Yongjung *et al*. (2009) noticed that CTX-M was predominantly the most common type of ESBL in *E. coli* compared with SHV and TEM enzymes. Furthermore, Feizabadi *et al*. (2010) mentioned in their study that CTX-M was considered as a common type of ESBLs that detected at Asia, Europe, North and South America among multidrug-resistant in *E. coli*. Also these result were farther than the study of Ali (2018) who found 73.3% of *E. coli* carried this gene while the results of the present study were incompatible with other studies in different places such as of AlBarraq *et al*. (2017) whose result was 10%.

The main reason for the prevalence of CTX-M β-lactamases in Mysan Province may be due to the wide use of certain third generation cephalosporins which led to high resistant in our study. A similar conclusion was reached in our study, there are high resistance against these antibiotic by identified *E. coli*. Additionally Pitout (2010) pointed in his study that *E. coli* producing CTX-M enzyme (especially CTX-M-15) have emerged worldwide as important causes of community-onest UTIs and BSIs.

Figure (3-6): Agarose gel electrophoresis of PCR chromosome encoded *bla*$_{CTX-M}$ gene, amplicon (550bp), where L: ladder (100bp) , Lane(2-19) positive results, C: Control (only primer+ Master Mix), the gel stained by ethidium bromide (0.5 µg/ml) and (65) volts for one hour.

3.5.1.2. *bla*$_{TEM}$ gene

The *bla*$_{TEM}$ gene which was their product size 374bp was 100% carried on chromosome of isolates as in figure (3-7). This result was strongly similar with the local study of Al-Jelehawy (2016) who clarified that 100% of *E. coli* isolated from different clinical cases in Najaf and Diwania cities which were carried this gene. The present study also in compatible with another local study of Alfatlawi *et al*. (2019) which recorded that 82.0% of *E. coli* carried this gene.

These results were somewhat like other studies in different places implemented by Yasir *et al*. (2018) where the results were 83.9% and pongpech *et al.* (2008) who found that *bla*$_{TEM}$ gene was present in 78% of the confirmed *E. coli* which were ESBLs producers in Thailand. Besides, there is a previous study conducted by Bradford (2001) who reported that up to 90% of *E. coli* was ampicillin resistance that due to the production of TEM enzyme, because this enzyme has the ability to hydrolyze penicillins and early cephalosporins.

Conversely, our findings don't agree with studies of Mawlood *et al*. (2018) who found that out of 21 *E. coli* isolate 6 (28.5%) were able to yield amplification products with TEM-PCR specific primers in study conducted in Erbil city, AL-Asady (2009) reported that out of 5 *E. coli* isolates only 1 (20%) was pos-

sess *bla*$_{TEM}$ genes , and AL-Muhannak (2010) in Najaf showed that 5 (25%) of *E. coli* carried *bla*$_{TEM}$ gene.

In a previous study originated from a variety of geographically locations, conducted by Tomanicek *et al*. (2010) they were concluded that the use of local antibiotics and practices may play an active role in encouraging the selection of point mutations in the genes *bla*$_{TEM}$.

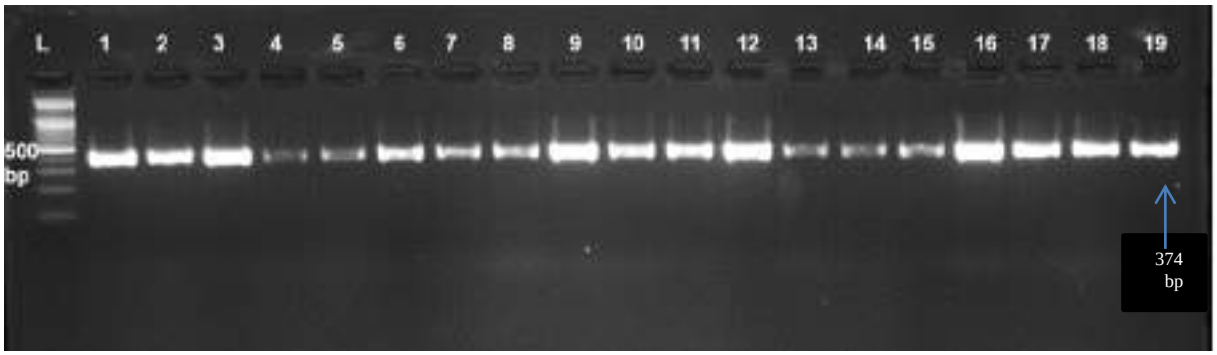


Figure (3-7): Agarose gel electrophoresis of PCR chromosome encoded *bla*$_{TEM}$ gene, amplicon (374bp), where L: ladder (100bp) , Lane(1-19) positive results ,the gel stained by ethidium bromide (0.5 µg/ml) and 65 volts for one hour.

3.5.1.3. *bla*$_{OXA}$ gene

Our study showed that high percentage of *bla*$_{OXA}$ gene which was their product size 619bp on chromosome was detected in 100% in *E. coli* as in figure (3-8). A similar conclusion was reached by Alsamarai *et al*. (2018) where the *bla*$_{OXA}$ gene was detected in all tested *E. coli*, and compatible with local studies in Diwania achieved by Al-Jelehawy (2016) in which the result was 91.3%, whereas the present study went beyond the study performed by Alfatlawi *et al*. (2019) in Al-Najaf where the results was 77.2%.

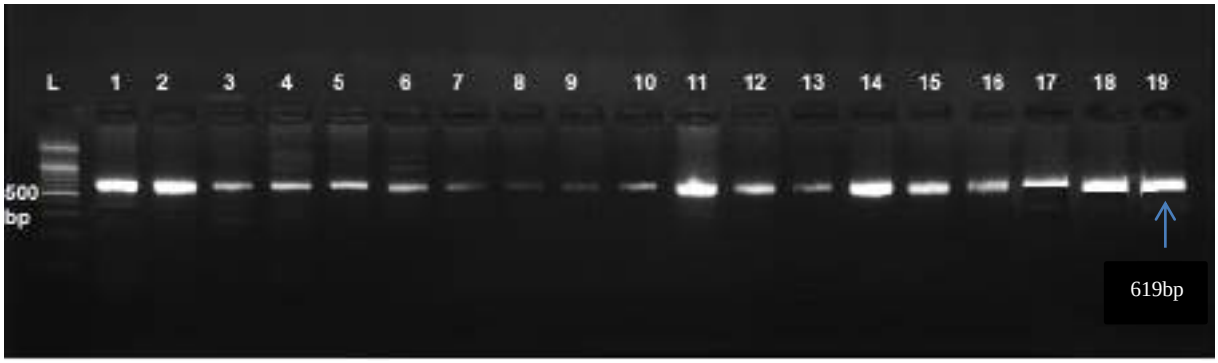


Figure (3-8): Agarose gel electrophoresis of PCR chromosome encoded *bla*$_{OXA}$ gene, amplicon (619bp), where L: ladder (100bp) , Lane(1-19) positive results ,the gel stained by ethidium bromide (0.5 µg/ml) and 65 volts for one hour.

The results of the current study were more than previous local study in Al-Najaf performed by AL-Muhannak (2010) who revealed that the *bla*$_{OXA}$ gene was detected in 5 (25%) of *E. coli* isolates, However, AL-Hilli (2010) found that all *E. coli* isolates from Merjan teaching hospital in Hilla city were negative in OXA-PCR.

Leylabadlo *et al.* (2015) found in their study that the diversity of carbapenemase enzymes depend on the country may be influenced by historical and cultural relationships and may also be due to wars and moving troops. Cross border transfer of patients, medical tourism, travelers and refugees might also play a significant role. Therefore, the guidelines and appropriate infection control measures are important to prevent such infections among patients.

3.5.1.4. *bla*$_{SHV}$ gene

The present study showed that prevalence ratio of *bla*$_{SHV}$ gene which their product 615 bp was 45.7% as in figure (3-9) which conform with a study in Iran for patients with urinary tract infection conducted by Seyedjavadi *et al.* (2016) in which the result was 45% and similar to a comparative study conducted by Al-Mayahie (2013) in Wasit for ESBLs production *E. coli* isolated from pregnant and non-pregnant women where the result was 44.7% in non-pregnancy women versus to 4.3% in pregnant, and very close to study of Rezai

et al. (2015) that was 44% of *E. coli* among uropathogens of pediatrics in north of Iran. Also another local study aimed to evaluate the occurrence of bla_{SHV} gene implemented by Alfatlawi *et al*. (2019) in Najaf where recorded that the prevalence of bla_{SHV} gene that was 36.6%.

The opposite of that, there are many local and international studies demonstrated a notable decline in the frequency of the bla_{SHV} gene such as a study for Al-Hilali (2015) and Dagi *et al*. (2015) where the result was 0%. Also in India a study of Akila *et al*. (2017) found that 5.56% of isolates carried this gene. while another study in Saudi Arabia for AlBarraq *et al*. (2017) reported that the bla_{SHV} gene was detected in 5 (7.1%) of isolates. Local studies for many researcher like Al-Hasnawy *et al*. (2018) who obtained the results that was 6.6% from different clinical samples carried this gene, furthermore, Lhwak and Abbas (2018) found in their studies that the frequency of bla_{SHV} gene was 15.8% of the *E. coli* isolated from urinary tract infection of pregnancy women in Thi-Qar Province.

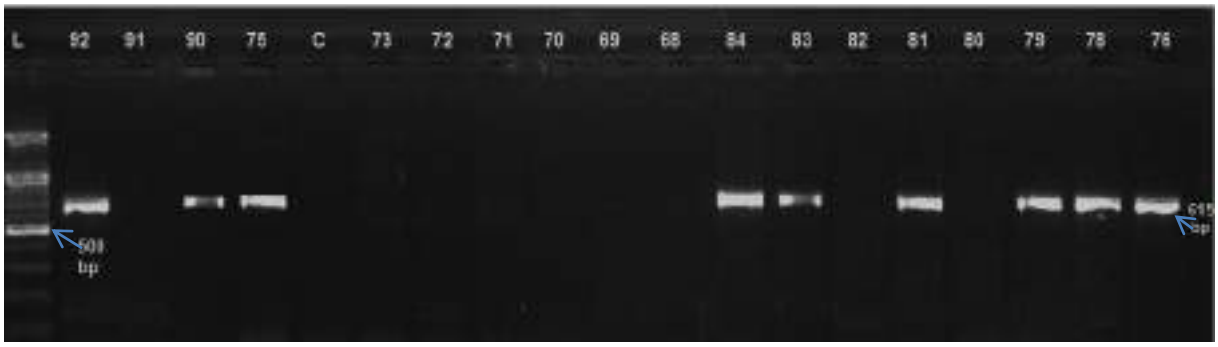


Figure (3-9): Agarose gel electrophoresis of PCR Chromosome encoded bla_{SHV} gene amplicon (615bp), where L: ladder (100bp), Lane (92, 90, 75, 84, 83, 81, 79, 78, 76) Positive results, Lane(91,73,72,71,70, 69, 68, 82, 80) Negative results, C:control (only primer+ Master Mix), the gel stained by ethidium bromide (0.5 µg/ml) and 65 volts for one hour.

3.5.2. Molecular detection of Plasmid encoded ESBL genes

The present study also focused on detecting isolates with possible plasmid-encoded ESBLs from 105 *E. coli* clinical isolates.

3.6.2.1. $bla_{CTX\text{-}M}$ gene

In the current study, the result revealed that (100%) of *E. coli* carried $bla_{CTX\text{-}M}$ gene on plasmid as shown in figure (3-10). These findings were strongly similar to a study for Kamel *et al*. (2013) in Egypt and Lohani *et al*. (2019) in Nepal where the results were 100% for both, and also very close to the results achieved by Moghaddam *et al*. (2012) in Mashhad who concluded that high prevalence of *CTX-M* mediated plasmid where the result was 94.6% and like to some degree with studies of Haghighatpanah *et al*. (2016) who found that 86.7% of *E. coli* carried $bla_{CTX\text{-}M}$ gene on plasmid. By surveying the literature, we recorded the increased prevalence of $bla_{CTX\text{-}M}$ among ESBLs producers in Norway was 90% conducted by Tofteland *et al*. (2007) and in a Swedish 92% by implemented by Fang *et al*. (2008).

Furthermore, Bush and Jacoby (2010) pointed that at the beginning of the 21st century, a new family of plasmid-mediated ESBLs called CTX-M, which preferentially hydrolyze cefotaxime, became predominant in European countries, and started to spread in southeast Asia including the Philippines. Also it has been reported about a study conducted in hospitals in the Philippines which done by Lucena *et al*. (2012) discovered the predominance of CTX-M type ESBL among clinical isolates.

Hopkins *et al*. (2006) concluded that wide spread appearance of this type of ESBLs genes could be attributed to either diversity of molecular platforms associated with it as not only plasmids but, also location of $bla_{CTX\text{-}M}$ gene near or within transposons. In addition to what was mentioned by Govinden *et al*. (2007) that the selective pressure exerted mainly by ceftriaxone and/or cefotaxime could be a reason behind wide spread of this type of genes. On the other

hand, the elevated rate of CTX-M β-lactamases in *E. coli* isolates suggested that the horizontal transfer of bla_{CTX-M} genes, mediated by plasmid and/ or mobile genetic element, contributes to ease with which these enzymes are spreading in *E. coli* isolates and the dissemination of CTX-M enzymes.

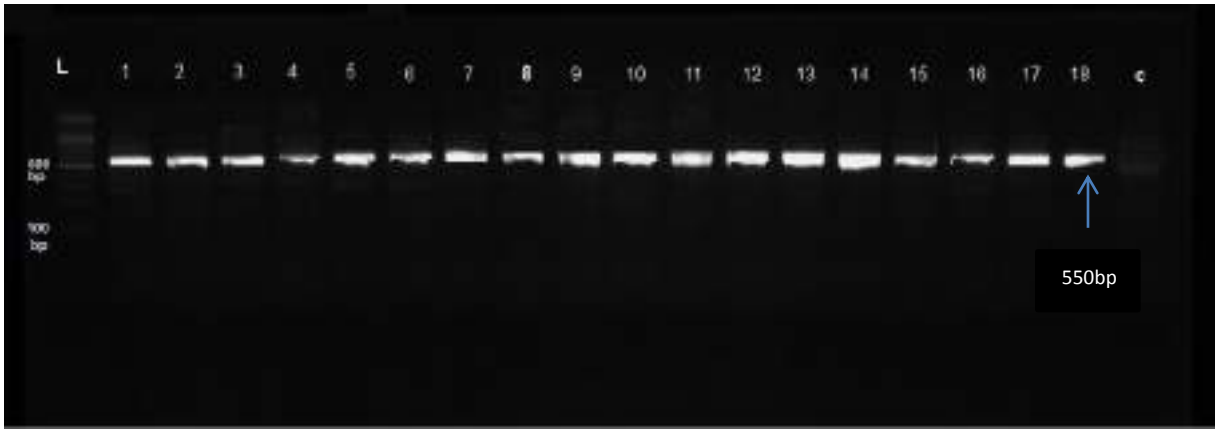


Figure (3-10): Agarose gel electrophoresis of PCR plasmid encoded bla_{CTX-M} gene, amplicon (550bp), where L: ladder (100bp), Lane(1-18) positive results, C: Control(only primer+Master Mix), the gel stained by ethidium bromide (0.5 µg/ml) and 65 volts for one hour.

3.5.2.2. bla_{TEM} gene

The present study exhibited 100% of *E.coli* isolates had bla_{TEM} gene on plasmid as shown in figure (3-11), this rate went beyond with a study conducted by Azargun *et al*. (2018) where the findings were 75.6% of *E. coli* isolating from patients with UTIs which is also more than another study in Sweden achieved by Fang *et al*. (2008) where the frequency of bla_{TEM} gene was 63% among *E. coli* isolates, while these results were contrary to the findings of Kamel *et al*. (2013) in Egypt, Haghighatpanah *et al*. (2016) in Iran and Lohani *et al*. (2019) in Nepal where the ratio reached to 50%, 32.5% and 34.6% respectively. The ratio of this gene in a study implemented by Moghaddam *et al*. (2012) for patients with UTIs was 56.8%.

TEM-type ESBLs are the first plasmid-mediated β-lactamase that is often found in general of Enterobacteriaceae (Mulvey *et al*., 2004). However, Lartigue

et al. (2007) noted that the transfer of bla_{TEM} gene on mobile plasmid led to spread rapidly to members of the same species or organisms of different genera.

Additionally, Bonnet, (2004) explained that TEM is a broad spectrum β-lactamase that is always combined with CTX-M in the same plasmid and the combinations of these genes are frequently seen in the ESBL producing strains, this conclusion may be one cause of prevalence TEM enzyme in our survey.

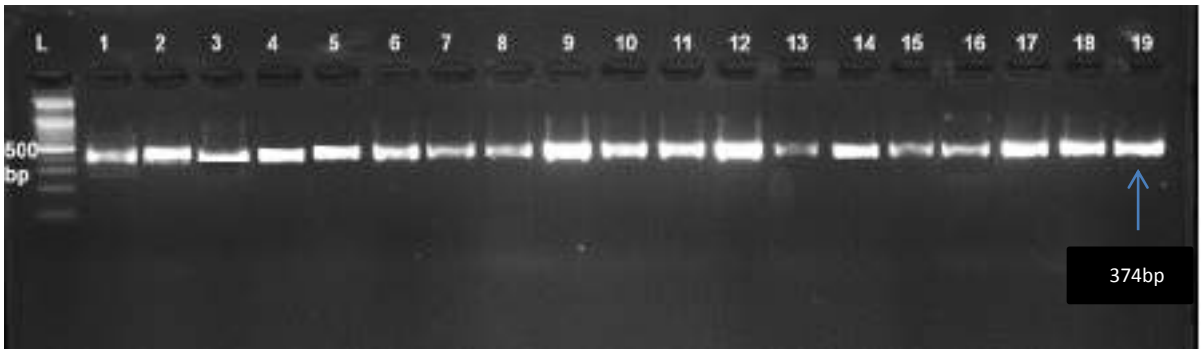


Figure (3-11): Agarose gel electrophoresis of PCR plasmid encoded bla_{TEM} gene amplicon (374bp), where L: ladder (100bp) , Lane(1-19) positive results, the gel stained by ethidium bromide (0.5 μg/ml) and 65 volts for one hour.

3.5.2.3. bla_{OXA} gene

In this study, (99.05%) of *E. coli* were reported to carry this gene on plasmid as shown in figure (3-12). This finding was broadly in line with that obtained by Bertini *et al*. (2010) who noticed that most of the groups of OXA-type -lactamases have been identified on plasmids. While the result of present study was more than the results of studies performed by Carrer *et al*. (2010) in Turkey, Rimrang *et al*. (2012) in Pakistan and Nasser (2017) in Baghdad where their results were 5.5%, 19.2% and 10% respectively, also our results went beyond a previous report of Abdulla *et al*. (2016) in Babylon who showed that 25% of *E. coli* encoded for this gene isolating from patients with UTIs.

Haciseyitoglu *et al*. (2017) observed that Iraq and its neighboring countries have a geographical significance to recognition of carbapenemase producing microorganisms and a high level of MDR Enterobacteriaceae were recorded.

On the other hand, Baran and Aksu (2016) mentioned that there are recently epidemiological information has emphasized that the commonest rate of OXA-48 variations are quickly expanding and that OXA-48 is at present turning into the widespread carbapenemase class in Enterobacteriaceae in numerous countries in the world. Moreover, Carrer *et al*. (2010) in their study concluded that besides class B (VIM and IMP) and class A (KPC) carbapenemases, the class D carbapenemase OXA-48 type might contribute significantly to carbapenems resistance in Enterobacteriaceae. Furthermore, Pulss *et al*. (2017) clarified in their study that plasmid-mediated resistance to carbapenems and colistin in Enterobacteriaceae represents an emerging public health threat. Therefore, caution should be taken with *E. coli* in the study area for the presence of this gene at that high rate, and quick recognition and of carbapenemes resistance in these bacteria for proper choice of antibiotics and avoidance multidrug-resistant pathogens.

Figure (3-12): Agarose gel electrophoresis of PCR plasmid encoded bla_{OXA} gene amplicon (619bp), where L: ladder (100bp) , Lane(1-18) positive results, C:control (only primer+ Master Mix), the gel stained by ethidium bromide (0.5 µg/ml) and 65 volts for one hour.

3.5.2.4. bla_{SHV} gene

The data of this study showed that the rate of bla_{SHV} gene encoded on plasmid was 41% as shown in Figure (3-13). This somewhat in concordance with studies for researchers from other countries Kamel *et al*. (2013), Azergun *et al* . (2018) and Lohani *et al*. (2019) where the percentage were 37.5%, 33.3% and

30.8% consecutively. These findings were different from a study for Fang *et al*. (2008) that was 6% and disagreed with a study performed by Moghaddam *et al*. (2012) where the result was 13.6%.

Schmitt *et al*. (2007) pointed to that different group of β- lactamase gene which are found either on chromosome DNA or plasmid, where the presence of the gene on plasmid further facilitated its transfer to different species of bacteria. This gene is responsible for plasmid mediated ampicillin resistance in bacteria which harbors it (Bradford, 2001). Additionally, Rimrang *et al*. (2012) noted that ESBL plasmid encoded genes were easily transferred through horizontal gene transfer mechanism therefore, the best results were obtained from PCR and DNA sequencing, where if somehow multiplex PCR of ESBL will applied in local diagnostic labs this helps in early detection and phenotypic antibiotic therapy against this infection.

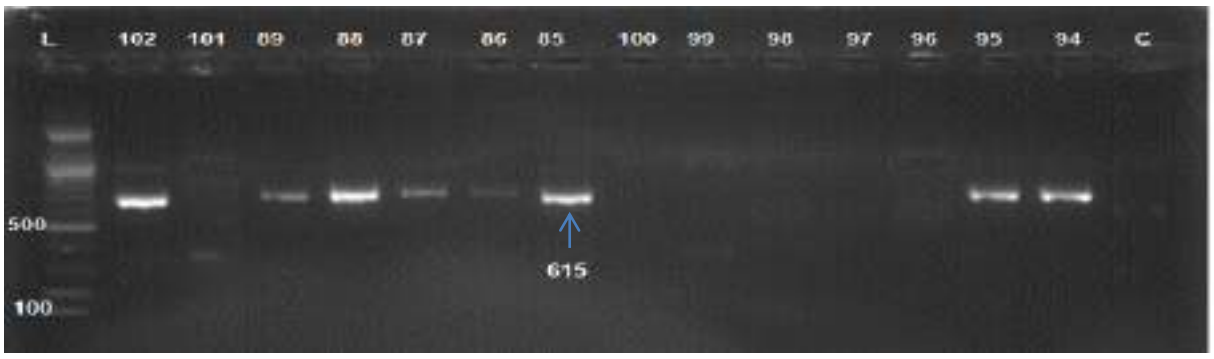


Figure (3-13): Agarose gel electrophoresis of PCR plasmid encoded bla_{SHV} gene amplicon (615bp), where L: ladder (100bp), Lane (102, 89, 88, 87, 86, 85, 95, 94) positive results, Lane (101, 100, 99, 98, 97, 96) Negative results, C:control (only primer+ Master Mix), the gel stained by ethidiume bromide (0.5 µg/ml) and 65 volts for one hour.

Conclusions and Recommendations

▪ Conclusions

1- There was a relationship between phenotypic screening test and genotypic detection of genes responsible for antibacterial resistance in *E. coli*.

2- *E. coli* became highly resistant to antibiotics especially to the third generation cephalosporins.

3- All identified *E. coli* have multidrug resistant might be due to the mobile genetic elements, these organisms represent a serious therapeutics challenge in patients especially in health care units.

4- Imipenem is the important antibiotic to treat these bacteria which are isolated from different clinical cases, followed by Amikacin**.**

5- High prevalence of ESBLs which represented of $bla_{CTX\text{-}M}$, bla_{OXA}, bla_{TEM} genes irrespective of the encoding origin.

6- The reduced rate of results concerning with confirmatory ESBLs test might be due to AmpC overexpression or might be mediated by alteration to membrane permeability.

▪ Recommendations

1- Using phenotypic and genotypic methods to identify the presence of an ESBL should be carried out routinely in all hospitals laboratories in our province.

2- Avoiding the use of third generation cephalosporins against pathogenic bacteria that appear resistant to these antibiotics (i.e. ESBL-producers) and it is important to determine the most effective antibiotic for treating infections caused by ESBL-producing isolates in patients.

3- Study prevalence of ESBLs in other types of Enterobacteraceae .

4- Advise to conducted a sequencing studies to identification and determination the different variants of ESBLs gene.

References

References

Abbasi, P., Kargar, M., Doosti, A., Mardaneh, J., Dehyadegari, M. A., and Ghorbani-Dalini, S. (2014). Multiplex real-time PCR assay for the detection of LT, STIa and STIb genes in enterotoxigenic *Escherichia coli*. *Electronic physician*, 2(1): 1-5.

Abdi, S., Ranjbar, R., Vala, M. H., Jonaidi, N., Bejestany, O. B., and Bejestany, F. B. (2014). Frequency of bla TEM, bla SHV, bla CTX-M, and qnrA among *Escherichia coli* isolated from urinary tract infection. *Archives of Clinical Infectious Diseases*, 9(1): 1-5.

Abdulla, A. A., Al-Dahmoshi, H. O. M., Abed, T. A., and Muttaleb, W. H. (2016). Characterization of multidrug resistant carbapenemases-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from urinary tract infection. *Journal of Chemical and Pharmaceutical Sciences*, 9(3): 1116-1120.

Abraham, S. (2011). *Molecular characterization of commensal and* pathogenic *Escherichia coli* that colonise human urinary tract and the porcine gastrointestinal tract and the development of whole cell biosensors to evaluate bacteriocin mediated bacterial interactions. M.SC. Thesis, School of Biological Sciences. University of Wollongong.

Abulreesh, H. H. (2014). Efficacy of two commercial systems for identification of clinical and environmental *Escherichia coli. International Journal of Biology*, 6(2): 31-41.

Adeyankinnu, F. A., Motayo, B. O., Akinduti, A., Akinbo, J., Ogiogwa, J. I., Aboderin, B. W., and Agunlejika, R. A. (2014). A multicenter study of beta-lactamase resistant *Escherichia coli* and *Klebsiella pneumoniae* reveals high level chromosome mediated extended spectrum β lactamase resistance in Ogun State, Nigeria. *Interdisciplinary perspectives on infectious diseases*, 2014: 7

Ahmadikiya, B. F., Mosadegh, A., Moradi, M., and Hossieni-Nave, H. (2017). Antimicrobial Resistance Patterns and Frequency of Extended-Spectrum

Beta-Lactamase Genes among *Acinetobacter Baumannii*. *Journal of Babol University of Medical Sciences*, 19(7): 28-34.

Akila, K., Sivasankari, S., Senthamarai, S., Anitha, C., Kumudavathi, M., and Muthulakshmi, K. (2017). Prevalence of TEM and SHV Genes among Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* Isolates from Infected Diabetic Foot Ulcers in a Tertiary Care Hospital. *International Journal of Current Microbiology and Applied Sciences*, 6(1): 645-648.

Akter, T., Hossain, M. J., Khan, M. S., Sultana, H., Fatema, K., Al Sanjee, S., and Datta, S. (2016). Isolation, identification and antimicrobial susceptibility pattern analysis of *Escherichia coli* isolated from clinical samples of Bangladesh. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 6(54): 13.

AL-Asady, F. M. H. (2009). Bacteriological study on ESBL-Producing Enterobacteriaceae that cause bacteremia in children in Hilla City. M.SC. Thesis, College of Medicine, University of BabylonL/Iraq.

AlBarraq, A. A., El-Badawy, M. F., Al-Qarni, M. A., Al-Saedi, S. S., Al-Sufyani, W. M., Osaimi, F. E., and Shohayeb, M. M . (2017). Prevalence of blaTEM, blaSHV and blaCTX-M among β-lactamase producing *Escherichia coli* isolated from tertiary care hospitals in Taif, Saudi Arabia**.** *Journal of Pharmacy and Biological Sciences*, 12(1):11-16

Al-Dulaimi, T. H., Aziz, H.W., Al-Marzoqi, A.H., Al-Aziz, S. A., and Mohsin, S. A. (2015). Molecular Characterization and Antibiotic Susceptibility of Diarrheagenic *Escherichia coli* from Children. *Medical Journal of Babylon*, 12(2): 541-550.

Ale, A., Crepin, V. F., Collins, J. W., Constantinou, N., Habibzay, M., Babtie, A. C., and Stumpf, M. P. (2017). Model of host-pathogen interaction dynamics links in vivo optical imaging and immune responses. *Infection and immunity*, 85(1): 16.

Alfatlawi, A. S., Alsaidi, M. A., Almaliky, N. K., and Alsherees, H. A. A. (2019). Molecular characterization of extended spectrum â-Lactamases (ESBLs) producing *Escherichia coli* isolated from Cholecystitis. *EurAsian Journal of BioSciences*, 13(1): 113-120.

Al-Hamadani, A. H., Al-Rikabi, A. M., and Al-Fatlawi, A. F. (2013). Detection of TEM and SHV genes in *Escherichia coli* and *Klebseilla species* isolated from cancer patients in Al-Diwaniya Governorate. *AL-Qadisiyah Medical Journal*, 9(16): 22-39.

Al-Hasnawy, H. H., Saleh, R. H., and Hadi, B. H. (2018). Existence of ESBL genes in *Escherichia coli* and *Acinetobacter baumannii* isolated from different clinical specimens. *Journal of Pharmaceutical Sciences and Research*, 10(5): 1112-1117.

Al-Hilali, S. A. M. (2015). Genetic affinities of multiple drug resistant uropathogenic *Escherichia coli* isolated from patients with urinary tract infection in Najaf. Najaf: Doctoral dissertation, Faculty of Medicine Department of Microbiology, University of Kufa/Iraq.

Al-Hilli, Z. B. (2010). Dissemination of β-lactamases in *Escherichia coli and Klebsiella spp.* isolated from Merjan teaching hospital in Hilla city. M.Sc. Thesis, College of Science, Kufa University/Iraq.

Al-Humam, A. N. (2016) .Special biochemical profiles of *Escherichia coli* strains isolated from humans and camels by the VITEK. *African Journal of Microbiology Research*, 10(22): 783-790.

Ali, E. A., Mohsin, I. H., and Jasim, I. M. (2017). Bacteriological Study of *Escherichia coli* Isolated from Different Infections in Diyala. *Diyala Journal For Pure Science*, 13(3-part 2): 249-261.

Ali, F. A. (2018). Distribution of CTX-M gene among *Escherichia coli* strains isolated from different clinical samples in Erbil City. *Iraqi Journal of Biotechnology*, 17(1): 78-90.

Ali, S. M. (2017)**.** Performance of VITEK 2 in the routine identification of bacteria from positive blood cultures in Sulaimani pediatrics' *hospital. Iraqi Journal of Science,* 58(1): 435-441.

Aljanaby, A. A. J., and Alfaham, Q. M. H. (2017). Phenotypic and molecular characterization of some virulence factors in multidrug resistance escherichia coli isolated from different clinical infections in Iraq. *American Journal of Biochemistry and Molecular Biology*, 7(2): 65-78.

Al-Jelehawy, A. H. j. (2016). Molecular Characterization of Antibacterial Resistant Genes in *Escherichia coli* Isolated from Clinical Samples. M.SC. Thesis, College of Medicine , University of Al-Qadisiyah/ Iraq.

Al-Jubouri, A . S., Mahmood, Y. A., and AL-Salihi, S. Sh. (2012). Pathogenicity of *Klebsiella pneumoniae* isolated from diarrheal cases among children in Kirkuk city. *Tikrit Journal of Pure Science*, 17(4): 17-25.

Al-Mayahie, S. M. (2013). Phenotypic and genotypic comparison of ESBL production by vaginal *Escherichia coli* isolates from pregnant and non-pregnant women. *Annals of clinical microbiology and antimicrobials*, 12(1): 7.

Al-Muhannak, F. H. N (2010). Spread of some extended-spectrum ß-lactamases in clinical isolates of Gram-negative Bacilli in Najaf. M.Sc. Thesis, College of Medicine , Kufa University/Iraq.

Al-Oebady, H. A. M. (2017). Identification of *E. coli* O157:H7 in Intestinal and Urinary Tract Infection in Samawah City. *Journal of Babylon University/Pure and Applied Sciences,* 25 (2): 544 -564 .

Alsamarai, A. M., Latif, I. A. R., and Abdul-Aziz, M. M. (2016). Antibiotic suceptibility of extended spectrum beta-lactamase [esbl] producing *Escerichia coli*. *World Journal of Pharmacy and Pharmaceutical Scinces,* 5(7): 195-215.

Alsamarai, A., Khorshed, S., and Weli, I. (2018). Molecular Characterisation of ESBL producer *E. coli*. *International Journal of Medical Sciences*, 1(2): 40-57.

Al-Shweely, S. H. (2018). Detection of Shiga Toxin Gene in *Escherichia coli* Serotype O157: H7 and O104:H4 Isolated from Clinical and Food Samples before and after Treatment with Probiotics. M.SC. Thesis Institute of Genetic Engineering, University of Baghdad/Iraq.

Alyamani, E. J., Khiyami, A. M., Booq, R. Y., Majrashi, M. A., Bahwerth, F. S., and Rechkina, E. (2017). The occurrence of ESBL-producing Escherichia coli carrying aminoglycoside resistance genes in urinary tract infections in Saudi Arabia. *Annals of clinical microbiology and antimicrobials*, 16(1): 1-13.

Al-Zubaidy, I. H. (2016). Study of Antimicrobial Activity for Hybrid Nanoantibiotics Against *E. coli* Isolated from Urinary Tract Infection in Sacred Karbala City. M.SC. Thesis Institute of Genetic Engineering, University of Baghdad/Iraq.

Amiri, F., Rooshan, M., Ahmady, M and Soliamani, M. (2009). Hygiene practices and sexual activity associated with urinary tract infection in pregnant women. *Mediterranean health journal*, 15(1):104–108.

Anago, E., Ayi-Fanou, L., Akpovi, C. D., Hounkpe, W. B., Tchibozo, M. A. D., Bankole, H. S., and Sanni, A. (2015). Antibiotic resistance and genotype of beta-lactamase producing *Escherichia coli* in nosocomial infections in Cotonou, Benin. *Annals of clinical microbiology and antimicrobials*, 14(1): 5**.**

Andrew, B., Kagirita, A., and Bazira, J. (2017). Prevalence of extended-spectrum beta-lactamases-producing microorganisms in patients admitted at KRRH, Southwestern Uganda. *International journal of microbiology*, 2017:1-5

Arenas-Hernández, M. M., Martínez-Laguna, Y., and Torres, A. G. (2012). Clinical implications of enteroadherent *Escherichia coli*. *Current gastroenterology reports*, 14(5): 386-394.

Ashrafian, F., Askari, E., Kalamatizade, E., Ghabouli-Shahroodi, M., and Naderi-Nasab, M. (2013). The Frequency of Extended Spectrum Beta Lactamase (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: A Report from Mashhad, Iran. *Journal of Medical Bacteriology*, 2(1-2): 12-19.

Aswani, S. M. Chandrashekar, U. K., Shivashankara, K. N. and Pruthvi, B.C. (2014). Clinical profile of urinary tract infections in diabetics and non-diabetics. *Journal of Australia Medical*, 7(1): 29-34**.**

Azargun, R., Sadeghi, M. R., Barhaghi, M. H. S., Kafil, H. S., Yeganeh, F., Oskouee, M. A., and Ghotaslou, R. (2018). The prevalence of plasmid-mediated quinolone resistance and ESBL-production in *Enterobacteriaceae* isolated from urinary tract infections. *Infection and drug resistance*, 11: 1007-1014.

Azekhueme, I., Moses, A. E., and Abbey, S. D. (2015). Extended spectrum beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from University of Uyo Teaching Hospital, Uyo-Nigeria. *Journal of Advances in Medical and Pharmaceutical Sciences*, 2(3): 117-125.

Bailey and Scott. (2002). *Diagnostic Microbiology*. (11th Ed), C.V. Mosby, Inc., pp: 122-125.

Bajaj, P., Singh, N. S., and Virdi, J. S. (2016). *Escherichia coli* β-lactamases: What really matters. *Frontiers in Microbiology*, 7: 417

Baker, K. S. (2015). Demystifying *Escherichia coli* pathovars. *Nature reviews Microbiology*, 13(5).

Bamidele, O., Jiang, Z. D., and Dupont, H. (2019). Occurrence of putative virulence-related genes, aatA, aggR and aaiC, of Enteroaggregative

Escherichia coli (EAEC) among adults with travelers' diarrhea acquired in Guatemala and Mexico. *Microbial pathogenesis*, 128: 97-99.

Baran, I., and Aksu, N. (2016). Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. *Annals of Clinical Microbiology and Antimicrobials*, 15(1): 20.

Barber, A. E., Norton, J. P., Spivak, A. M., and Mulvey, M. A. (2013). Urinary tract infections: current and emerging management strategies. *Clinical infectious diseases*, 57(5): 719-724.

Barcella, L., Barbaro, A. and Rogolino, S. (2016). Colonial morphology of *Escherichia coli*: impact of detection in clinical specimens, *Microbiologia Medica*. 31(2): 51-55.

Bardiau, M. Szalo, M. and Mainil, J.G. (2010). Initial adherence of EPEC, EHEC and VTEC to host cells. *Veterinary research*, 41(5): 57.

Barer, M. R., and Irving, W. L. (2018**).** *Medical Microbiology E-Book: A Guide to Microbial Infections*. *Elsevier Health Sciences*.

Bartolini, A., Frasson, I., Cavallaro, A., Richter, S. N., and Palù, G. (2014). Comparison of phenotypic methods for the detection of carbapenem non-susceptible Enterobacteriaceae. *Gut pathogens*, 6(1): 13.

Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by standard single disc method. *American journal of clinical pathology*, 45(4): 493-496

Bavaro, M. F. (2012). *E. coli* O157:H7 and other toxigenic strains: the curse of global food distribution. *Current Gastroenterology Reports*, 14(4): 317-323.

Bedenić, B., Vraneš, J., Bošnjak, Z., Marijan, T., Mlinarić-Džepina, A., Kukovec, T., Knežević, J., Anušić, M., Beader, N., Barl, P., Leskovar, V. and Kalenić S. (2010). Emergence of CTX-M group 1 extended-spectrum ß-lactamase–producing *Klebsiella pneumoniae* strains in the community. *Medical Glass*, **7**(1): 32-39.

Belotserkovsky, I., and Sansonetti, P. J. (2018)**.** Shigella and Enteroinvasive *Escherichia Coli*. In *Escherichia coli*, a Versatile Pathogen, Springer Cham, 416: 1-26.

Bergstrom, K. S., Sham, H. P., Zarepour, M., and Vallance, B. A. (2012). Innate host responses to enteric bacterial pathogens: a balancing act between resistance and tolerance. *Cellular microbiology*, 14(4): 475-484.

Bertini, A., Poirel, L., Mugnier, P. D., Villa, L., Nordmann, P., and Carattoli, A. (2010). Characterization and PCR-based replicon typing of resistance plasmids in *Acinetobacter baumannii*. *Antimicrobial agents and chemotherapy*, 54(10): 4168-4177.

Bettelheim, K. and Goldwater, P. (2014). Serotypes of Non-O157 Shigatoxigenic *Escherichia coli* (STEC). *Advances in Microbiology*, 4(7): 377-389.

Beyrouthy, R., Robin, F., Cougnoux, A., Dalmasso, G., Darfeuille-Michaud, A., Mallat, H., and Bonnet, R. (2013). Chromosome-mediated OXA-48 carbapenemase in highly virulent *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*, 68(7): 1558-1561.

Bhattacharjee, A., Sen, M. R., Anupurba, S., Prakash, P., and Nath, G. (2007). Detection of OXA-2 group extended-spectrum-β-lactamase-producing clinical isolates of *Escherichia coli* from India. *Journal of antimicrobial chemotherapy*, 60(3): 703-704.

Birbrair, A., and Frenette, P. S. (2016). Niche heterogeneity in the bone marrow. *Annals of the new York Academy of Sciences*, 1370(1): 82-96.

Black, J. G. (2012). *Microbiology*. (8th Ed). John Wiley and Sons, Inc., Jefferson city. USA. 212-239.

Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., Piddock, L. J. V., (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(1): 42-51.

Blazevic, D., Mackay, D. and Warwood, N. (1979). Comparison of micro- ID and API 20E systems for identification of Enterobacteriaceae. *Journal of Clinical Microbiology*, 9(5): 605-608.

Blount, Z. D. (2015). The natural history of model organisms: The unexhausted potential of *E. coli*. *Elife*, p4.

Bok, E., Mazurek, J., Stosik, M., Wojciech, M., and Baldy-Chudzik, K. (2015). Prevalence of virulence determinants and antimicrobial resistance among commensal *Escherichia coli* derived from dairy and beef cattle. *International journal of environmental research and public health*, 12(1): 970-985.

Bonnet, R. (2004). Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrobial agents and chemotherapy*, 48(1): 1-14.

Bonnet, M., Buc, E., Sauvanet, P., Darcha, C., Dubois, D., Pereira, B., and Darfeuille-Michaud, A. (2014). Colonization of the human gut by *E. coli* and colorectal cancer risk. *Clinical Cancer Research*, 20(4): 859-867.

Bora, A., Hazarika, N. K., Shukla, S. K., Prasad, K. N., Sarma, J. B., and Ahmed, G. (2014). Prevalence of blaTEM, blaSHV and blaCTX-M genes 15 in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. *Indian journal of Pathology and Microbiology*, 57(2): 249-254.

Bradford, P. A. (2001). Extended spectrum beta-lactamases in the 21st century: characterization, epidemiology and the detection of this important resistance threat. *Clinical Microbiology Reviews*, 14(4): 933-951.

Brenner, D. J., Krieg, N. R., Staley, J. T., Garrity Sc. D, G. M., Boone, D. R., DeVos, P., and Schleifer, K.-H. (2005). *Bergey's Manual® of Systematic Bacteriology*.(2nd Ed.)*:* Volume Two The Proteobacteria Part B The Gammaproteobacteria. Springer Science and Business Media

Brooks, Melnick and delberg's. (2016). *Medical Microbiology*. (27th Ed.). American Lange, 470.

Brook, G., Butel, J., Carroll, K., and Morse, S. (2007). *Jawetz, Melnick and Adelberg's Medical Microbiology*. (24th Ed). McGraw-Hill. NewYork. U.S.A.

Brooks, G. F., Carroll, K. C., Butel, J. S. and Morse, S. A. (2010). *Jawetz, Melnick and Adelbergs Medical Microbiology*. (25th Ed.). McGraw - Hill. New York.

Brooks, G. F., Carroll, K. C., Butel, J. S., Morse, S. A., and Mietzner, T. A. (2013). *Jawetz, Melnick and Adelberg's Medical Microbiology*. (26th Ed.). *Journal of Chemical Information and Modeling*.

Bush, K. (2010). Alarming b-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. *Current opinion in microbiolog*, 13(5): 558-564.

Bush, K. (2013). The ABCD's of β-lactamase nomenclature. *Journal of Infection and Chemotherapy*, 19(4): 549–559.

Bush, K., Jacoby, G. A. (2010). Updated functional classification of β-lactamases. *Antimicrobial Agents and Chemotherapy,* 54(3): 969-976.

Carrer, A., Poirel, L., Yilmaz, M., Akan, Ö. A., Feriha, C., Cuzon, G., and Nordmann, P. (2010). Spread of OXA-48-encoding plasmid in Turkey and beyond. *Antimicrobial agents and chemotherapy*, 54(3), 1369-1373.

Carroll, K. C., Hobden , J. A., Miller, S., Morse, S. A., Mietzner, T. A., Detrick , B., Mitchell ,T. G., McKerrow, J. M. and Sakanari , J. A. (2016). *Jawetz, Melnick, and Adelberg's Medical Microbiology*. 27th ed.

Center for Disease Controle and Prevention (CDC), (2015). About antimicrobial resistance [WWW Document]. URLhttps://www.cdc.gov/drugresistance/about.html (accessed 12.12.16).

Cebeci, T., Keskin, D., and Gökal, A. A. (2019). Comparison of Antibiotic Sensitivity Pattern of Escherichia coli which Produce Extended Spectrum Beta-Lactamase Strains Isolated from Various Clinical Specimens in Intensive Care Unit. *Middle Black Sea Journal of Health Science*, 5(1): 33-38.

Chang, Y. H., Han, J. I., Chun, J., Lee, K. C., Rhee, M. S., Kim, Y. B., and Bae, K. S. (2002). Comamonas koreensis sp. nov., a non-motile species from wetland in Woopo, Korea. *International journal of systematic and evolutionary microbiology*, 52(2): 377-381.

Chaudhary, U. A. R. (2004). Extended-spectrum B-lactamases (ESBL)- An emerging threat to clinical therapeutics. *Indian Journal of Medical Microbiology*, 22(2): 75-80.

Cirit, O. S., Fernández-Martínez, M., Yayla, B., and Martínez-Martínez, L. (2019). Aminoglycoside resistance determinants in multiresistant Escherichia coli and Klebsiella pneumoniae clinical isolates from Turkish and Syrian patients. *Acta microbiologica et immunologica Hungarica*, 66(3): 1-9.

Clinical and Laboratory Standards Institute (CLSI). (2017). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M100*. (27th Ed.). Wayne, PA, USA.

Conway, T., and Cohen, P. S. (2015). Commensal and pathogenic *Escherichia coli* metabolism in the gut. *Microbiology spectrum*, 3(3).

Coque, T. M., Baquero, F., and Cantón, R. (2008). Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Europe Surveilllance*, 13(47).

Cordonnier, C., Etienne-Mesmin, L., Thévenot, J., Rougeron, A., Rénier, S., Chassaing, B., Livrelli, V. (2017). Enterohemorrhagic *Escherichia coli* pathogenesis: Role of long polar fimbriae in peyer's patches interactions. *Scientific reports*, 7: 44655.

Costa, E. V., da Cruz, P. E. O., de Lourenço, C. C., de Souza Moraes, V. R., de Lima Nogueira, P. C., and Salvador, M. J. (2013). Antioxidant and antimicrobial activities of aporphinoids andother alkaloids from the bark of Annona salzmannii A. DC. (Annonaceae). *Natural product research*, 27(11): 1002-1006

Cruz, M. C., and Hedreyda, C. T. (2017). Detection of Plasmid-Borne β-Lactamase Genes in Extended-Spectrum β-Lactamase (ESBL) and Non-ESBL-Producing *Escherichia coli* Clinical Isolates. *Philippine Journal of Science*, 146(2): 167-175.

Cuzon, G., Naas, T., Bogaerts, P., Glupczynski, Y., Huang, T. D., and Nordmann, P. (2008). Plasmid-encoded carbapenem-hydrolyzing β-lactamase OXA-48 in an imipenem-susceptible *Klebsiella pneumoniae* strain from Belgium. *Antimicrobial agents and chemotherapy*, 52(9): 3463-3464.

D'Andrea, M. M., Arena, F., Pallecchi, L., and Rossolini, G.M. (2013). CTX-M-type b-lactamases: asuccessful story of antibiotic resistance. *International Journal of Medical Microbiology*, 303(6-7): 305-317.

Dagi, H. T., Al Dulaimi, D. A. A., Kus, H., Seyhan, T., Findik, D., Tuncer, I., and Arslan, U. (2015). Genotype distribution of extended Spectrum?-Lactamase producing *Escherichia coli and Klebsiella pneumonia. An International Journal of Medical Sciences*, 26(2).

Dagil, R., O'shea, C., Nykjær, A., Bonvin, A. M., and Kragelund, B. B. (2013). Gentamicin Binds to the Megalin Receptor as a Competitive Inhibitor Using the Common Ligand Binding Motif of Complement Type Repeats insight from the NMR structure of the 10th complement type repeat domain alone and in complex with gentamicin. *Journal of Biological Chemistry*, 288(6): 4424-4435.

Davey, P., Wilcox, M., Irving, W. and Thwaites, G. (2015). *Antimicrobial Chemotherapy*. (7th Ed.). Oxford University Press. United Kingdom.

Day, M. J., Rodríguez, I., van Essen-Zandbergen, A., Dierikx, C., Kadlec, K., Schink, A. K., and Helmuth, R. (2016). Diversity of STs, plasmids and ESBL genes among *Escherichia coli* from humans, animals and food in Germany, the Netherlands and the UK. *Journal of Antimicrobial Chemotherapy*, 71(5): 1178-1182.

Dhillon, R. H. P., and Clark, J. (2012). ESBLs: a clear and present danger?. *Critical care research and practice*, 2012: P11

Distefano, M. D. (2015). Role of the commensal microbiota in normal and pathogenic host immune responses. *American Journal Reproductive Immunology*, 10(5): 213-223.

Donnenberg, M. S. (2002). *Escherichia coli: virulence mechanisms of a versatile pathogen*. (Ed.) Amsterdam; Boston: Academic press(pp 20-25).

Drawz, S. M., and Bonomo, R. A. (2010). Three decades of β-lactamase inhibitors. *Clinical microbiology reviews*, 23(1): 160-201.

Dubreuil, J. D., Isaacson, R. E., and Schifferli, D. M. (2016). Animal Enterotoxigenic *Escherichia coli*. *EcoSal Plus*, 7(1).

DuPont, H. L. (2014). Bacillary Dysentery: *Shigella* and Enteroinvasive *Escherichia coli*. *In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 2: 2569-2574.

Džidić, S., Šušković, J., and Kos, B. (2008). Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. *Food Technology and Biotechnology*, 46(1): 11-21.

Ehmann, D. E., and Lahiri, S. D. (2014). Novel compounds targeting bacterial DNA topoisomerase/DNA gyrase. *Current opinion in pharmacology*, 18: 76-83.

Escherich, T. (1988). The intestinal bacteria of the neonate and breast-fed infant. *Clinical Infectious Diseases*, 10(6): 1220-1225.

European Centre for Disease Prevention and Control. (2014). Systematic review of the effectiveness of infection control measures to prevent the transmission of extended-spectrum Enterobacteriaceae through cross-border transfer of patients. Stockholm, Sweden.

Evans, B. A., and Amyes, S. G. (2014). OXA β-lactamases. *Clinical microbiology* reviews, 27(2): 241-263.

Fang, H., Ataker, F., Hedin, G., and Dornbusch, K. (2008). Molecular epidemiology of ESBLs among *Escherichia coli* isolates collected in a

Swedish Hospital and its associated health care facilities from 2001 to 2006. *Journal of Clinical Microbiol*ogy, 46(2): 707-712.

Feizabadi, M. M**.,** Delfani, S., Raji, N., Majnooni, A., Aligholi, M., Shahcheraghi, F., and Yadegarinia, D. (2010). Distribution of bla TEM, bla SHV, bla CTX-M genes among clinical isolates of Klebsiella pneumoniae at Labbafinejad Hospital, Tehran, Iran. *Microbial drug resistance*, 16(1): 49-53.

Fernando, D., and Kumar, A. (2011). Growth phase-dependent expression of RND efflux pump-and outer membrane porin-encoding genes in Acinetobacter baumannii ATCC 19606. *Journal of antimicrobial chemotherapy*, 67(3): 569-572.

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

Gashe, F., Mulisa, E., Mekonnen, M., and Zeleke, G. (2018). Antimicrobial resistance profile of different clinical isolates against third-generation cephalosporins. *Journal of pharmaceutics*, 2018 : 1-7

George, H.L. and Prasad, M. P. (2015). Determination of genetic diversity among antibiotic resistant *E. coli* strains using RAPD molecular markers. *European Journal of Biotechnology and Bioscience*, 3 (2): 20-25.

Gerritsen, J., Smidt, H., Rijkers, G. T., and De Vos, W. M. (2011). Intestinal microbiota in human health and disease: The impact of probiotics. *Genes and Nutrition*, 6(3): 209-240.

Ghafourian, S., Sadeghifard, N., Soheili, S., and Sekawi, Z. (2014). Extended spectrum beta-lactamases: definition, classification and epidemiology. *current issues in molecular biology*, 17(1): 11-22.

Gharajalar, S. N. and Sofiani, V. H. (2017). Patterns of Efflux Pump Genes Among Tetracycline Resistance Uropathogenic *Escherichia coli* Isolates Obtained from Human Urinary Infections. *Jundishapur Journal of Microbiology*, 10(2): 1-5.

Giguère, S. (2013). *Antimicrobial drug action and interaction: an introduction*. Fifth(Ed). *Antimicrobial therapy in veterinary medicine*, 1-10.

Gillespie, S., and Hawkey, P. M. (2006). *Principles and practice of clinical bacteriology*. (2nd Ed.). John Wiley and Sons.

Goldman, E., and Lorrence, H. G. (2009). *Practical Handbook of Microbiology*. (2nd Ed). Printed in the United States of America.

Gomes, T. A., Elias, W. P., Scaletsky, I. C., Guth, B. E., Rodrigues, J. F., Piazza, R. M., and Martinez, M. B. (2016). Diarrheagenic *Escherichia coli*. *Brazilian journal of microbiology*, 47: 3-30.

Govinden, U., MocktMoodley, P., Sturm, AW. (2007) Geographical evolution of the CTX-M β-lactamase – an update. *African Journal of Biotechnology*, 6(7): 831-839.

Guran, H. S., Vural, A., Erkan, M. E., and Durmusoglu, H. (2017). Prevalence and some virulence genes of *Escherichia coli* O157 isolated from chicken meats and giblets. *Annals of animal science*, 17(2): 555-563.

Haciseyitoglu, D., Dokutan, A., Abulaila, A., Erdem, F., Cag, Y., Ozer, S., and Aktas, Z. (2017). The First Enterobacter cloacae Co-Producing NDM and OXA-48 Carbapenemases and Interhospital Spread of OXA-48 and NDM-Producing *Klebsiella pneumoniae* in Turkey. *Clinical laboratory*, 63(7): 1213-1222.

Haghighatpanah, M., Nejad, A. S. M., Mojtahedi, A., Amirmozafari, N., and Zeighami, H. (2016). Detection of extended-spectrum β-lactamase (ESBL) and plasmid-borne blaCTX-M and blaTEM genes among clinical strains of *Escherichia coli* isolated from patients in the north of Iran. *Journal of global antimicrobial resistance*, 7: 110-113.

Hall, M. M., Finnoff, J. T., and Smith, J. (2011). Musculoskeletal complications of fluoroquinolones: guidelines and precautions for usage in the athletic population. *Physical medicine and rehabilitation*, 3(2): 132-142. .

Hamdoon, A. M. (2011). Detection of extended spectrum B-lactamase in *E. coli* from clinical samples. *Annals of the College of Medicine Mosul*, 37(1&2): 122-128.

Hano, B. K., Tareef, M. F., and Rasheed, E. M. (2018). Antibiotic Resistant Gene Exchanged Between *Escherichia coli* and *Staphylococcus aureus. Iraqi Journal of Science*, 59(1C): 456-462.

Hassan, M. M., Gaber, A., Alsanie, W. F., El-Hallous, E. I., Mohamed, A. A., Alharthi, A. A., and Ibrahim, A. M. (2018)**.** Phylogeny and Detection of blaTEM, blaSHV, blaCTX-M Genes in *Escherichia coli* Isolates from Patients with Urinary Tract Infections in Taif Hospitals, Saudi Arabia. *Annual Research and Review in Biology*, 25(1): 1-8.

Hawkey, P. M. (2008). Prevalence and clonality of extended-spectrum β-lactamases in Asia. *Clinical Microbiology and Infection*, 14: 159-165.

Helmy, M. M., and Wasfi, R. (2014). Phenotypic and molecular characterization of plasmid mediated AmpC β-lactamases among *Escherichia coli*, *Klebsiella spp.*, and *Proteus mirabilis* isolated from urinary tract infections in Egyptian hospitals. *BioMed research international*, 2014.1-8

Hemraj, V., Diksha, S. and Avneet, G. (2013). A Review on Commonly Used Biochemical Test for Bacteria. *Innovare Journal of Life Sciences*,1(1): 1-7.

Hopkins, K. L., Liebana, E., Villa, L., Batchelor, M., Threlfall, E. J., and Carattoli, A. (2006). Replicon typing of plasmids carrying CTX-M or CMY β-lactamases circulating among Salmonella and *Escherichia coli* isolates. *Antimicrobial Agents and Chemotherapy*, 50(9): 3203-3206.

Huddleston, J. R. (2014). Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. *Infection and Drug Resistance* 7: 167-176.

Hujer, K. M., Hujer, A. M., Hulten, E. A., Bajaksouzian, S., Adams, J. M., Donskey, C. J., and Thomson, J. M. (2006). Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter sp*. isolates from

military and civilian patients treated at the Walter Reed Army Medical Center. *Antimicrobial agents and chemotherapy*, 50(12): 4114-4123**.**

Hussein, A. R. (2018). Molecular Typing of Uropathogenic and Normal Flora *Escherichia coli* Isolated from Immunosuppression Patients. M.SC. Thesis College of Science the University of Thi-Qar/Iraq.

Huttner, A., Verhaegh, E. M., Harbarth, S., Muller, A. E., Theuretzbacher, U., and Mouton, J. W. (2015). Nitrofurantoin revisited: a systematic review and meta-analysis of controlled trials. *Journal of Antimicrobial Chemotherapy*, 70(9): 2456-2464.

Insan, N. G., Payal, N., Singh, M., Yadav, A., Chaudhary, B. L., and Srivastava, A. (2013). Post-operative wound infection: Bacteriology and antibiotic sensitivity pattern. *International Journal of Current Research and Review*, 5(13): 74-79.

Inwezerua, C., Mendonça, N., Calhau, V., Domingues, S., Adeleke, O. E., and Da Silva, G. J. (2014). Occurrence of extended-spectrum beta-lactamases in human and bovine isolates of Escherichia coli from Oyo state, Nigeria. *The Journal of Infection in Developing Countries*, 8(06): 774-779.

Jacoby, G. A. (2009). AmpC b-lactamases. *Clinical Microbiology Reviews*, 22: 161-182.

Jamali, S., Shahid, M., Sobia, F., Singh, A., and Khan, H. M. (2017). Phenotypic and molecular characterization of cefotaximases, temoniera, and sulfhydryl variable β-lactamases in *Pseudomonas* and *Acinetobacter* isolates in an Indian tertiary health-care center. *Indian Journal of Pathology and Microbiology*, 60(2): 196-201**.**

Janssen, H., Janssen, I., Cooper, P., Kainyah, C., Pellio, T., Quintel, M., and Schulze, M. H. (2018). Antimicrobial-Resistant Bacteria in Infected Wounds, Ghana, 2014. *Emerging infectious diseases*, 24(5): 916-919.

Jawetz, E., Melnick, J. A. and Adelberg, E. A. (2016). *Review of Medical Microbiology*. (27th Ed.). McGraw-Hill education, incorporated company: 851p.

Jena, J., Sahoo, R. K., Debata, N. K., and Subudhi, E. (2017). Prevalence of TEM, SHV, and CTX-M genes of extended-spectrum β-lactamase-producing *Escherichia coli* strains isolated from urinary tract infections in adults. *3 Biotech*, 7(4): 244-251.

Jena, J., Debata, N. K., Sahoo, R. K., Gaur, M., and Subudhi, E. (2018). Molecular characterization of extended spectrum β-lactamase-producing Enterobacteriaceae strains isolated from a tertiary care hospital. *Microbial pathogenesis*, 115: 112-116.

Juma, B. W., Kariuki, S., Waiyaki, P. G., Mutugi, M. M., and Bulimo, W. D. (2016). The prevalence of TEM and SHV genes among Extended-Spectrum Beta-Lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli. African Journal of Pharmacology and Therapeutics*, 5(1).

Kaber, J. B., Nataro, J. P., and Mobley, H . L. (2004). Pathogenic *Escherichia coli*. Nature Reviews. Microbiology, 2(2): 132-140.

Kaira, S. S., and Pai, C. (2017). Study of uropathogenic *Escherichia coli* with special reference to its virulence factors. *International Journal Of Community Medicine And Public Health*, 5(1): 177-181.

Kamel, N. A., Aboshana, K. M., Abouelwafa, M. M., and El-tayeb, W. N. (2013). Plasmid mediated extended spectrum beta-lactamase producing strains of Enterobacteriaceae isolated from diabetic foot infections in Egypt. *Archives of Clinical Microbiology*, 4(4):1.

Kao, C. C., Liu, M. F., Lin, C. F., Huang, Y. C., Liu, P. Y., Chang, C. W., andShi, Z. Y. (2010). Antimicrobial susceptibility and multiplex PCR screening of AmpC genes from isolates of *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens. Journal of Microbiology, Immunology and Infection*, 43(3): 180-187.

Karami, N., Hannoun, C., Adlerberth, I., and Wold, A. E. (2008). Colonization dynamics of ampicillin-resistant *Escherichia coli* in the infantile colonic microbiota. *Journal of antimicrobial chemotherapy*, 62(4): 703-708.

Karanika, S., Karantanos, T., Arvanitis, M., Grigoras, C., and Mylonakis, E. (2016). Fecal colonization with extended-spectrum beta-lactamase–producing Enterobacteriaceae and risk factors among healthy individuals: A systematic review and metaanalysis. *Reviews of Infectious Diseases*, 63(3): 310-318.

Kassakian, S. Z., and Mermel, L. A. (2014). Changing epidemiology of infections due to extended spectrum beta-lactamase producing bacteria. *Antimicrobial resistance and infection control*, 3(1): 9.

Katouli, M. (2010). Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections, 2(2): 59-72.

Katzung, B. G.(2001). *Basic and Clinical Pharmacology*. (8th Ed.). Lange Medical Books/ McGraw Hill. NewYork: 222.

Kaye, K. S., Gold, H. S., Schwaber, M. J., Venkataraman, L., Qi, Y., De Girolami, P. C.,and Tenover, F. C. (2004). Variety of β-lactamases produced by amoxicillin-clavulanate-resistant *Escherichia coli* isolated in the northeastern United States. *Antimicrobial agents and chemotherapy*, 48(5) 1520-1525.

Khosravi, A. D., Khaghani, S., Sheikh, A. F., Ahmadzadeh, A., and Shamsizadeh, A. (2016). Prevalence of *Escherichia coli* O157: H7 in children with bloody diarrhea referring to Abuzar teaching hospital, Ahvaz, Iran. *Journal of clinical and diagnostic research*, 10(1): DC13- DC15.

Kibret, M., and Abera, B. (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *African health sciences*, 11(3): 40-45.

Kiratisin, P., Apisarnthanarak, A., Saifon, P., Laesripa, C., Kitphati, R., and Mundy, L. M. (2007). The emergence of a novel ceftazidime-resistant CTX-M extended-spectrum β-lactamase, CTX-M-55, in both community-onset and hospital-acquired infections in Thailand. *Diagnostic microbiology and infectious disease*, 58(3): 349-355.

Klesiewicz, K., Karczewska, E., Nowak, P., Mrowiec, P., Skiba-Kurek, I., Bialecka, J., and Zajdel, P. (2018). Comparative in vitro studies of furazidin and nitrofurantoin activities against common uropathogens including multidrug-resistant strains of *E. coli* and *S. aureus. Acta Poloniae Pharmaceutica*, 75(3): 803-812.

Koga, V. L., Tomazetto, G., Cyoia, P. S., Neves, M. S., Vidotto, M. C., Nakazato, G., and Kobayashi, R. K. (2014). Molecular screening of virulence genes in extraintestinal pathogenic *Escherichia coli* isolated from human blood culture in Brazil. *BioMed research international*, 2014:1-9

Koroska, F., Göttig, S., Kaase, M., Steinmann, J., Gatermann, S., Sommer, J., et al. (2017). Comparison of phenotypic tests and an immune chromatographic assay and development of a new algorithm for detection of OXA-48-like carbapenemases. *Journal of Clinical Microbiology*, 55(3): 877-883.

Korzeniewska, E., Korzeniewska, A., and Harnisz, M. (2013). Antibiotic resistant *Escherichia coli* in hospital and municipal sewage and their emission to the environment. *Ecotoxicology and environmental safety*, 91: 96-102.

Kuenzli, E. (2016). Antibiotic resistance and international travel: causes and consequences. *Travel Medicine and Infectious Disease*, 14(6): 595-598.

Kukanur, S., Meundi, M., Bajaj, A.and Kotigadde, S. (2015). Co- Relation between Virulence Factors and Antibiotic Resistance of *E. coli,* With Special Reference to Uropathogenic *E. coli*. *IOSR Journal. of Dental and Medical Sciences,* 14(3): 15-21.

Kumar, N., Gupta, N., and Kishore, J. (2012). Kuppuswamy's socioeconomic scale: updating income ranges for the year 2012.

Kumar, D., Singh, A. K., Ali, M. R., and Chander, Y. (2014). Antimicrobial susceptibility profile of extended spectrum β-lactamase (ESBL) producing *Escherichia coli* from various clinical samples. *Infectious Diseases*, 7: 1-8.

Kumburu, H. H., Sonda, T., Mmbaga, B. T., Alifrangis, M., Lund, O., Kibiki, G., and Aarestrup, F. M. (2017). Patterns of infections, aetiological agents and antimicrobial resistance at a tertiary care hospital in northern Tanzania. *Tropical Medicine and International Health*, 22(4): 454-464.

Kwak, M. J., Kim, M. S., Kwon, S. K., Cho, S. H., and Kim, J. F. (2016). Genome sequence of *Escherichia coli* NCCP15653, a group D strain isolated from a diarrhea patient. *Gut Pathogens*, 8(1): 7.

Laehnemann, D., Pena-Miller, R., Rosenstiel, P., Beardmore, R., Jansen, G., and Schulenburg, H. (2014). Genomics of rapid adaptation to antibiotics: convergent evolution and scalable sequence amplification. *Genome biology and evolution*, 6(6): 1287-1301.

Lahlaoui, H., Khalifa, A. B. H., and Moussa, M. B. (2014). Epidemiology of Enterobacteriaceae producing CTX-M type extended spectrum β-lactamase (ESBL). *Medecine et maladies infectieuses*, 44(9): 400-404.

Lamy, B., Dargère, S., Arendrup, M. C., Parienti, J. J., and Tattevin, P. (2016). How to optimize the use of blood cultures for the diagnosis of bloodstream infections? A state-of-the art. *Frontiers in microbiology*, 7: 697.

Lartigue, M. F., Zinsius, C., Wenger, A., Bille, J., Poirel, L., and Nordmann, P. (2007). Extended-spectrum β-lactamases of the CTX-M type now in Switzerland. *Antimicrobial agents and chemotherapy*, 51(8): 2855-2860.

Leylabadlo, H. E., Asgharzadeh, M., and Aghazadeh, M. (2015). Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. *Iranian journal of microbiology*, 7(5): 226-246.

Lhwak, N. S., and Abbas, Y. A. (2018). Detection of Extended Spectrum β–Lactmase GeneeCTX-M-1 in *Escherchia coli* and *Klebseilla pneumonia* Isolated from Urinary Tract Infection of Pregnant Women in Al-Nassyriah City. *University of Thi-Qar Journal of Science*, 6(4): 92-96.

Liakopoulos, A., Mevius, D., and Ceccarelli, D. (2016). A review of SHV extended-spectrum β-lactamases: neglected yet ubiquitous. *Frontiers in microbiology*, 7: 1374.

Livermore, D. M. (1995). beta-Lactamases in laboratory and clinical resistance. *Clinical microbiology reviews*, 8(4): 557-584.

Livermore, D. M. (2008). Defining an extended-spectrum β-lactamase. *Clinical Microbiology and Infection*, 14(1): 3-10.

Livermore, D. M. (2012). Fourteen years in resistance. *International journal of antimicrobial agents*, 39(4): 283-294.

Lohani, B., Thapa, M., Sharma, L., Adhikari, H., Sah, A. K., Khanal, A. B., and Aryal, M. (2019). Predominance of CTX-M Type Extended Spectrum β-lactamase (ESBL) Producers Among Clinical Isolates of Enterobacteriaceae in a Tertiary Care Hospital, Kathmandu, Nepal. *The Open Microbiology Journal*: 13(1): 28-33.

Lota, M. M., and Latoree, A. E. (2014). A retrospective study on extended-spectrum beta-lactamase bacteria in the Philippines from 1999-2013. *Acta Medica Philippina* 48(1): 28-35.

Lucena, M. A, Metillo, E. B, Oclarit, J. M. (2012). Prevalence of CTX-M extended-spectrum beta-lactamase-producing Enterobacteriaceae at a private tertiary hospital in Southern Philippines. *Philippine Journal of Science*, 141(1): 117-121.

Mac Faddin, J. F. (2000). *Biochemical tests for identification of medical bacteria*. (3rd Ed.). Lippincott Williams and Wilkins USA.

Magdy, A., Elhadidy, M., Ellatif, M. A., El Nakeeb, A., Abdallah, E., Thabet, W., and Farid, M. (2015). Enteropathogenic *Escherichia coli* (EPEC): Does it have a role in colorectal tumourigenesis? A Prospective Cohort Study. *International journal of surgery*, 18: 169-173.

Magiorakos, A. P. A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, and D. L. Monnet**. (**2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an International expert proposal for

interim standard definitions for acquired resistance. *Clinical microbiology and infection* 18: 268-281.

Mahon, C. R., Lehman, D. C., and Manuselis, G. (2014). *Textbook of diagnostic microbiology-e-book*. (5th Ed.). Elsevier Health Sciences.

Majeed, H. T., and Aljanaby, A. A. J. (2019). Antibiotic Susceptibility Patterns and Prevalence of Some Extended Spectrum Beta-Lactamases Genes in Gram-Negative Bacteria Isolated from Patients Infected with Urinary Tract Infections in Al-Najaf City, Iraq. *Avicenna journal of medical biotechnology*, 11(2): 192-201.

Malema, M., Abia, A., Tandlich, R., Zuma, B., Mwenge Kahinda, J. M., and Ubomba-Jaswa, E. (2018). Antibiotic-resistant pathogenic *Escherichia coli isolated* from rooftop rainwater-harvesting tanks in the eastern cape, South Africa. *International journal of environmental research and public health*, 15(5): 892.

Markus, M. (2017). What You Should Know About Diarrhea. medical news today, University of Illinois-Chicago, School of Medicine,p1-11

Mawlood, A. H., Omer, S. A., Jalal, S. T., and Haji, S. H. (2018). Molecular detection of SHV-Type ESBL in *E. coli* and *K. pneumoniae* and their antimicrobial resistance profile. *Zanco Journal of Medical Sciences*, 22(2): 262-272.

Melnyk, A. H., Wong, A., and Kassen, R. (2015). The fitness costs of antibiotic resistance mutations. *Evolutionary applications*, 8(3): 273-283.

Meza-Segura, M., and Estrada-Garcia, T. (2016). *Diffusely adherent Escherichia coli* . In *Escherichia coli* in the Americas. Cham, Switzerland, Springer: 125-147.

Mishra, A. K., Sharma, K., Misra, R. S. (2010). Isozyme and PCR-based genotyping of epidemic *Phytophthora colocasiae* associated with taro leaf blight. *Archives of Phytophathology and Plant Protection*. 43(14): 1367-1380.

Moghaddam, M. N., Forghanifard, M. M., and Moshrefi, S. (2012). Prevalence and Molecular Characterization of Plasmid-mediated Extended-Spectrum β-Lactamase Genes (balaTEM, blaCTX and blASHV) Among Urinary *Escherichia coli* Clinical Isolates in Mashhad, Iran. *Iranian journal of basic medical sciences*, 15(3): 833-839.

Moghaddam, M. N., Beidokhti, M. H., Jamehdar, S. A., and Ghahraman, M. (2014). Genetic properties of blaCTX-M and blaPER β-lactamase genes in clinical isolates of Enterobacteriaceae by polymerase chain reaction. *Iranian journal of basic medical sciences*, 17(5): 378-383.

Moghnieh, R. A., Kanafani, Z. A., Tabaja, H. Z., Sharara, S. L., Awad, L. S., and Kanj, S. S. (2018). Epidemiology of common resistant bacterial pathogens in the countries of the Arab League. *The Lancet Infectious Diseases*, 18(12): 379-394

Mohammed, Y., Gadzama, G. B., Zailani, S. B., and Aboderin, A. O. (2016). Characterization of extended-spectrum beta-lactamase from *Escherichia coli* and *Klebsiella species* from North Eastern Nigeria. *Journal of clinical and diagnostic research*, 10(2): DC07-DC10.

Moretti, E., Capitani, S., Figura, N., Pammolli, A., Federico, M. G., Giannerini, V., and Collodel, G. (2009). The presence of bacteria species in semen and sperm quality. *Journal of assisted reproduction and genetics*, 26(1): 47-56.

Mulvey, M. R., Bryce, E., Boyd, D. A., Ofner, A. M., Christianson, S., Simor, A. E., and Paton, S. (2004). Ambler class A extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella spp*. In Canadian hospitals. *Antimicrobial Agents and Chemotherapy*. 48(4): 1204-1209.

Munoz-Davila, M. (2014). Role of Old Antibiotics in the Era of Antibiotic Resistance. Highlighted Nitrofurantoin for the Treatment of Lower Urinary Tract Infections. *Antibiotics*, 3(1): 39-48.

Murray, P. R. Ken S. Rosenthal, K. S. and Pfaller, M. A. (2013). Medical Microbiology. (7th Ed.). with student consult online access, *Elsevier Health Sciences*, pp.874.

Murthy, K. (2006). Phenotypic Characterization of *Escherichia coli* Strains Taken from Human Intestinal and Urinary Tracts. M.SC. Thesis Institute of Applied Sciences**,** Wright State University**.**

Nanakali, Z. G., and Ahmad, Z. F. (2015). Antibiotic resistance study and detection of virulence gene among Uropathogenic *E. coli*. *Kirkuk University Journal-Scientific Studies*, 10(3): 205-229.

Nasser, I. J. (2017). Identification and Characterization of OXA-48 Carbapenemase-Producing Enterobacteriaceae Clinical Isolates in Baghdad. *Mustansiriya Medical Journal*, 16(3): 11-18.

Navidinia, M., Peerayeh, S. N., Fallah, F., and Sajadinia, R. S. (2014). Phylogenetic grouping and pathotypic comparison of urine and fecal *Escherichia coli* isolates from children with urinary tract infection. *Brazilian Journal of Microbiology*, 45(2): 509-514.

Neamati, F., Firoozeh, F., Saffari, M. and Zibaei, M. (2015). Virulence Genes and Antimicrobial Resistance Pattern in Uropathogenic *Escherichia coli* Isolated from Hospitalized Patients in Kashan, Iran. *Jundishapur Journal of Microbiology*, 8(2): 1-6 .

Nepal, K., Pant, N. D., Neupane, B., Belbase, A., Baidhya, R., Shrestha, R. K., and Jha, B. (2017). Extended spectrum beta-lactamase and metallo beta-lactamase production among *Escherichia coli* and *Klebsiella pneumoniae* isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. *Annals of clinical microbiology and antimicrobials*, 16(1): 62.

Niumsup, P. R., Tansawai, U., Na-Udom, A., Jantapalaboon, D., Assawatheptawee, K., Kiddee, A., and Walsh, T. R. (2018). Prevalence and risk factors for intestinal carriage of CTX-M-type ESBLs in Enterobacteriaceae from a Thai community. *European Journal of Clinical Microbiology and Infectious Diseases*, 37(1): 69-75.

Nordmann, P., Naas, T., Poirel, L., (2011). Global spread of carbapenemase producing Enterobacteriaceae. *Emerging Infectious Diseases*, 17(10): 1791-1798.

Nugent, R., Back, E., Beith, A editorsm .(2010). *The Race against Drug Resistance*. Washington, DC: Center for Global Development,116.

Oliphant, C. M., and Green, G. M. (2002). Quinolones: a comprehensive review. *American family physician*, 65(3): 455-464.

Oliveira, R. V., Oliveira, M. C., and Pelli, A. (2017). Disease infection by Enterobacteriaceae family in fishes: a review. *Journal of Microbiology and Experimentation,* 4(5): 00128.

Olowe, B. M., Oluyege, J. O., Famurewa, O., Ogunniran, A. O., and Adelegan, O. (2017). Molecular identification of *Escherichia coli* and new emerging enteropathogen, *Escherichia fergusonii*, from drinking water sources in Ado-Ekiti, Ekiti State, Nigeria. *Journal of Microbiology Research*, 7(3): 45-54.

Omololu-Aso, J., Oluwatoyin, O., Omololu-Aso., Egbedokun, A., Olufunmilayo, O., Otusanya, Owolabi, A. T. and Oluwasanmi, A. V. (2017). Antibiotic Susceptibility Pattern of *Escherichia coli* Isolated from Out-patient Individuals Attending the University College Hospital (UCH), Ibadan, Nigeria. *Journal of Infectious Diseases and Treatment*, 3(1).

Ouedraogo, A. S., Sanou, M., Kissou, A., Sanou, S., Solaré, H., Kaboré, F., and Nacro, B. (2016). High prevalence of extended-spectrum ß-lactamase producing Enterobacteriaceae among clinical isolates in Burkina Faso. *BMC infectious diseases*, 16(1): 326.

Pasqua, M., Michelacci, V., Di Martino, M. L., Tozzoli, R., Grossi, M., Colonna, B., and Prosseda, G. (2017). The intriguing evolutionary journey of enteroinvasive *E. coli* (EIEC) toward pathogenicity. *Frontiers in microbiology*, 5(8).

Paterson, D. L. (2006). Resistance in gram-negative bacteria: Enterobacteriaceae. *American journal of infection control*, 34(5): S20-S28.

Paterson, D. L., and Bonomo, R. A. (2005). Extended-spectrum β-lactamases: a clinical update. *Clinical microbiology reviews*, 18(4): 657-686.

Paterson, D. L., Hujer, K. M., Hujer, A. M., Yeiser, B., Bonomo, M. D., Rice, L. B., and Bonomo, R. A. (2003). Extended-spectrum β-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV-and CTX-M-type β-lactamases. *Antimicrobial agents and chemotherapy*, 47(11): 3554-3560.

Perilli, M., Segatore, B., Mugnaioli, C., Celenza, G., Rossolini, G. M., Stefani, S., and Amicosante, G. (2011). Persistence of TEM-52/TEM-92 and SHV-12 extended-spectrum β-lactamases in clinical isolates of Enterobacteriaceae in Italy. *Microbial Drug Resistance*, 17(4): 521-524.

Philippon, A., Slama, P., Dény, P., and Labia, R. (2016). A structure-based classification of class A β-lactamases, a broadly diverse family of enzymes. *Clinical microbiology reviews*, 29(1): 29-57.

Philippon, A., Arlet, G., and Jacoby, G. A. (2002). Plasmid-determined AmpC-type b-lactamases. *Antimicrobial Agents and Chemotherapy*, 46: 1-11.

Pincus , D. H. (2006). Microbial identification using the bioMerieux VITEK ® 2 system Encyclopedia of Rapid Microbiological Methods.pp1-32.

Pitout, J. D. (2010). Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae. *Drugs*, 70(3): 313-333.

Pitout, J. D. (2012a). Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. *Frontiers in microbiology*, 3: 9.

Pitout, J. D. (2012b). Extraintestinal pathogenic *Escherichia coli*: an update on antimicrobial resistance, laboratory diagnosis and treatment. *Expert review of anti-infective therapy*, 10(10): 1165-1176

Poirel, L., Bonnin, R. A., and Nordmann, P. (2012). Genetic support and diversity of acquired extended-spectrum β-lactamases in Gram-negative rods. *Infection, Genetics and Evolution*, 12(5): 883-893.

Poirel, L., Kieffer, N., Liassine, N., Thanh, D., and Nordmann, P. (2016). Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of Escherichia coli. *The Lancet infectious diseases*, 16(3): 281.

Polse, R. F., Yousif, S. Y., and Assafi, M. S. (2016). Prevalence and molecular characterization of extended spectrum beta-Lactamases-producing uropathogenic *Escherichia coli* isolated in Zakho, Iraq. *Journal of Microbiology and Infectious Diseases*, 6(4): 163-167.

Pongpech, P., Naenna, P., Taipobsakul, Y., Tribuddharat, Ch. and Srifuengfung, S. (2008). Prevalence of Extended-spectrum beta- lactamase and class1 integron integrase gene INTII In *Escherichia coli* from that patients and healthy adults southeast asian. *Journal of Tropical Medicine. Public. Health*., 39(3): 425-433.

Poolman, J. T., and Wacker, M. (2016). Extra intestinal pathogenic *Escherichia coli*, a common human pathogen: challenges for vaccine development and progress in the field. *Journal of Infection Disease*., 213(1): 6-13.

Pulss, S., Semmler, T., Prenger-Berninghoff, E., Bauerfeind, R., and Ewers, C. (2017). First report of an *Escherichia coli* strain from swine carrying an OXA-181 carbapenemase and the colistin resistance determinant MCR-1. *International journal of antimicrobial agents*, 50(2): 232-236.

Quiros, Y., Vicente-Vicente, L., Morales, A. I., López-Novoa, J. M., and López-Hernández, F. J. (2010). An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin. *Toxicological sciences*, 119(2): 245-256.

Rahman, S., Ali, T., Ali, I., Khan, N. A., Han, B., Gao, J. (2018). The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases. *BioMed research international*, 2018:1-14.

Raju, G. K. and Cooney, C. L. (1993). Media and Air Sterilization. In Stephanopoulos G. *Biotechnology*, 3: 157-184.

Randall, L. P., Clouting, C., Horton, R. A., Coldham, N. G., Wu, G., Clifton-Hadley, F. A., and Teale, C. J. (2010). Prevalence of *Escherichia coli*

carrying extended-spectrum β-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. *Journal of Antimicrobial Chemotherapy*, 66(1): 86-95.

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M. and Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982–2002. *Emerging Infectious Diseases*. 11(4): 603-609.

Ray, B. and Bhunia, A. (2014). *Fundamental Food Microbiology*. (5th Ed.). CRC Press. Taylor and Francis Group., USA. p.139-156.

Redgrave, L. S., Sutton, S. B., Webber, M. A., and Piddock, L. J. (2014). Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends in Microbiology*, 22(8): 440-445.

Reimer, S. (2019). Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae (ESBL). Report MMIC 7050-Microbial Pathogenicity.

Reinthaler, F. F., Feierl, G., Galler, H., Haas, D., Leitner, E., Mascher, F., and Marth, E. (2010). ESBL-producing *E. coli* in Austrian sewage sludge. *Water Research*, 44(6): 1981-1985.

Reppin, F., Cochet, S., El Nemer, W., Fritz, G., and Schmidt, G. (2017). High affinity binding of *Escherichia coli* cytotoxic necrotizing factor 1 (CNF1) to Lu/BCAM adhesion glycoprotein. *Toxins*, 10(1): 3.

Rezai, M. S., Salehifar, E., Rafiei, A., Langaee, T., Rafati, M., Shafahi, K. and Eslami, G. (2015) **.** Characterization of multidrug resistant extended-spectrum beta-lactamase producing *Escherichia coli* among uropathogens of pediatrics in north of Iran. *Biomed Research International*, 23: 4847-4861.

Rimrang, B., Chanawong, A., Lulitanond, A., Wilailuckana, C., Charoensri, N., Sribenjalux, P., and Chetchotisakd, P.(2012). Genotypic Distribution of Plasmid Encoded Extended Spectrum Beta-lactamases from Lahore, Pakistan. *International Journal of Infectious Diseases,* 16S (2012): e317-e473

Rivas, L., Mellor, G. E., Gobius, K. and Fega, N. (2015). *Detection and Typing Strategies for Pathogenic Escherichia coli*. Springer. New York. 110pp.

Roy, S., Ahmed, M. U., Uddin, B. M. M., Ratan, Z. A., Rajawat, M., Mehta, V., and Zaman, S. B. (2017). Evaluation of antibiotic susceptibility in wound infections: A pilot study from Bangladesh. *F1000Research*, 6.

Rubin, J. E., and Pitout, J. D. (2014). Extended-spectrum β-lactamase, carbapenemase and AmpC producing Enterobacteriaceae in companion animals. *Veterinary microbiology*, 170(1-2): 10-18.

Ruppé, É., Woerther, P. L., and Barbier, F. (2015). Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Annals of intensive care*, 5(1): 21.

Sáez-lópez, E., Cossa, A., Benmessaoud, R., Madrid, L., and Moraleda, C. (2018). Characterization of Vaginal *Escherichia coli* Isolated from Pregnant Women in Two Different African Sites. *Public Library of Science* 11(7): 1-10.

Saleh, M. B., and Hussain, A. R. (2019). Determination of Phylogenetic Groups and Antimicrobial Susceptibility Patterns for *Escherichia coli* Isolated from patients with urinary tract infection. *Journal of Education for Pure Science-University of Thi-Qar*, 9(1): 71-81.

Sambrook, J. and Russell, D. (2001). *Molecular Cloning: a Laboratory Manual*. (3rd Ed.). Cold Spring Harbour Laboratory Press, 3.

Sambrook, J. F. and Russell, D. W. (2006). *Detection of DNA in agarose gels*. Cold Spring Harbor Protocols. 2006(1).

Sanders, J. W., Riddle, M. S., Taylor, D. N., and DuPont, H. L. (2019). *Epidemiology of Travelers' Diarrhea*. (4th Ed.). In Travel medicine. Elsevier. 187-198

Sandhu, S. S. (2010). *Recombinant DNA Technology*. I.K. International Publishing House PVT. Ltd., New Delhi. India. P. 59-80.

Saravanan, M., Ramachandran, B., and Barabadi, H. (2018**).** The prevalence and drug resistance pattern of extended spectrum β–lactamases (ESBLs)

producing Enterobacteriaceae in Africa. *Microbial pathogenesis*, 114: 180-192.

Saravanan, R., and Raveendaran, V. (2013). Antimicrobial resistance pattern in a tertiary care hospital: An observational study. *Journal of basic and clinical pharmacy*, 4(3): 56-63.

Sasaki, T., Hirai, I., Niki, M., Nakamura, T., Komalamisra, C., Maipanich, W., Yamamoto, Y. (2010). High prevalence of CTX-M β-lactamase producing Enterobacteriaceae in stool specimens obtained from healthy individuals in Thailand. *Journal of Antimicrobial Chemotherapy*, 65(4): 666-668.

Schmidt, T. and Relman, D. (1994). phylogenetic identification of uncultured pathogens using ribosomal RNA sequences. *Methods in Enzymol*ogy. 235: 205-222.

Schmitt, J., Jacobs, E., Schmidt,T. H. (2007). Molecular characterization of extended-spectrum beta-lactamases in Enterobacteriaceae from patients of two hospitals in Saxony, Germany. *Journal of Medical Microbiology* 56: 241-249.

Seyedjavadi, S. S., Goudarzi, M., and Sabzehali, F. (2016). Relation between blaTEM, blaSHV and blaCTX-M genes and acute urinary tract infections. *Journal of Acute Disease*, 5(1): 71-76.

Shabana, I. I. (2014). *Escherichia coli* pathotypes associated with diarrhea in human and domestic animals. *American Journal of Animal and Veterinary Sciences*, 9(3): 155-161.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D., and Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi journal of biological sciences*, 22(1): 90-101.

Shanthi, B., Selvi, R. and Madhumathy, A. (2018). Antimicrobial susceptibility pattern of *Escherichia coli* from patients with urinary tract infections in a tertiary care. *International Journal of Current Microbiology and Applied Sciences;*7(1): 289-294.

Sharma, J., Sharma, M., and Ray, P. (2010). Detection of TEM and SHV genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital from India. *Indian Journal of Medical Research*, 132(3): 332-337.

Sharp, C., Boinett, C., Cain, A., Housden, N. G., Kumar, S., Turner, K., and Kleanthous, C. (2019). O-antigen-dependent colicin insensitivity of uropathogenic *Escherichia coli*. *Journal of bacteriology*, 201(4): e00545-18.

Singh, N., Pattnaik, D., Neogi, D. K., Jena, J., and Mallick, B. (2016). Prevalence of ESBL in *Escherichia Coli* isolates among ICU patients in a tertiary care hospital. *Journal of Clinical and Diagnostic Research*,, 10(9): DC19-DC22.

Skurnik, D., Le Menac‟h, A., Zurakowski, D., Mazel, D., Courvalin, P., Denamur, E., Ruimy, R. (2005). Integron-associated antibiotic resistance and phylogenetic grouping of *Echerichia coli* isolates from healthy subjects free of recent antibiotic exposure. *Antimicrobial Agents and Chemotherapy*, 49(7): 3062-3065.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Claeys, G., and Butaye, P. (2010). Characterization of extended-spectrum β-lactamases produced by *Escherichia coli* isolated from hospitalized and nonhospitalized patients: emergence of CTX-M-15-producing strains causing urinary tract infections. *Microbial Drug Resistance*, 16(2): 129-134.

Sohail, M., Khurshid, M., Saleem, H., Javed, H. and Khan, A. (2015). Characteristics and antibiotic resistance of urinary tract pathogens isolated from punjab, pakistan. *Jundishapur journal of microbiology*. 8(7): e19272.

Solomon, M., and Henkel, R. (2017). Semen culture and the assessment of genitourinary tract infections. *journal of the Urological Society of India*, 33(3): 188-193.

Soltani, S., Emamie, A. D., Dastranj, M., Farahani, A., Davoodabadi, A., and Mohajeri, p. (2018). Role of Toxins of Uropathogenic *Escherichia coli* in

Development of Urinary Tract Infection. *Journal of Pharmaceutical Research International*, 21(1): 1-11.

Søraas, A. V. L. (2014). Extended spectrum beta-lactamase producing Enterobacteriaceae. Aspects on carriage, infection and treatment. M.SC. Thesis of Department of Medical Microbiology, University of Oslo, p122.

Spaulding, C. N., Klein, R. D., Ruer, S., Kau, A. L., Schreiber, H. L., Cusumano, Z. T., Dodson, K. W., Pinkner, J. S., Fremont, D. H., Janetka, J. W., Remaut, H., Gordon, J. I. and Hultgren, S. J. (2017). Selective Depletion of Uropathogenic *E. coli* from the Gut by a *FimH* Antagonist. *Nature*, 546(7659): 528-532 .

Steward, C. D., Wallace, D., Hubert, S. K., Lawton, R., Fridkin, S. K., Gaynes, R. P., and Tenover, F. C. (2000). Ability of laboratories to detect emerging antimicrobial resistance in nosocomial pathogens: a survey of project ICARE laboratories. *Diagnostic microbiology and infectious disease*, 38(1): 59-67.

Subash, S., Hazen, T., Brumbaugh, A., Himpsl, S., Smith, S. and Ernst, R. (2014). Host-specific induction of *Escherichia coli* fitness genes during human urinary tract infection. *Proceedings of the National Academy of Sciences*, 111(51): 18327-18332.

Tabassum, S. N. (2018). Study of antibiotic resistance gene transfer between isolates from patients with urinary tract infection via conjugation. Doctoral dissertation, BRAC University.

Talbot, G. H. (2013). β-Lactam antimicrobials: what have you done for me lately?. Annals of the New York Academy of Sciences, 1277(1): 76-83.

Tamerat, N., Muktar, Y., and Shiferaw, D. (2016). Application of molecular diagnostic techniques for the detection of E. coli O157: H7: a review. *Journal* of *Veterinary Science* and *Technology*, 7(362): 1-9.

Tan, T. Y., Yong Ng, L. S., He, J., Koh, T. H., and Hsu, L. Y. (2009). Evaluation of screening methods to detect plasmid-mediated ampc in

Escherichia coli, *Klebsiella pneumoniae*, and *Proteus mirabilis*. *Antimicrobial Agents and Chemothrapy*., 53(1): 146-149.

Tanimoto, Y., Tamai, S., Matsuzaki, T., Takeuchi, N., Noju, T., Yanagida, S., and Motooka, D. (2019). Diffusely adherent *Escherichia coli* strains isolated from healthy carriers suppress cytokine secretions of epithelial cells stimulated by inflammatory substances. *Infection and immunity*, 87(1): 1-13.

Tchaptchet, S., and Hansen, J. (2011). The Yin and Yang of host-commensal mutualism. *Gut Microbes*, 2(6): 347-352.

Terlizzi, M. E., Gribaudo, G. and Maffei, M. E. (2017). UroPathogenic *Escherichia coli* (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-Antibiotic Antimicrobial Strategies. *Frontiers in microbiology*, 8(1566): 1-23.

Tham, J. (2012). *Extended- spectrum beta- lactamase- producing Enterobacteriaceae: epidemiology, risk factors, and duration of carriage* . Department of Clinical Sciences, Lund University. 2012 (65**).**

Thiong'o, L. N. (2014). Molecular characterization of multi-drug resistant *Escherichia coli* isolated from urine samples. Doctoral dissertation in Mater Hospital, Nairobi, Kenya.

Tofteland, S., Haldorsen, B., Dahl, K. H., Simonsen, G. S., Steinbakk, M., Walsh, T. R., and Norwegian ESBL Study Group. (2007). Effects of phenotype and genotype on methods for detection of extended-spectrum-β-lactamase-producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Norway. *Journal of clinical microbiology*, 45(1): 199-205.

Tomanicek, S. J., Blakeley, M. P., Cooper, J., Chen, Y., Afonine, P. V., and Coates, L. (2010). Neutron diffraction studies of a class a β- lactamase Toho-1 E166A/R274N/R276N triple mutant . *Journal of Molecular Biology*, 396(4): 1070-1080.

Topaloglu, R., Er, I., Dogan, B. G., Bilginer, Y., Ozaltin, F., Besbas, N., Gur, D. (2010). Risk factors in community-acquired urinary tract infections

caused by ESBL-producing bacteria in children. *Pediatric Nephrology*, 25(5): 919-925.

Tortora, G. J., Funke, B.R. and Case, C.L. (2016). *Microbiology an introduction*. (12th Ed). Benjamin. Cummings publishing Company. United States. America.

Tschudiu-sutter, S. Frei, R., Battegay, M. Hoesli, I and Widmer, A. F. (2010). Extended spectrum β-lactamase – producing *Escherichia coli* in neonatal care unit. *Emerging Infectious Diseases journal*, 16(11): 1758-1760.

Tulara, N. K. (2018). Nitrofurantoin and Fosfomycin for Extended Spectrum Beta-lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae*. *Journal of Global Infectious Diseases*, 10(1): 19-21.

Tumbarello, M., Sali, M., Trecarichi, E. M., Leone, F., Rossi, M., Fiori, B., Cauda, R. (2008). Bloodstream infections caused by extended-spectrum-β-lactamase-producing *Escherichia coli*: risk factors for inadequate initial antimicrobial therapy. *Antimicrobial agents and chemotherapy*, 52(9): 3244-3252.

Van Elsas, J. D., Semenov, A. V, Costa, R., and Trevors, J. T. (2011). Survival of*Escherichia coli* in the environment: fundamental and public health aspects. *The ISME Journal*, 5(2): 173-183.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics, 40(4): 277.

Verhaegen, B. , De Reu, K. , Heyndrickx, M. and De Zutter, L. (2015). Comparison of six chromogenic agar media for the isolation of a broad variety of non-157 Shiga toxin producing *Escherichia coli* (STEC) serogroups. *International Journal of Environmental Research and Public Health*,12: 6965-6978.

Villegas, J. V., Boguen, R., and Uribe, P. (2017). Effect of Uropathogenic *Escherichia coli* on Human Sperm Function and Male Fertility. In *Escherichia coli-Recent Advances on Physiology, Pathogenesis and Biotechnological Applications*. In tech Open.

Vilvanathan, S., Kandasamy, B., Jayachandran, A. L., Sathiyanarayanan, S., Tanjore Singaravelu, V., Krishnamurthy, V., and Elangovan, V. (2016). Bacteriospermia and its impact on basic semen parameters among infertile men. *Interdisciplinary perspectives on infectious diseases*, 2016:1-6

Vuotto, C., Longo, F., Balice, M., Donelli, G., and Varaldo, P. (2014). Antibiotic resistance related to biofilm formation in *Klebsiella pneumoniae*. *Pathogens*, 3(3): 743-758.

Wagle, S., Khanal, B. R., and Tiwari, B. R. (2018). High susceptibility of fosfomycin to uropathogenic *Escherichia coli* isolated at Tertiary Care Hospital of Nepal. *Journal of Advances in Microbiology*, 13(2): 1-8.

Walther-Rasmussen, J., and Høiby, N. (2004). Cefotaximases (CTX-M-ases), an expanding family of extended-spectrum β-lactamases. *Canadian journal of microbiology*, 50(3): 137-165.

Wang, L., Pulk, A., Wasserman, M. R., Feldman, M. B., Altman, R. B., Cate, J. H. D., and Blanchard, S. C. (2012). Allosteric control of the ribosome by small-molecule antibiotics. *Nature structural and molecular biology*, 19(9): 957.

Wanger, A., Chavez, V., Huang, R. S. P., Wahed, A., Actor, J. K. and Dasgupta, A. (2017). *Microbiology and Molecular Diagnosis in Pathology*. Elsevier Inc. All Rights Reserved. 300pp.

Wassef, M. A., El Sherif, R. H., El Shenoufy, A. E., and Ghaith, D. M.(2010). Phenotypic and genotypic patterns of aminoglycosides resistance in gram negative bacilli . *Journal of American Sciences*, 6 (9): 781-786 .

World Health Organization (WHO). (2014). Antimicrobial resistance: global report on surveillance. World Health Organization.

Yadav, K. K., Adhikari, N., Khadka, R., Pant, A. D., and Shah, B. (2015). Multidrug resistant Enterobacteriaceae and extended spectrum β-lactamase producing *Escherichia coli*: a cross-sectional study in National Kidney Center, Nepal. *Antimicrobial resistance and infection control*, 4(1): 42.

Yang, S., Xi, D., Jing, F., Kong, D., Wu, J., Feng, L., and Wang, L. (2018). Genetic diversity of K-antigen gene clusters of *Escherichia coli* and their molecular typing using a suspension array. *Canadian journal of microbiology*, 64(4): 231-241.

Yasir, M., Ajlan, A. M., Shakil, S., Jiman-Fatani, A. A., Almasaudi, S. B., Farman, M., and Ismaeel, N. A. (2018). Molecular characterization, antimicrobial resistance and clinico-bioinformatics approaches to address the problem of extended-spectrum β-lactamase-producing *Escherichia coli* in western Saudi Arabia. *Scientific reports*, 8(1): 14847.

Yongjung, P., M. D., Hyun-Kyung, K. Ph. D.; Kwon B. Ph. D., Juwon K. Jae Seok, M. D., Kim, M. D., Young Uh, M. D., Seok-Hoon, J. M. D., and Kyungwon, L. M. D. (2009). Prevalence of the extended-spectrum β-lactamase and *qnr* genes in clinical isolates of *Escherichia coli*. *Korean Journal* of *Laboratory Medicine*, 29: 218-23.

Yousef, S., Ammar, A. and Ahmed, D. (2013). Serological and molecular typing of avian pathogenic *E. coli* originating from outbreaks of colibacillosis in chicken flocks. *International Journal of Science and Research*. 4: 2082-2088.

Yun, K. W., Kim, H. Y., Park, H. K., Kim, W., and Lim, I. S. (2014). Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children. *Journal of Microbiology, Immunology and Infection*, 47(6): 455-461.

Zandi, H., Tabatabaei, S. M., Ehsani, F., Zarch, M. B., and Doosthosseini, S. (2017). Frequency of Extended-Spectrum Beta-lactamases (ESBLs) in strains of *Klebsiella* and *E. coli* isolated from patients hospitalized in Yazd. *Electronic physician*, 9(2): 3810-3815.

Zaniani, F. R., Meshkat, Z., Nasab, M. N., Khaje-Karamadini, M., Ghazvini, K., A., Esmaily, H., Nabavinia, M. S. and Hoseini, M .D. (2012). The Prevalence of TEM and SHV Genes among Extended-Spectrum Beta-

Lactamases Producing *Escherichia Coli* and *Klebsiella Pneumoniae*. *Iranian Journal of Basic Medical Sciences*, 15(1): 654-660.

Zeynudin, A., Pritsch, M., Schubert, S., Messerer, M., Liegl, G., Hoelscher, M., and Wieser, A. (2018). Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. *BMC infectious diseases*, 18(1): 524.

Zhang, R., Gu, D. X., Huang, Y. L., Chan, E. W. C., Chen, G. X., and Chen, S. (2016). Comparative genetic characterization of Enteroaggregative *Escherichia coli* strains recovered from clinical and non-clinical settings. *Scientific reports*, 6: 24321.

Zhao, W. H., and Hu, Z. Q. (2013). Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Critical reviews in microbiology*, 39(1): 79-101.

Zowawi, H. M., Harris, P. N., Roberts, M. J., Tambyah, P. A., Schembri, M. A., Pezzani, M. D., and Paterson, D. L. (2015). The emerging threat of multidrug-resistant Gram-negative bacteria in urology. *Nature Reviews Urology*, 12(10): 570-584.

http://WWW.lahey.org/studies/temtable.asp last access on 14 of January 2017.

http://WWW.lahey.org/studies/temtable.asp last access on 14 of January 2018.

Appendices

Appendix(1): The component of kits that used in the present study

1. The component of MaximeTM PCR PreMix

1- Taq DNA polymerase.

2- dNTPs (dATP, dCTP, dGTP, dTTP).

3- Tris-HCl pH 9.0, KCl, MgCl2.

4- Stabilizer and Tracking dye.

2. The component of AccuPrep® Plasmid Mini extraction kit

1- Buffer (1) 60 ml

2- Buffer (2) 60 ml

3- Buffer (3) 80 ml

4- Buffer (D) 75 ml

5- Buffer (4) 32 ml

6- Buffer (5) 24 ml

7- RNase A powder

8- DNA binding column 200

3. The components of API System

1- Incubation Box

2- Mineral oil dropper

3- Chemical reagents

a- James reagent

b- Voges- Proskauer reagent

This reagent consisted of two solutions (α- naphthol and 40% KOH)

c- Ferric chloride (TDA)

4- Saline suspension medium (NaCl 0.85% suspension)

Appendix (2): The component of kits that used in the present study

2.1.5.4. The components of Gram' stain Kit

1- Crystal Violet stain.

2- Decolourizer (acetone 30 ml with alcohol 70 ml).

3- Iodine stain.

4- Safranin stain**.**

2.1.5.5. The component of Genomic DNA Extraction and Purification kit

1- GT buffer 30 ml.

2- GB buffer 40 ml.

3- W1 buffer 45 ml.

4- Wash buffer 25 ml +100 ml Ethanol.

5- Elution buffer 30 ml.

6- GD column.

7- Collection tube 2ml.

In addition to Proteinase K, Absolute ethanol and RNase A.

The kit contents were stored at 22-25ºC.

2.1.5.6. The component of Vitek 2- GN Kit

1- cassette identification of Gram negative.

2- Saline suspension medium (NaCl 0.45% suspension).

Appendix (3): The phenotypic test of *E. coli*

No	Screen test	Confirmatory test
1	+Ve	-Ve
2	+Ve	-Ve
3	-Ve	-Ve
4	-Ve	-Ve
5	+Ve	-Ve
6	+Ve	-Ve
7	-Ve	-Ve
8	+Ve	-Ve
9	+Ve	-Ve
10	-Ve	-Ve
11	+Ve	+Ve
12	+Ve	-Ve
13	-Ve	-Ve
14	+Ve	+Ve
15	+Ve	+Ve
16	-Ve	-Ve
17	+Ve	-Ve
18	+Ve	+Ve
19	-Ve	-Ve
20	+Ve	-Ve
21	+Ve	-Ve
22	+Ve	+Ve
23	-Ve	-Ve
24	+Ve	-Ve
25	+Ve	+Ve
26	-Ve	-Ve
27	+Ve	+Ve
28	+Ve	-Ve
29	+Ve	+Ve
30	+Ve	+Ve
31	+Ve	-Ve
32	+Ve	-Ve
33	-Ve	-Ve
34	+Ve	-Ve
35	+Ve	-Ve
36	+Ve	-Ve
37	-Ve	-Ve
38	-Ve	-Ve
39	+Ve	-Ve
40	-Ve	-Ve
41	-Ve	-Ve

42	+Ve	+Ve
43	+Ve	+Ve
44	+Ve	-Ve
45	+Ve	+Ve
46	+Ve	-Ve
47	-Ve	-Ve
48	+Ve	+Ve
49	+Ve	-Ve
50	+Ve	-Ve
51	+Ve	-Ve
52	+Ve	+Ve
53	+Ve	-Ve
54	+Ve	-Ve
55	+Ve	-Ve
56	+Ve	-Ve
57	+Ve	-Ve
58	+Ve	-Ve
59	+Ve	+Ve
60	+Ve	-Ve
61	+Ve	-Ve
62	+Ve	-Ve
63	+Ve	-Ve
64	+Ve	-Ve
65	+Ve	-Ve
66	+Ve	-Ve
67	+Ve	-Ve
68	+Ve	-Ve
69	+Ve	-Ve
70	+Ve	+Ve
71	-Ve	-Ve
72	+Ve	-Ve
73	+Ve	-Ve
74	+Ve	+Ve
75	-Ve	-Ve
76	+Ve	-Ve
77	+Ve	-Ve
78	+Ve	-Ve
79	+Ve	+Ve
80	+Ve	-Ve
81	+Ve	-Ve
82	+Ve	-Ve
83	+Ve	-Ve
84	+Ve	-Ve
85	+Ve	-Ve
86	+Ve	-Ve
87	+Ve	-Ve
88	+Ve	-Ve
89	+Ve	+Ve

90	+Ve	-Ve
91	+Ve	-Ve
92	+Ve	-Ve
93	+Ve	-Ve
94	+Ve	-Ve
95	+Ve	-Ve
96	+Ve	-Ve
97	+Ve	-Ve
98	+Ve	-Ve
99	-Ve	-Ve
100	+Ve	-Ve
101	+Ve	-Ve
102	+Ve	-Ve
103	+Ve	-Ve
104	+Ve	-Ve
105	+Ve	-Ve

Appendix (4): The results of molecular tests of ESBLs of *E. coli* isolated from clinical cases

Type of isolate	No of *E.coli* isolate	CTX-M		TEM		OXA		SHV	
		Chrom-osome	Plasmid	Chrom-osome	Plasmid	Chrom-osome	Plasmid	Chrom-osome	Plasmid
Seminal fluid	1	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Blood	2	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	3	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Blood	4	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	5	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	6	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	7	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	8	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	9	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Blood	10	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve
Urine	11	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	12	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	13	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	14	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	15	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	16	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Wound swab	17	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Wound swab	18	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Blood	19	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	20	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	21	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	22	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	23	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	24	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	25	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	26	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	+Ve
Urine	27	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	28	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	29	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	30	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	31	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	32	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	33	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	34	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve

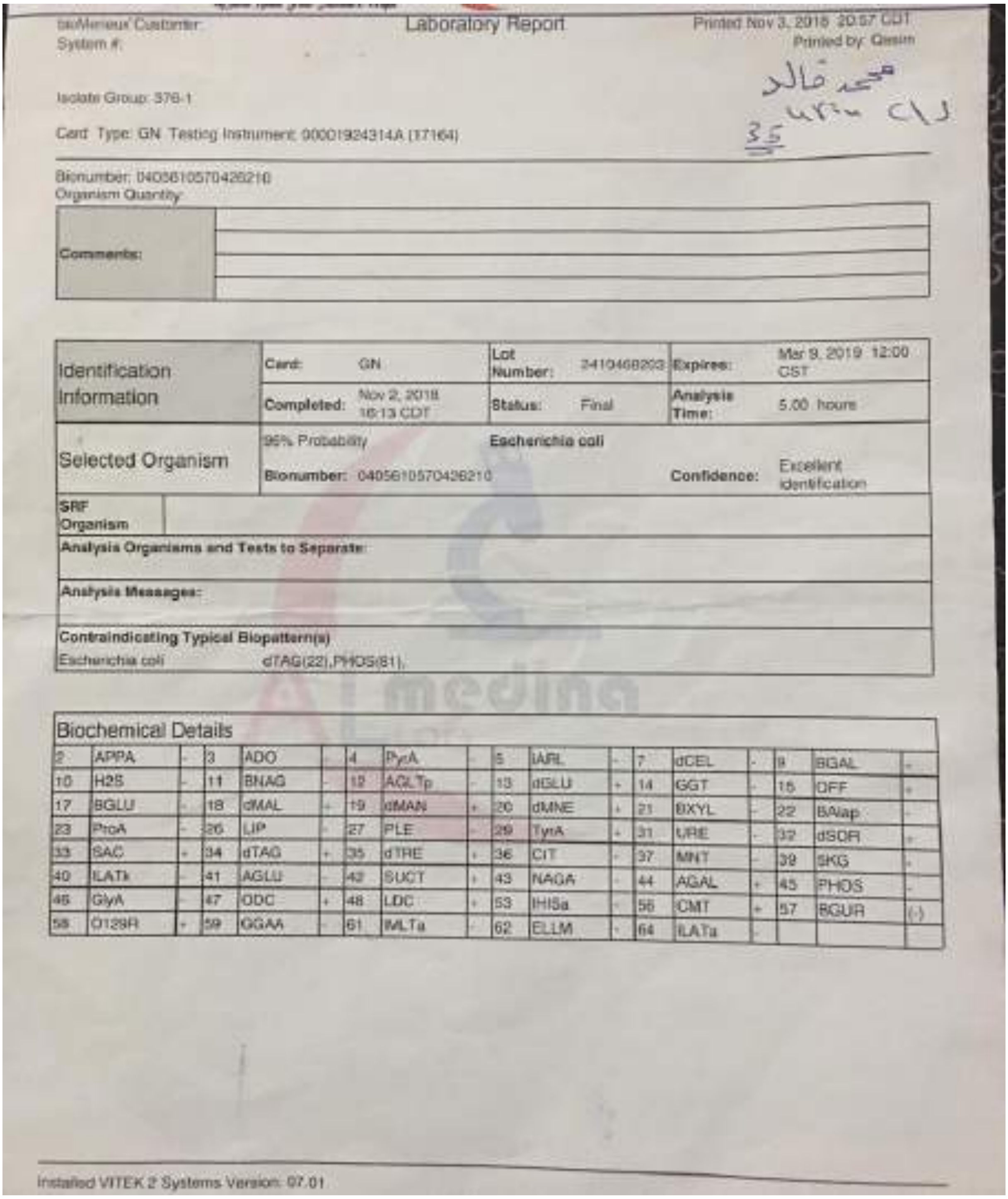
Urine	35	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	36	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	37	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	38	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	39	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	40	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	41	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	42	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	43	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	44	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	45	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	46	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	47	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	48	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	49	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	50	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve
Urine	51	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	52	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	53	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	54	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	55	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	56	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	57	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	58	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	59	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	60	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	61	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	62	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve
Urine	63	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	64	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	65	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	66	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	67	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	68	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	69	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	70	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	71	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	72	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	73	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	74	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	75	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	76	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Seminal fluid	77	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve
Urine	78	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	79	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Blood	80	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Blood	81	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve

Urine	82	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Seminal fluid	83	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Seminal fluid	84	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	85	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	86	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	87	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	88	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	89	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	90	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	91	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	92	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	93	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Blood	94	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	95	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Urine	96	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	97	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	98	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve	-Ve
Urine	99	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve
Urine	100	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Wound swab	101	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	102	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Wound swab	103	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve
Urine	104	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve
Urine	105	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	-Ve

Appendix (5): Results of API 20 E for *E. coli*:

NO	Test	Material	Result
1	ONPG	Ortho-nitrophenyle galactoside	+
2	ADH	Arginine	+
3	LDC	Lycine	+
4	ODC	Ornithine	+
5	CIT	Sodium citrate	-
6	H2S	Sodium thiosulfate	-
7	URE	Urea	-
8	TDA	Tryptophane	-
9	IND	Tryptophane	+
10	VP	Sodium pyruvate	-
11	GEL	Gelatine	-
12	GLU	Glucose	+
13	MAN	Mannitol	+
14	INO	Inositol	-
15	SOR	Sorbitol	+
16	RHA	Rhaminose	+
17	SAC	Sucrose	-
18	MEL	Milibiose	-
19	AMY	Amygdalin	-
20	ARA	Arabinose	+

Appendix(6): Examination report by VITEK2 device to one sample under study



bioMerieux Customer:
System #:

Laboratory Report

Printed Nov 3, 2018 20:57 CDT
Printed by: Qasim

محمد خالد
35

Isolate Group: 376-1

Card Type: GN Testing Instrument: 00001924314A (17164)

Bionumber: 0405610570426210
Organism Quantity:

Comments:	

Identification Information	Card:	GN	Lot Number:	2410468203	Expires:	Mar 9, 2019 12:00 CST
	Completed:	Nov 2, 2018 16:13 CDT	Status:	Final	Analysis Time:	5.00 hours
Selected Organism	96% Probability		Escherichia coli			
	Bionumber:	0405610570426210			Confidence:	Excellent identification
SRF Organism						

Analysis Organisms and Tests to Separate:

Analysis Messages:

Contraindicating Typical Biopattern(s)
Escherichia coli dTAG(22),PHOS(61),

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	-			

Installed VITEK 2 Systems Version: 07.01

الخلاصة

اجريت هذه الدراسة لتحديد انتشار انزيمات البيتا لاكتام واسعة الطيف بواسطة الطرق المظهرية والجزيئية لبكتريا الاشيريشيا القولونية *Escherichia coli* المعزولة من حالات سريرية مختلفة (ادرار، دم، سائل منوي، مسحات جروح) إذ أخذت هذه العينات من المستشفيات الرئيسية لمحافظة ميسان (مستشفى الصدر التعليمي، مستشفى الزهراوي الجراحي ومستشفى الطفل والولادة) واستمر جمع العينات من بداية شهر تشرين الاول الى نهاية شهر كانون الاول (2018).

جمعت 291 عينة من حالات سريرية مختلفة زرعت هذه العينات على اوساط (الدم والماكونكي الصلب) كعزل اولي حيث تم بعد ذلك زرع العينات على اوساط اختيارية أخرى مثل وسط الكروم آكار والايوسين مثيلين الازرق، بعدها اجريت الفحوصات المجهرية والمظهرية والاختبارات الكيموحيوية وأستخدم API ونظام الفايتك (Vitek System) لتأكيد التشخيص النهائي للعينات. أما فحص حساسية المضادات لهذه البكتريا فقد تم باستخدام طريقة أنتشار الاقراص. علاوة على ذلك ، تم استخدام اختبار الغربلة واختبار تآزر الاقراص المزدوج (DDST) لتحديد الاشيريشيا القولونية المنتجة لانزيمات البيتا لاكتام واسعة الطيف.

بينت النتائج ان الزرع الموجب ظهر في 235 عينة موزعة بين 138 عينة ادرار،28 سائل منوي، 30 مسحات جروح، 39 عينة دم. حيث تم تشخيص 105 عزلة منها على انها اشيريشيا قولونية. وكانت نتيجة العينات المنتجة لأنزيمات البيتالاكتام لفحص الغربلة هي (82.9%)87 أما نتيجة فحص التآزر كانت (21.8%) 19.

وأوضحت نتائج فحص الحساسية للمضادات الحيوية ان العينات كانت 100% حساسة لمضاد imipenem ويليه amikacin بنسبة 90.5%، أما مضادات ampicillin, pipracillin, augmentin, oxacillin فكانت الأقل تأثيرا على البكتريا حيث كانت النتائج 96.2% , 98.1% , 90.5%, 93.3% على التوالي، بينما كانت نسبة المقاومة للسيفالوسبورينات Cefotaxime, ceftazidime, cefpodoxime ,ceftriaxone, cefipeme ,cefoxitin هي 94.3% , 86.7%, 92.4% , 87.6% , 93.3%, 93.3% وعلى التوالي. بينما اظهرت المضادات الاخرى درجات متفاوتة من المقاومة وهي 34.3%, 35.2%, 46.7%, 53.3%, 68.6%, 81.9% لل aztreonam, trimethoprim/sulphamethoxazole, gentamycin, norfloxacin, nitrofuranition and ciprofloxacin على التوالي.

خضعت كل العزلات المعزولة للفحص الجزيئي لتحديد بعض الجينات التي تشفر لمقاومة المضادات الحياتية المحمولة على الكروموسوم و البلازميد مثل TEM, CTX-M, OXA, SHV, حيث أثبتت

الدراسة ان جينات bla_{TEM}, bla_{CTX-M}, bla_{OXA} محمولة على الكروموسوم بنسبة 100% اما جين bla_{SHV} كانت نسبته 45.7%. أما على البلازميد كانت نسبة وجود جينات bla_{TEM}, bla_{CTX-M} 100% ونسبةbla_{OXA} هي 99.05%، بينما كان جين bla_{SHV} محمول على البلازميد بنسبة 41%.

أظهرت الدراسة الحالية أن هنالك أنتشار واسع لمقاومة المضادات الحيوية وخصوصا لمضادات البيتالاكتام التي تتوسطها أنزيمات البيتالاكتاميز واسعة الطيف التي أكدتها فحوصات الغربلة، أضافة الى ذلك الدراسة الجزيئية بينت مستويات عالية من جينات المقاومة للمضادات الحيوية واسعة الطيف متمثلة بجينات bla_{CTX-M}, bla_{OXA}, bla_{TEM} بغض النظر عن أصل تشفيرها.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

دراسة مظهرية وجزيئية لبعض جينات البيتالاكتاميز واسعة الطيف لبكتريا الاشريشيا القولونية المعزولة من حالات سريرية في محافظة ميسان/العراق

رسالة مقدمة الى

مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قِبل

رباب نعيم علك

بكالوريوس علوم حياة/ الجامعة المستنصرية

(2004)

بإشراف

أ.م.د. زاهد سعدون عزيز

محرم 1441 هـ

أيلول 2019م