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Molecular Detection of Transposable Elements Genes among Bacterial Isolates of Antibiotic Resistance

A Thesis

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By

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بسم الله الرحمن الرحيم

۞ وَيَرْزُقْهُ مِنْ حَيْثُ لَا يَحْتَسِبُ ۚ وَمَنْ يَتَوَكَّلْ عَلَى اللَّهِ فَهُوَ حَسْبُهُ ۚ إِنَّ اللَّهَ بَالِغُ أَمْرِهِ ۚ قَدْ جَعَلَ اللَّهُ لِكُلِّ شَيْءٍ قَدْرًا ۞

صدق الله العلي العظيم

الطلاق (٣)

Certification

I certify that the preparation of this thesis **(Molecular Studyof Transposable Elements Genes among Bacterial Isolates of Antibiotic Resistance)** was made by **(Ola Adnan Hamza Hassan)** under my supervision at the University of Babylon, College of Science, Department of Biology, as partial fulfillment of the requirements for the Degree of Master of Science Biology

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Date: / / 2023

Dedication

My Great parent …

My Wonderful sister…

My Dear Brothers…

My Hasband ,My Beautiful children:

Abdullah, Fatima

OLA

Acknowledgments

I would like to express my thanks to "**Allah**" the Most Gracious and Most Merciful, and to His prophet "**Mohammad**", God's Blessings and Peace Be Upon Mohammed and His Family for enabling me to complete this study.

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Summary

Escherichia coli and *Klebsiella spp*. It commonly spreads in the gastrointestinal tract of humans and animals and may reason opportunistic infections, especially virulent and multidrug-resistant strains that pose a severe public health risk. Tn7-like transposons play an vital role in spreading antibiotic resistance. This study aimed to investigate the molecular detection of Tn7-like transposons and their association with multidrug resistance in *Escherichia coli* and *Klebsiella* isolated from feces of healthy children, children with gastroenteritis, and patients with urinary tract infections.

The present study was conducted in Biotechnology and Genetic Engineering Laboratory at the University of Babylon. The study involved 240 stool specimens and 90 urine specimens collected from subjects (aged 1- 40 years) of both genders who attended the hospitals of Hillah city and the Public Health Laboratory in Babylon, during a period that extended from November 2021 to January 2022. *Escherichia coli* and *Klebsiella* were isolated from the specimens and diagnosed according to Cultural, Microbial and Biochemical characteristics. Also, they confirmed by a VITEK® 2 system and 16S rRNA sequence. The antibiotic susceptibility tested by Kirby-Bauer disc diffusion technique. Tn7-like transposons and *intI2* genes were identified by polymerase chain reaction (PCR) - sequencing , then phylogenetic tree contracted.

The results showed that *Escherichia coli* of the intestinal microbiota strains were isolated from 60% of healthy stool specimens and the rest samples (15%) harbor a normal *Klebsiella spp*. Whereas the percentage of isolation of *E. coli* and *Klebsiella spp* from the patient stool with gastroenteritis was 9% and 5% respectively and the rest samples (86%) harbor other pathogenic bacteria or other pathogens. While the percentage of isolation of pathogenic *E. coli* and *Klebsiella spp*. from the patient urine with UTI was 35.56% and 11.11% respectively, and the rest samples (53.33%) contained other pathogens which were excluded.

The isolates of *E. coli* and *Klebsiella spp*. appeared to have varying abilities to resist the studied antimicrobials, including 14 antibiotics belonging to nine different classes that have different patterns or mechanisms in stopping the growth or killing of microorganisms. All bacterial isolates showed high antimicrobial resistance to almost all antibiotics except for carbapenems. About 72% of the isolates were multidrug resistant (MDR) because they appeared to be resistant to at minimum three antibiotic classes. Only four isolates of *E. coli* were sensitive to all antibiotics, including two (8.3%) isolates that were recovered from healthy stool specimens and the other two (6.25%) isolates from urine. In spite of both species were isolated from healthy people, they showed high resistance to antibiotics and one of them (*E. coli*) resist to eight antimicrobial classes, which indicates the ability of normal flora to receive antimicrobial resistance genes from other resistance bacteria by genetic transfer such as transformation, transduction or conjugation in intestine. Both bacterial species (74 isolates of *E. coli* and 26 isolates of *Klebsiella spp*.) had the greatest rates of drug resistance, but *E. coli* isolates were more resistance than

Klebsiella with Amoxiclline-clavulanate (70.58%), Cefotaxime (58.96%) and Ceftazidime (57.81%) . although the lowest resistance frequency was meropenem (4.86%), and all isolates were sensitive to imipenem (100%). About 75.68% of *E. coli* isolates were MDR-phenotypes and 61.53 % MDR- phenotype of *Klebsiella* and the rest isolates were non-MDR phenotype, and most MDR- bacteria isolated from urine specimens. Overall, compared to non-MDR strains and MDR bacteria were substantially more likely to exhibit beta-lactam resistance. Amoxiclline-clavulanate ($P < 0.002$), Ceftazidime ($P <0.014$), Piperacilline ($P <0.028$) and other antibiotic classes such as macrolids (azithromycin: $P <0.001$), quinolones/ fluoroquinolones (ciprofloxacin and levofloxacin),tetracyclines and chloramphenicol ($P<0.005$).

The presence of Tn7 is associated with multidrug resistance of the studied bacteria ($P < 0.001$; Fishers exact test). which revealed most MDR *E. coli* and *Klebsiella* were harbor different patterns of Tn 7 like transposons and intI2 gene. There are correlation between the presence Tn7 like transposon and class 2 integron with the studied antibiotic resistances ($P < 0.029$). This study also detected a considerable correlation between class 2 integron and resistance to Amoxiclline-clavulanate (AMC), Piperacilline (PRL), Cefotaxime (CTX), Gentamicin (CN), Azithromycin (AZM), Nitrofurantoin (NIT) ($P < 0.05$).

The molecular analysis appeared there were eight patterns of Tn7 like transposons, five patterns of Tn7 like transposon, including *(tnsABC, tnsAB ,tnsAC, tnsA ,tnsB)* distributed among 62% of total isolates, whereas 24% of total isolates were negative which were not detected the presence of Tn7 like transposon nor *intI2* gene. These

patterns are distributed among *E. coli* and *Klebsiella* isolates. Generally, *E. coli* and *Klebsiella* isolates harbored different Tn7 like transposons (complete or truncated) that were associated with the source of isolation (*P* 0.029; Fishers exact test) and they related with pathogenic bacteria in spite of some normal flora harboring the mobile genetic elements. Some *E. coli* and *Klebsiella* isolates were harboring integron genes in addition to Tn7- transposition genes. These isolates appeared variable patterns of transposon genes., three of them associated with the presence of *intI2* gene, including (*tnsABC& intI2* , *tnsAB & intI2* , *tnsB& intI2).* The frequency of integrons of class 2 was estimated as 14% that associated with Tn7 like transposon for total studied isolates and as 18.42% for positive isolates that carried Tn7 genes.

Finally, it was observed These results partly explain the high prevalence of antibiotic resistance observed in Iraq due to drug misuse. Most of the bacterial strains were multidrug-resistant, and they spread more in pathogenic strains than in commensal strains. In this study appeared there is a correlation between the presence of Tn7 like transposon and class 2 integron with multidrug resistance of the studied bacteria.

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List of Abbreviations

Symbol	**Description**
AMR	Antimicrobial resistance
attTn7	attachment site
bp	Base Pair
BSI	Blood stream infections
β -Lactamase	Beta-Lactamase
BaCl2. 2H2O	Barium Chloride
CLSI	Clinical and Laboratory Standards Institute Instructions
°C	Degree Celsius
D.W	Distilled Water
ddH_2O	Deionized Sterile Distal Water
DNA	Deoxyribonucleic Acid

E.coli	*Escherichia coli*
EDTA	Ethylene diamine tetra acetic acid
EMB	Eosin Methylene Blue Agar
$ESBL_S$	Extended-spectrum β-lactamases
GI	gastrointestinal tract
h.	hour
In	Integrons
IS	Insertion sequence
K.	*Klebsiella*
MDR	Multiple Drugs Resistant
MGE	Mobile genetic element
MR-VP	Methyl Red-Vogues Proskauer
µm	Micrometers
NCBI	National Center For Biotechnology Information
NS	Normal Saline
OD	Optical Density
PAIs	Pathogenicity islands
PCR	Polymerase chain reaction
RND	Restanse nodulation divison
TBE	Tris-Borate EDTA
TEs	Transposable elements

T_m	Melting Temperature
TN7	The bacterial transposon
TniQ	larger family of proteins
tnsABCDE	Transposase Tn7 gene
UTI	Urinary Tract Infection
UV Light	Ultra Violet Light
WHO	World Health Organization
16SrRNA	16 Svedberg Ribosomal Ribonucleic Acid

Chapter One

Introduction

Introduction

Transposable elements (TEs) are mobile DNA sequences with the ability to change their positions within a genome. TEs and the associated transposase genes are the most abundant and ubiquitous genes in nature and are essential for adaptation and biological diversification (Fan *et al.*, 2019). One significant finding over the past 10 years is the appreciation of the dissemination of Tn7, and related elements called Tn7-like elements that contain homologs of the Tn7 transposition proteins, in highly diverged bacteria adapted to a remarkable number of different environments (He *et al*., 2020). Tn7 is typically large, often 14 kb in size (Benler *et al.*, 2021). However, comparative analysis of some derivatives of the Tn7, Tn7-like, shows that these elements are very diverse in terms of genetic structure and have accumulated several accessory resistance regions. The Tn7-like transposon , as an important mobile genetic element of transposons, transfers various resistance genes between bacteria through its transposase, promotes the horizontal spread of drug resistance in bacteria (He *et al*., 2020).

The sequences of the two ends of the Tn7 transposon are highly conserved, which are respectively transposition module and class 2 integron system. Transposition module encodes five proteins required for two transposition path-ways, *tnsA, tnsB, tnsC, tnsD, and tnsE* (*tnsABCDE*)(He *et al*., 2020). Tn*7* transposition proteins *tnsA* (required for second strand cleavage), *tnsB* (the transposase), *tnsC* (transposase accessory protein), *tnsD* (required for high frequency transposition to the preferred site downstream of *glmS*) and *tnsE* (required for broader dissemination)(Hamidian *et al*., 2021).

Integrons form a complex mobilome in the majority of environments and, in addition, they are capable of moving between species over evolutionary periods, and have a vast pool of new genes available whose functions are not still

transparent (Pormohammad *et al.*, 2019) .

Tn7-like transposons have been found in several species of the Enterobacterales such as *Escherichia coli. E. coli* is a facultative anaerobic, non-spore forming, Gram-negative bacteria within the Enterobacteriaceae family. It forms part of the humans and warm-blooded animals natural gastrointestinal flora (Aijuka *et al*., 2019). Although most *E. coli* are harmless commensal organisms several pathogenic strains can cause a variety of diseases (Meredith *et al*., 2022).

Klebsiella is a Gram-negative, encapsulated, non-motile bacterium found in the environment and has been associated with pneumonia in patient populations with alcohol use disorder or diabetes mellitus. The bacterium typically colonizes human mucosal surfaces of the oropharynx and gastrointestinal (GI) tract. Once the bacterium enters the body, it can display high degrees of virulence and antibiotic resistance (Jondle *et al.*, 2018 ; Aghamohammad *et al*., 2020).

Besides their pathogenesis and virulence, *E. coli* strains also acquire antibiotic resistance over time. Mobile DNA elements, temperate bacteriophage, and transmissible plasmid have all served as carriers for antibiotic resistance genes in *E. coli* (Ghita *et al*., 2020). In fact, *E. coli* belongs to the group I enterobacteria, characterized by a phenotype naturally sensitive to all β-lactams. It is also naturally sensitive to other classes of antibiotics including aminoglycosides, quinolones, sulfonamides, trimethoprim, tetracycline, and chloramphenicol. However, this bacterium is a good example of antibiotic resistance evolution, and multidrug-resistant (MDR) strains are currently spreading in both community and hospital settings (Rozwadowski *et al*., 2022).

Antimicrobial resistance (AMR) is widely acknowledged as a major public health concern worldwide . Antibiotic-resistant *E. coli* and *Klebsiella pneumoniae* are among the most dangerous drug-resistant bacteria. Both species are members of the Enterobacterales family and are clinically important since they regularly

cause infections in individuals of all ages. Urinary tract infection, cholangitis, and sepsis are all infections caused by these bacteria. Several antibiotics have become more resistant to both species (Foudraine *et al*., 2021).

The aim of the present study:

Molecular detection of Tn7- like transposon and genotypic analysis in *E. coli and Klebsiella* isolated from healthy and patient with enterogastritis and urinary tract infections.

Through the following objectives:

1. Isolation and identification *E. coli and Klebsiella* from stool and urine of patients with urinary tract infections.

2. Determine antibiotics susceptibility pattern.

3. Molecular detection for Tn7- like transposon using specific primers for *tns A, tns B, tns C, and inl2* genes by PCR.

4. Detection of nucleotide heterogeneity for transposable elements .

Chapter Two

Literature Review

2.1 *Escherichia coli* and *Klebsiella*

The bacterium *Escherichia coli* was discovered by the German-Austrian pediatrician Dr. Theodor Escherich (1857–1911) in 1885 (Escherich, 1988). He conducted examinations of neonate's meconium and feces of breast-fed infants with the aim to gain insight into the development of intestinal "flora." In preparations of meconium and stool samples under the microscope, he observed "slender short rods" of the size of 1–5 μm in length and 0.3–0.4 μm in width, which he named Bacterium coli commune (Erjavec, 2019).

Escherichia coli is a member of the family Enterobacteriaceae, which are Gram- negative facultative anaerobic rods (possessing both a fermentative and respiratory metabolism) and do not produce the enzyme oxidase. They can be either motile or non-motile, and when motile produce lateral, rather than polar flagella. In addition to flagella, many strains produce fimbriae or pili, which are proteinaceous structures (or appendages or fibers) that extend outwards from the bacterial surface and play a role in attachment of the cells to other cells or to host tissues (Desmarchelier and Fegan, 2003).

Escherichia coli is commonly found in the lower intestine of warm-blooded organisms (endotherms) . Cells are typically rod- shaped, and are about 2.0 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm3 (Panchangam and Chandana, 2015). It is known to be a part of normal intestinal flora but can also be the cause of intestinal and extra intestinal illness in humans. There are hundreds of identified *E. coli* strains, resulting in a spectrum of disease from mild, self-limited gastroenteritis to renal failure and septic shock. Its virulence lends to *E. coli's* ability to evade host defenses and develop resistance to common antibiotics (Mueller, 2022).

Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora in addition these bacteria is one of the most common hospital-acquired pathogens which could cause urinary tract infections, abdominal infections, bloodstream infections (BSI), etc. (Li *et al*.,2021) .

However, there are some strains that have evolved into pathogenic *E. coli* by acquiring virulence factors through plasmids, transposons, bacteriophages, and/or pathogenicity islands. These pathogenic *E. coli* can be categorized based on serogroups, pathogenicity mechanisms, clinical symptoms, or virulence factors (Kaper *et al*., 2004).

Escherichia coli is classified taxonomically in the genus Escherichia (named after its discoverer Theodor Escherich), family Enterobacteriaceae, order Enterobacteriales, class Gammaproteobacteria, phylum Proteobacteria. Currently, the genus Escherichia comprises five recognized species: *E. albetii*, *E. coli* (the type species), *E. fergusonii*, *E. hermanii* and *E. vulneris*. Two former *Escherichia* species, *E. adecarboxylata*, and *E. blattae*, have been reclassified as *Leclercia adecarboxylata* and *Shimwellia blattae*, respectively (Schmidt, 2019).

The existing 16S rRNA gene databases provide (partial or complete) gene sequences for more than 1.7 million bacteria and archaea (Quast *et al*., 2013), and are detailed enough to classify bacteria at different taxonomic levels, from phylum (high taxonomic level) to genus (low taxonomic level). Yet, these databases contain unresolved information for some sequences, so species-level identification is not attainable for some microorganisms (Cole *et al*., 2009).

In 1882, Carl Friedlander first described *Klebsiella* as an encapsulated bacillus after isolating the bacterium from the lungs of those who had died from pneumonia. Originally named Friedlander's bacillus, it was not until 1886 when the bacterium garnered the name *Klebsiella* (Friedlander,1882). *Klebsiella spp.* are among the most frequently reported pathogens associated with the opportunistic infections in immunocompromised individuals and patients hospitalized for prolonged periods, and commonly implicated in nosocomial urinary tract infections and sepsis in infants from sub-Saharan Africa. Given the importance of *Klebsiella spp*. in nosocomial infection and the continuous increase and spread of community-acquired multi-drug resistant (MDR) *Klebsiella pneumoniae*, including Extended-Spectrum β-Lactamase (ESBL) producers, the

associated high mortality is causing a grave concern (Massinga *et al.*, 2021).

Klebsiella species are considered opportunistic pathogens colonizing mucosal surfaces without causing pathology; however, from mucosae *Klebsiella* may disseminate to other tissues causing life-threatening infections including pneumonia, UTIs, bloodstream infections and sepsis (Paczosa and Mecsas 2016).

Klebsiella pneumoniae infections are particularly a problem among neonates, elderly and immunocompromised individuals within the healthcare setting (Magill *et al*., 2014). This organism is also responsible for a significant number of community- acquired infections worldwide (Ko *et al*., 2002). Defining features of these infections are the ability to metastatic ally spread and their significant morbidity and mortality (Paczosa and Mecsas 2016). *Klebsiella* strains associated with these infections are regarded as hyper virulent, and recent epidemiological studies indicate that these strains share specific genetic characteristics (Holt *et al*., 2015).

Klebsiella is a non-spore-forming, non-motile, facultative anaerobic Gram-negative straight rod, 0.3–1.0 μm in diameter and 0.6–6.0 μm in length. The rods are arranged singly, in pairs or in short chains. The cells are capsulated. The optimal temperature for growth is 37°C. *Klebsiella sp*. are chemoorganotrophic, having both a respiratory and a fermentative type of metabolism. Glucose is fermented with the production of acid and gas (more CO_2 is produced than H_2). Most strains produce 2,3-butanediol as a major end product of glucose fermentation, whereas lactic acid, acetic acid and formic acid are formed in smaller amounts and ethanol in larger amounts than in typical mixed acid bacterial fermentations. No special growth factor requirements are known. Some strains have the ability to fix molecular nitrogen under anaerobic conditions (Bennett *et al*.,2019).

Klebsiella pneumoniae is resides in the mucosal surfaces of mammals and the environment (soil, water, etc.). In humans, *K. pneumoniae* colonizes the gastrointestinal tract and less frequently the nasopharynx, whence it gains entry to

the circulation and other tissues causing infection (Piperaki *et al*.,2017). The organism's polysaccharide capsule is the most important virulence factor and can prevent the host organism from developing opsonophagocytosis and bacterium eradication (Sun *et al*.,2020). *Klebsiella* is classified under the Enterobacteriaceae family which contained a large array of biochemically distinct genus, including the model organism *E. coli* and the notorious human pathogens *Salmonella, Yersinia, Serratia*, *Enterobacter*, *Citrobacter, Kluyvera, Leclercia, Raoultella, Cronobacter* ,…..etc (Figure 2-2) (Dong *et al*., 2022).

Klebsiella spp. are opportunistic pathogens which are normally found in the flora of healthy individuals' nose, throat, skin, and intestinal tract, but can also cause a range of infections, including pneumonia, soft tissue and surgical wound infections, urinary tract infections, bloodstream infections and sepsis (Holt *et al*., 2015). The *Klebsiella* genus comprises a wide diversity of species, including the *Klebsiella pneumoniae* species complex (KpSC) and several more genetically distant species (Wyres *et al*.,2020). A large proportion of infections caused by *Klebsiella* spp. are due to two major path types, namely the multidrug-resistant (MDR) and hyper virulent (hv) clones (Chen *et al*.,2018).

2.2 Antibiotic Susceptibility of *Escherichia coli* and *Klebsiella*

Antimicrobial resistance is a serious public health problem worldwide. Inappropriate use of antibiotics by humans, factories, and farms, poor hygiene and sanitation, and inefficient prevention and control of infections in health-care settings are considered important reasons in the emergence and distribution of antibiotic resistant bacteria (Bonnedahl *et al*., 2010; Yang *et al*., 2009; Boonyasiri *et al*., 2014). Extended-spectrum β-lactamases (ESBLs) are enzymes that confer resistance to most β-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. Infections with ESBL producing organisms have been associated with poor outcomes (Bryce *et al*., 2016; Bhoomika *et al*., 2016; Hashemi *et al*., 2018).

Escherichia coli is a commensal microorganism of the intestinal microbiota of healthy humans and animals, as well as an important opportunistic pathogen, which may be implicated in many types of infections. Intestinal bacteria (as is the case of *E. coli*) are exposed to the effect of antimicrobial agents used in humans and animals, and the emergence and dissemination of resistance could occur, with antimicrobial resistance bacteria (AMR) being disseminated in the environment (Vila *et al*., 2016; Jang *et al*., 2017) One of the most relevant mechanisms of resistance in *E. coli* that causes great clinical concern is the expression of extended-spectrum beta- lactamases (ESBLs), which confer resistance to penicillins, narrow and broad- spectrum cephalosporins (such as ceftazidime and cefotaxime), and monobactams (Canton *et al*., 2012).

Many of the genes encoding ESBLs are hosted on plasmids that facilitate the transfer among bacterial species (Alonso *et al*., 2017; Dandachi *et al*., 2018). Multiple genetic mechanisms are implicated in the acquisition and dissemination of AMR. The *E. coli* mobilome includes a variety of mobile and mobilizable genetic elements, including plasmids, transposons, insertion sequences, and integrons (*intI*) (Gillings, 2014). The latter are well known to be involved in the spread of antibiotic resistance, notably among Gram-negative bacteria. Integrons are genetic structures containing AMR genes in their variable region (as gene cassettes) and have been detected in poultry farms in different studies (Pérez-Etayo *et al*., 2018; Kalantari *et al*., 2021).

The Pathogenic bacteria *Klebsiella pneumoniae* are developing multidrug resistant (MDR) strains and commonly pose a serious threat to the patients because of an increased fatality rate due to the reduced effectiveness of therapy options. *K. pneumoniae* is known to be responsible for community acquired infections although recently it is routinely observed as a major cause of hospital acquired pathogens (Munita and Arias, 2016). *K. pneumoniae* has been observed to develop resistance to antibiotics more easily than most bacteria through the production of enzymes such as Extended Spectrum β- Lactamase (ESBLs) and

Carbapenemases (Bengoechea and Pessoa, 2019).

K. pneumoniae also plays an important role in spreading antimicrobial resistance genes from bacteria in the environment to clinically important bacteria (Wyres and Holt, 2018). There are multiple mechanisms of antimicrobial resistance which will negatively affect the therapeutic outcomes. The World Health Organization (WHO) declared antibiotic resistance as one of the three major problems in the world (Bengoechea and Pessoa, 2019).

The resistance mechanism of *K. pneumonia* mainly includes the production of Beta- lactamase, the lack of membrane porin proteins, and the active efflux of antibacterial drugs. Extended-spectrum Beta-lactamase (ESBL)-producing *K. pneumoniae* has a high degree of drug resistance, which can simultaneously present with multiple resistance mechanisms, often resulting in multidrug resistance (Chong *et al*., 2018). Carbapenem antibiotics are commonly used in the clinic for the ESBL-producing *K. pneumoniae*. However, their overuse has led to significant increases in *K. pneumonia* resistance rates in recent years (Hu *et al*., 2020).

2.3 Transmission of *Escherichia coli* and *Klebsiella*

The main transmission routes of *E. coli* can be explained by the figure (2-1)

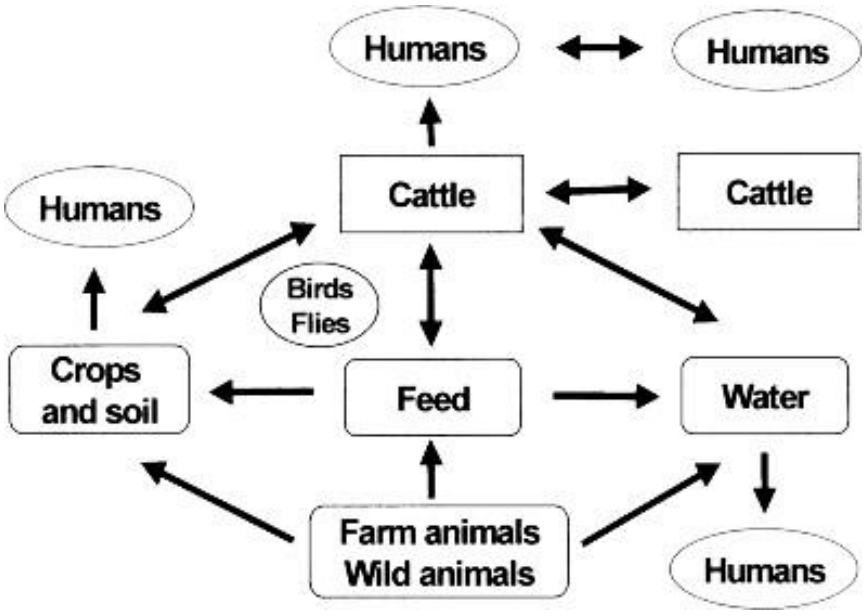


Figure 2-1: Transmission of *E. coli* (Bach *et al*.,2002)

Whereas, the main sources for *Klebsiella* transmission in hospitals are the gastrointestinal tract of patients and hands of the hospital staff and are contaminated by faulty hygiene procedures (Petrosillo *et al*., 2019). The capacity of the organism, particularly in neonatal units, to spread rapidly frequently causes nosocomial diseases (Ramos-Castañeda *et al*., 2018). In addition to being a potential source of autoinfection, the acquisition of a strain in the bowel during hospitalization provides a possible source for transmission of the organism. Person-to-person spread is the most common mode of transmission of *Klebsiella* species in hospital infections, and hands are the main vehicles for transmission. *Klebsiella* species are isolated from the hospital kitchens that prepared ice creams, nasogastric feeds, cold meat, and salads (Gundogan, 2014).

2.4 Pathogenicity of *Escherichia coli* and *Klebsiella*

Escherichia coli typically colonizes the gastrointestinal tract of human infants within a few hours after birth. Usually, *E. coli* and its human host coexist in good health and with mutual benefit for decades (Denamur *et al*., 2021). Based on this latter classification, *E. coli* can be distinguished into two main groups based on the ability to cause infection of the gastrointestinal system (Intestinal Pathogenic *Escherichia coli*, IPEC) or outside this (Extra intestinal Pathogenic *Escherichia coli*, ExPEC) (Who, 2020) (Figure 2-2).

Among IPEC, *E. coli* strains are categorized into the following path types: Enter pathogenic *E. coli* (EPEC), which causes diarrhea in children and animals; Enter hemorrhagic *E. coli* (EHEC) responsible for hemorrhagic colitis and hemolytic uremic syndrome (Dell'Orco *et al*., 2019); Enter toxigenic *E. coli* (ETEC), the main cause of traveler's diarrhea and porcine and bovine diarrhea (Manges *et al*., 2019); Enter aggregative *E. coli* (EAEC), that can cause persistent diarrhea in humans, and Diffusely adherent *E. coli* (DAEC), a subclass of Enter aggregative *E. coli* which causes diarrhea in children; Enter invasive *E. coli* (EIEC), which causes watery diarrhea and dysentery. EIEC and EAEC strains

were found only in humans and not in animals (Sora *et al*., 2021).

The ExPEC group incorporates the following variants: uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), those isolates responsible for septicemia (SEPEC), avian pathogenic *E. coli* (APEC) and mammary pathogenic *E. coli* (MPEC), (Kong *et al*., 2017). These path types are also isolated from animal diseases such as fatal pneumonia in pigs, mastitis in cows and pigs, plyometric, and urinary tract infections in dogs (Mü¸stak *et al*., 2015).

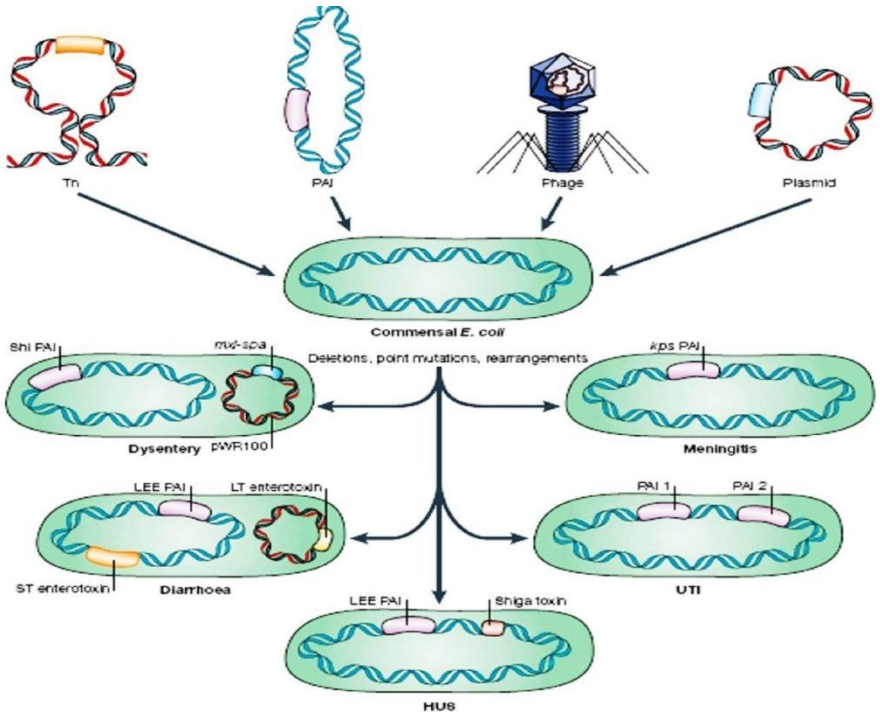


Figure 2-2: Contribution of mobile genetic elements to the evolution of pathogenic *E. coli* (Denamur *et al*., 2021)

Klebsiella sp. is one of the important pathogenic strain of MDR bacteria that is a Gram-negative bacterium associated with many infection Such as (HAI), community-acquired, urinary tract Urinary tract infections (UTIs) and wound infections. *K. pneumonia* can harbor and express beta lactamases, most importantly carbapenemases capable of hydrolyzing newer carbapenems drugs used in the treatment of MDR bacterial infections (Bassetti *et al*., 2018).

An array of virulent factors responsible for pathogenesis such as endotoxins, capsules, iron-scavenging systems, siderophores and adhesions can be expressed by *K. pneumoniae.* A capsule is a vital virulence factor, because it confers two pathogenic mechanisms; shielding the invading bacteria from phagocytosis, and neutralizing the host immune response (Ferreira *et al*., 2019). *Klebsiella* capsular serotyping (K typing) differentiates *K. pneumoniae* into approximately 77 K types . Several capsular (K) types, predominantly K1, K2, K54, K57, K20, and K5, are frequently linked to community-acquired invasive septicemia, pyogenic liver abscess syndrome and pneumonia . K3 is the usual cause of rhinoscleroma (Moradigaravand *et al*., 2017).

Klebsiella pneumoniae is an opportunistic pathogen that is widely found in the mouth, skin and intestines, as well as in hospital settings and medical devices. Opportunistic *K. pneumoniae* mostly affects those with compromised immune systems or who are weakened by other infections. Colonization of the GI tract by opportunistic *K. pneumoniae* generally occurs prior to the development of nosocomial infections, and *K. pneumoniae* colonization can be further found in the urinary tract, respiratory tract and blood (Munoz-Price *et al*.,2013).

Klebsiella pneumoniae biofilms that form on medical devices (e.g., catheters and endotracheal tubes) provide a significant source of infection in catheterized patients . Nosocomial infections caused by *K. pneumoniae* tend to be chronic due to the two following major reasons: *K. pneumoniae* biofilms formed in vivo protect the pathogen from attacks of the host immune responses and antibiotics; and nosocomial isolates of *K. pneumoniae* often display multidrug- resistance phenotypes that are commonly caused by the presence of extended- spectrum β-lactamases or carbapenemases, making it difficult to choose appropriate antibiotics for treatment (Li *et al*., 2014).

2.5 Mobile genetic elements

Mobile genetic element (MGE), also known as transposable element (TE), can be defined as repetitive DNA sequences able to move/transpose throughout their host genome. They were first discovered in maize by Barbara McClintock in the 1940s as controlling elements able to modify gene expression and change their location upon genomic stress, such as chromosomal double-strand breaks With the development of molecular biology and sequencing technologies, the detection of mobile genetic elements has been generalized to almost all living organisms (Mhiri *et al*., 2022).

Transposable element (TE) have served as a rich source of novel mutations on which selection can operate and have contributed to gene evolution and phenotypic diversification (Chuong *et al*., 2017). Despite this, changes induced by TEs are primarily neutral or deleterious to their hosts, and Test are kept under strict control by host immune systems. Overlapping transcriptional and post-transcriptional silencing mechanisms have evolved in plants and animals as layered defenses that have evolved to repress TE expression and amplification (Blumenstiel *et al*., 2014). Although this system is highly efficient and results in epigenetic silencing of most Test in most genomes, it is clear that Test can also undergo rapid increases in copy number, and currently or recently active Test have been identified in a wide variety of organisms (Sultana *et al*., 2017). DNA transposons transpose via a 'cut-and-paste' mechanism, in which an element is physically excised from one position and reintegrated at a second position. DNA transposons, require to integrate into the genome. Instead, a transposon-encoded protein called a transposase recognizes the terminal inverted repeats (TIRs) that flank the TE, excises the TE out of the donor position, and then integrates the transposon into the new acceptor site (Ochmann *et al*., 2021).

Insertion sequence (IS) elements are small (~0.7 to ~2.5 kbp), simplest type of mobile genetic elements are found in prokaryotic cells, and consist largely of a transposase gene surrounded by inverted repeats (Brkljacic *et al*., 2022).There is

tremendous variability in the types and copy numbers of the different IS element families that bacteria harbor. Both their presence and activities affect genome structure and gene expression, and they can thus impact fitness (Consuegra, *et al*., 2021). IS elements play a crucial role in mediating large DNA sequence variation in bacterial genome evolution and mutagenicity. Their mobility in the genome can lead to detrimental, advantageous or neutral effects on the bacteria fitness (Nzabarushimana and Tang, 2018).

Pathogenicity islands (PAIs) are distinct genetic elements on the chromosomes of a large number of bacterial pathogens. PAIs encode various virulence factors and are normally absent from non-pathogenic strains of the same or closely related species(Qayyum, 2019). PAIs carry genes encoding one or more virulence factors such as toxins, adhesions, invasions, iron uptake systems, and type III and IV protein secretion systems. These elements play a pivotal role in the virulence of bacterial pathogens of humans and are also essential for virulence in pathogens of animals and plants (Balasubramanian, *et al*., 2022).

Transposons a class of mobile genetic elements are widespread in nature, being present in virtually every organism examined. Indeed, the sequencing of the human genome has shown that 35–50% of the genome consists of transposable elements. Although some transposons might encode beneficial functions such as drug resistance or pathogenicity determinants in bacteria more often they seem to provide little discernible benefit to the host (Peters and Craig, 2001). A transposon is a discrete DNA segment that can move in a cell between sites that lack homology by using a self-encoded recombinase called a transposase (Minkley, 2018).

Integrons are genetic elements found in bacteria, which can capture and express antimicrobial resistance gene cassettes integrons are strongly associated with the dissemination of antimicrobial genes among different genera of bacteria several types of integron classes have been found nowadays in bacteria integrons

are often located on transmissible plasmids or transposons, which can facilitate the transferability of antimicrobial genes among bacteria integrons, especially class 1 and 2 ones, have a worldwide distribution in Enterobacteriaceae (Huang *et al*., 2020).

2.6 Tn7-like Transposon

Transposons are mobile elements that can move between positions in a genome. Transposon Tn7 and similar elements are common reservoirs for antibiotic resistance and pathogenesis functions in clinical settings, as well as encoding other adaptive functions in natural environments (Peters *et al*., 2014). The success of these elements likely stems from the amount of control they have over when and where transposition occurs. The Tn7 system has evolved mechanisms to almost completely avoid integrating into important host genes, but also maximize dispersal of the element by recognizing mobile plasmids and bacteriophage capable of moving Tn7 between host bacteria (Peters *et al*., 2017; Faure *et al.*, 2019).

The sequences in Tn7 on which the transposition machinery acts lie in the ends of Tn7. The necessary segment at the left end (Tn7-L) is about 150 base pairs prolong, and the necessary segment at the right end of Tn7 (Tn7-R) is about 90 bp long (Figure 2-3). Any piece of DNA flanked by these end segments can transpose in the presence of the Tn7 transposition proteins. Although the Tn7-L and Tn7-R segments are related, they are not identical, and Tn7 shows a preferential orientation of insertion at particular targets, which is dictated by the differences in the left and right ends of Tn7. Each end of Tn7 contains a series of 22-bp transposase-binding sites that are recognized by the Tn7 recombination machinery three non-overlapping binding sites in Tn7-L, and four overlapping binding sites in Tn7-R. In addition Tn7 also encodes an integron is a DNA segment containing several cassettes of antibiotic-resistance genes. Although these cassettes are fixed in Tn7 owing to a mutation of the cognate recombinase,

they can undergo rearrangements in hosts that express a related recombinase, leading to alternative combinations of antibiotic- resistance genes (Peters and Craig, 2001).

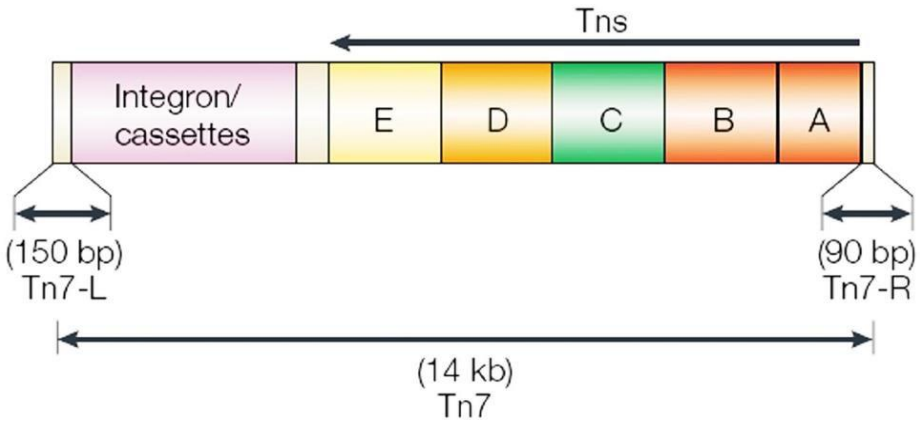


Figure 2-3 :The structure of Tn7 (Peters and Craig, 2001).

The bacterial transposon Tn7 and its relatives are distributing among bacteria in a wide variety of medical and environmental settings (Parks and Peters, 2007). Tn7 has served as a model system for transposition, especially for the understanding of transposon target-site selection (Craig *et al*., 2002). most transposable elements possess a weak target DNA sequence preference that guides target site selection, Tn7 uses two distinct target site selection pathways. In one pathway a sequence-specific DNA binding protein directs transposition into a single site within the bacterial chromosome and in the other a separate protein recognizes a process associated with DNA replication. These two target selection pathways optimize vertical and horizontal transmission of the transposable element, respectively (Parks and Peters, 2009). As mobile plasmids enter a new host cell, they replicate in a single direction by a discontinuous process, similar to lagging-strand DNA synthesis . In both mobile plasmids and in the chromosome, transposition events occur in a single orientation correlating with the direction of replication progression (Peters and Craig, 2001).

The bacterial transposon Tn7 is a particularly sophisticated mobile element, and it has developed several alternative lifestyles to promote its propagation.

In one transposition pathway, Tn7 resembles many other transposable elements in that it transposes to many different sites at low frequency. Although these sites are unrelated in their DNA sequence, there is a marked preference for Tn7 insertion into certain Replicons. Tn7 transposes preferentially to conjugal plasmids when they enter the cell (Figure 2-4). This preferential targeting to such plasmids by Tn7, and the generally broad host range of such plasmids, contributes to the dispersal of Tn7 among bacterial populations. Tn7, like many other bacterial transposons, carries genes that encode antibiotic resistance determinants. So, this plasmid-dispersal pathway can lead to the acquisition of antibiotic resistance by many different bacteria (Li *et al*., 2013).

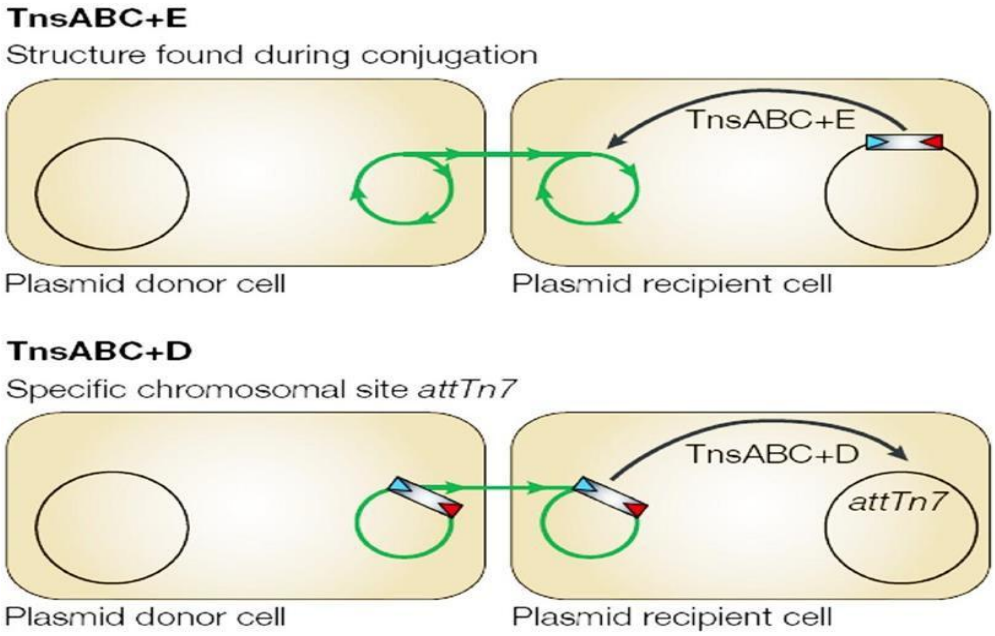


Figure 2-4:Tn7 has two pathways of transposition to facilitate its safe propagation in diverse hosts (Li *et al*.,2013).

Tn7 was discovered over four decades ago and it remains the prototype for the many Tn7-like elements (Peters *et al*., 2014). Bacterial transposons that use a recombinase from the retroviral integrase superfamily are common (Nowotny, 2009). The majority of these elements have a relatively simple make up, often only encoding the transposase needed for excision and integration at a

new position flanked by the cis- acting sequences it recognizes ,While these elements can have highly adapted mechanisms of regulation for when transposition occurs, they tend to move with little control over the target sites they choose (Craig *et al*., 2015). The transpososomes that promote transposition of the bacterial transposon Tn7 are particularly elaborate. Tn7 transposase genes (*tnsA, tnsB, tnsC, tnsD, and tnsE)* encodes five transposition proteins includes TnsABCDE (Li *et al*., 2011).

The TnsA protein, an endonuclease (encodes by *tnsA*), 273 amino acids, cleaves at the 5′ ends of the element allowing it to be completely excised from a donor DNA (Turlan and Chandler, 2000).

The TnsB (702 amino acids) is from the large family of bacterial transposase. TnsB with TnsA and a regulator protein TnsC form a core heteromeric transposase. The TnsB cleaves at the 3′ ends of the element and directly joins these strands to the target DNA (Turlan and Chandler, 2000). The TnsB is responsible for recognizing the cis-acting left and right ends of the element via a series of conserved TnsB-binding sites with Tn7, the heteromeric TnsA + TnsB transposase further coordinates with additional proteins allowing transposition to only occur when a suitable target site has been identified (Peters, 2019).

The TnsC as regulator protein (555 amino acids) is responsible for recruiting the element to the target site by interacting with the TnsA + TnsB transposase (Choi *et al*., 2013). Prototypic Tn7 has a second pathway of transposition capable of preferentially targeting transposition into mobile plasmids and filamentous bacteriophage, facilitating the spread of Tn7 between bacterial hosts (Finn *et al*., 2007). A regulator protein, TnsC, functions with different target site selecting proteins to recognize different targets, TnsC binds non-specifically to DNA in the presence of ATP ,TnsC is required for Tn7 transposition. The TnsC is an AAA protein that communicates to the TnsAB transposase that an appropriate insertion site has been recognized (Stellwagen and

Craig, 1998; Li *et al*., 2013). The TnsC is important for another regulatory role in which it inhibits transposition into a plasmid or a region of the chromosome that already has a copy of the element in a process called target immunity (Stellwagen and Craig, 1997; Skelding *et al*., 2003).

The TnsD, 508 amino acids, a member of the larger TniQ family of proteins (Fricker, 2015). The TnsD recruits TnsC, a AAA + ATPase (Li *et al*., 2013) to the attTn7 site using a combination of protein–protein interactions and a distortion imposed by TnsD-binding that is recognized by TnsC (Kuduvalli *et al*., 2001). Tn7 transposition will occur at a high frequency into a single site found in bacteria called its attachment site (attTn7) located downstream of the *glmS* gene, which encodes a highly conserved and essential protein .The attTn7 site is recognized by the Tn7- encoded sequence-specific DNA binding protein (Mitra *et al*., 2010).

The TnsE recognizes features of discontinuous DNA replication during conjugation, 3′ recessed ends (Parks *et al*., 2009). A structural analysis of the C-terminal portion of TnsE indicates that the protein has a completely novel DNA binding mechanism without discernable sequence or structural homology with known proteins, including TnsD (TniQ) (Shi *et al.*, 2015). The large family of Tn7 elements possessing TnsABCDE have diverged significantly and are found in a broad variety of bacteria which all appear to use the glmS attTn7 site as recognized by TnsD (TniQ) and presumably benefit from maximizing horizontal transfer via mobile plasmids targeted by TnsE (Peters *et al*., 2017).

Tn7 mediate transposition to two classes of target sites (Li *et al.*, 2011). TnsABC+D promote target site-specific insertion of Tn7 into its preferred chromosomal target site attTn7, whereas TnsABC+E promote Tn7 insertion into non- attTn7 sites on conjugal plasmids (Parks *et al*., 2009). Thus, TnsABC form the core of the transposition machinery. Although TnsABC alone do not promote transposition, transposition with TnsABC can occur when *tnsC* is activated by gain-of-function mutations that allow TnsC target binding and transposase

activation in the absence of TnsD or TnsE . In contrast, other characterized transposition systems involve only one or two transposition proteins (Craig et al.,2002), the TnsABC+E transposition shown in table 2-1.

Table 2-1: Tn7 proteins and their roles in transposition (Craig *et al.*, 2002)

protein	Function	Biochemical activities	Stricture homologues
TnsA	Transposase subunit	Cutting at the 5 end of Tn7	Type II restriction Enzyme
TnsB	Transposase subunit	Cutting and joining at th 3end of Tn7	Retroviral integrase And transposase DDE motif
TnsC	Regulator/connector	ATP-dependent DNA Binding and ATP hydrolysis	Nucleotide-binding Motif
TnsD	Target selection-recognition of att Tn7	Sequence-specific DNA binding	None
TnsE	Target selection-congeal Replication/lagging Strand DNA synthesis	Stricture-specific DNA Binding 3 recessed end	None

2.7 Integron with *Escherichia coli* and *Klebsiella*

Integrons are versatile genetic platforms involved with the acquisition, stockpiling, excision, and rearrangements of gene cassettes by site-specific recombination events mediated by integrase activity. Cassettes are circular mobilizable structures comprising a gene bounded to a cassette-associated recombination site, called *attC* (Figure 2-7). In general, genes included in cassettes are devoid of promoter sequences due to the proximity of their initiation codons and the 50 boundary of the cassette (Cambray *et al*., 2010). Several

classes of integrons have been recognized based on integrase protein sequences, which shared 45–59% amino acid identity. Three major integron classes (classes 1, 2, and 3) are featured by their strong link with antibiotic resistance gene cassettes and the emergence of resistance phenotypes and, therefore, these elements have been considered resistance (Fonseca and Vicente, 2022).

Class 2 integron is commonly found to be associated with the Tn7 transposon family (Tn7 and its derivatives, such as Tn1825, Tn1826 and Tn4132), carrying both of its recombination site *attI2* and promoter Pc found within such transposons (Labbate *et al*., 2009). Its 3′ conserved segment (3′-CS) contains 5 *tns* genes (*tnsA, tnsB, tnsC, tnsD and tnsE*) functioning in the movements of transposon, which mediates the mobility of class 2 integron via a preferential insertion into a unique site within bacterial chromosomes (Deng *et al*., 2015).

Commensal *E. coli* strains efficiently exchanging genetic materials with other bacteria such as *Shigella, Salmonella, Yersinia* and *Vibrio*, as well as pathogenic *E. coli* (Oluyege *et al*., 2015). Recently this exchange of many different and diverse genes responsible for antibiotic resistance have been correlated to genetic structures named integrons, that integrate and mobilize individual gene cassettes encoding antimicrobial resistance determinants (Cury *et al*., 2016). There are many classes of integron have been indicated and clarified by their respective integrase (*IntI)* genes. Class 2 integron *(IntI2*)were present among *Acinetobacter, Shigella, Escherichia coli* and *Salmonella* isolates (Mazurek *et al*., 2015) also may be detected in other bacteria in subsequent studies. The class 2 integron is related to the class 1 integron (46% amino acid identity) and both these integrons are also present in resistant intestinal *E. coli* isolated from different hosts in the community (Manal and Meraim, 2018).

Integrons which are one of the kind mobile genetic elements presumed to be involved in the dissemination of these MDR strains (Deng *et al*., 2015) Integrons are considered powerful mobile genetic elements that are located on plasmids,

transposons and pathogenicity islands which facilitate their transferring among different bacteria. According to reports available, integrons have a wide distribution among clinically isolated bacteria; also, their mobility has become a major problem in antibiotic resistance in clinical specimens (Firoozeh *et al*., 2019).

An integron, which can be located either on the bacterial chromosome or on a plasmid, includes the gene for an integrase site (*int*) and for an adjacent recombination site (*attI*) (Figure 2-7). So far three classes of antibiotic-resistance-encoding integrons have been identified. Each class has its own integrase. Among the antibiotic–resistance integrons, class 1 integrons are the most common integron type, class 2 integrons are embedded in Tn7-family transposons and only one example of a class 3 integron is known (Derakhshan *et al.*, 2014).

The acquisition of mobile genetic elements including plasmids, transposons, and integrons among Gram negative bacteria plays an important role in the development of antibiotic resistance (Tabar *et al*., 2016). Up to now, more than 9 classes of integrons have been identified based on the differences in the integrase gene in Gram- negative bacteria, but there are only four main classes associated with clinical isolates. Class I and Class II integrons, respectively, are the most common classes among clinical isolates (Stalder *et al* ., 2012). Integron of Class I carry more than 40 resistance genes related to resistance of aminoglycosides, chloramphenicol, beta- lactams, sulfonamides, macrolides and disinfectants. Integron of Class II has been found in Tn7 transposons and affiliated transposons. Since the integrons have promoter sequences, they can express the genes in the genetic cassettes. Therefore, the integrons classes especially class II can act as vector of gene expression and as a natural cloning system in *Klebsiella pneumoniae* isolates (Mirkalantari *et al*., 2017).

Class 1 integrons are the most prevalent and have been frequently reported in clinical isolates of Gram negative bacteria including *K. pneumoniae*. The structure of class 1 integrons is consisted of two conserved regions, including 3′

conserved segment (3′ CS) and 5′ conserved segmen (5′ CS), as well as internal gene cassettes that encode antimicrobial resistance genes (CLSI,2018). Class 2 integrons found sometimes and class 3 integrons are rarely documented in *K. pneumoniae* (Xu *et al.*,2017). Up to now, more than 130 different cassettes which confer resistance against a wide range of antibiotics including all ß- lactams, all aminoglycosides, quinolones, fluoroquinolones, macrolides, and many other antibiotics classes have been detected (Xu *et al.*, 2018).

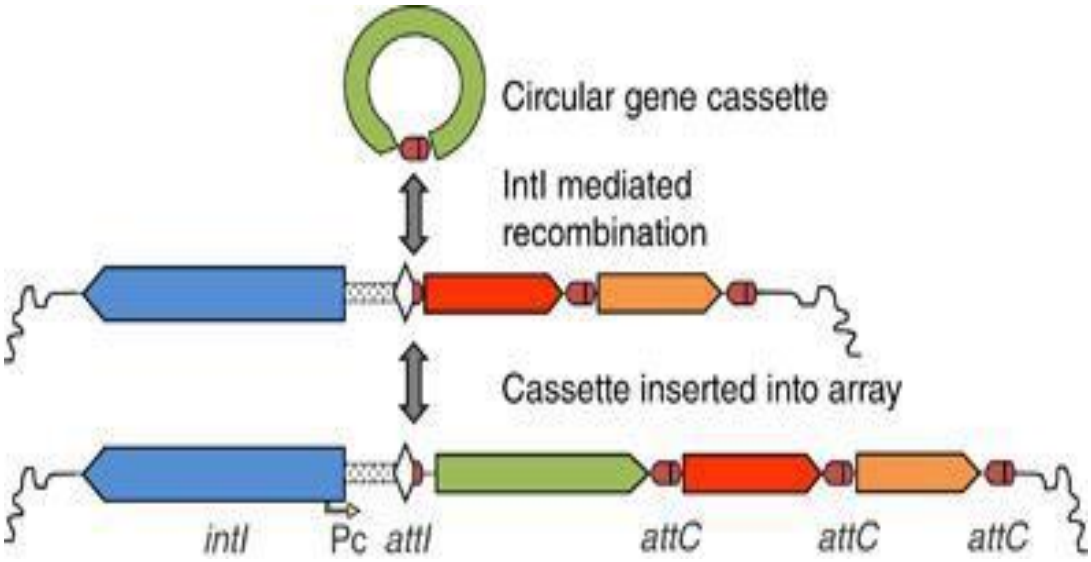


Figure 2-5: Integron structure and function (Gillings *et al.*,2015)

Chapter three

Materials and Methods

3.Materials and Methods

3.1Materials

3.1.1 Instrument and Equipment

The equipment and Instrument were used in this study are listed in table (3-1).

Table (3-1): The equipment and Instrument were used in this study

Apparatus and Tools	Manufacturer (Origin)
Autoclave	YX-280 B-China
Centrifuge	Hermle/Labor Technik–Germany
Eppendorf Centrifuge	Hettich /Germany
Eppendrof tube	Sigma /UK
Gradient PCR Thermal Cycler	Techne /UK
Horizontal gel electrophoresis System	Cleaver Scientific /UK
Incubator	Memmert/Germany
Laboratory Distillation Unit	GFL/Germany
Laminar Flow Hood	Cryste /Korea
Nanodrop	Implen (Germany)
Para film	Afco-Dispo /Jordan
PCR tube 1.5 ml and 0.2 ml	Biobasic /Canada
Refrigerator	LG/India
Sensitive Balance	Sartorius/Germany
Sterilized needles (5,3 ml)	Shanchuan /China
Swab	Lab. Servic /Spain
Tips	Sterellin Ltd /UK
Volumetric cylinder	HDA /China
Vortex	Griffin /England
Water bath	Memmert / Germany

3.1.2 Biological and Chemical Materials

The chemical materials, kits, media and antibiotic disks were used in this study are listed in the followed tables (3-2) and (3-3).

Table (3-2): Chemical Materials and molecular kits

Chemical Materials	**Manufacturer company (Origin)**
Agarose	Condalab /Spain
DNA Loading Buffer Blue	Eurx/Poland
Ethanol absolute	J.T. Baker /Netherland
Glycerol	Sigma/ USA
Gram stain 's	BHD /England
Hydrogen peroxide 3%	BHD /England
Normal Saline	Mehico /India
Nuclease Free Water	Bioneer /Korea
Simply Red Safe	Eurx /Poland
Tris-Borate-EDTA Buffer	Condalab /Spain
Culture Media	
Brain heart infusion broth	Biolife/ Italian
Eosin methylene blue	Biolife/ Italian
MacConkey agar	Biolife/ Italian
MR–VP broth	Biolife/ Italian
Muller Hinton agar	Biolife/ Italian
Nutrient agar	Biolife/ Italian
Nutrient broth	Biolife/ Italian
Peptone water	Biolife/ Italian
Simmons citrate Agar	Biolife/ Italian
Tryptic soy agar, Tryptic soy broth	Biolife/ Italian
Molecular Kits	
DNA extraction Kit	Favorgen /Taiwan
DNA ladder(1kb)	IntronBio /Korea
Green master mix	USA/ Promega
Primers	Macrogen /Korea

Table (3-3): Antibiotic Disks, Symbol, and Potency

Types of Antibiotics	Antibiotic disks	Symbol	Potency (µg/disk)	Manufacture (Origin)
Pencillins	Piperacilline	PRL	100	Bioanalysis/ Turkey
B-Lactamase inhibitor	Amoxicillin-clavulanate	AMC	30	Bioanalysis /Turkey
Extended Spectrum 3rd generation Cephalosporis	Ceftazidime Cefotaxime	CAZ CTX	30 30	Himedia/ India
Carbapenems	Imipenem Meropenem	IPM MEM	10 10	Bioanalysis/ Turkey
Aminoglycosides	Gentamicin Amikacin	CN AK	10 30	Bioanalysis/ Turkey
Macrolides	Azithromycin	AZM	15	Bioanalysis/ Turkey
Tetracyclines	Tetracycline	TE	30	Bioanalysis/ Turkey
Quinolones/ Fluoroquinolones	Ciprofloxacin-Levofloxacin	CIP LE	5 5	Himedia/ India
Chloramphenicol	Chloramphenicol	C	30	Himedia/ India
Nitrofurantoins	Nitrofurantoin	NIT	300	Biomaxima/ Poland

3.1.3 Primer Pairs

The primer pairs used in this study are listed in the table (3-4)

Table (3-4): Primer Pairs, Sequences, Product Size and References

Gene	Nucleotide sequence (5'→3')	Product size (bp)	Refer.
tnsA	F: TGGCTAACAGTACAAGAAGT R: CGCAACTCCTCCATATTCA	713	(He *et al.*, 2020)
tnsB	F: GGCTGAGTTGTTGCTAATG R: CCACCACATAAGACGGATT	845	(He *et al.*, 2020)
tnsC	F: TCGCATAATGGTTCGCTAA R: CTTGTCATCGTTGGATTCTG	893	(He *et al.*, 2020)
intI2	F: CACGGATATGCGACAAAAAGGT R: GTAGCAAACGAGTGACGAAATG	789	(Mazel *et al.*, 2000)
16S rDNA E.coli	F: GGATGACCAGCCACACTGGA R: CGCTTGCACCCTCCGTATTA	250	Designed

3.2 Study Design

The study design was showed in figure (3-1).

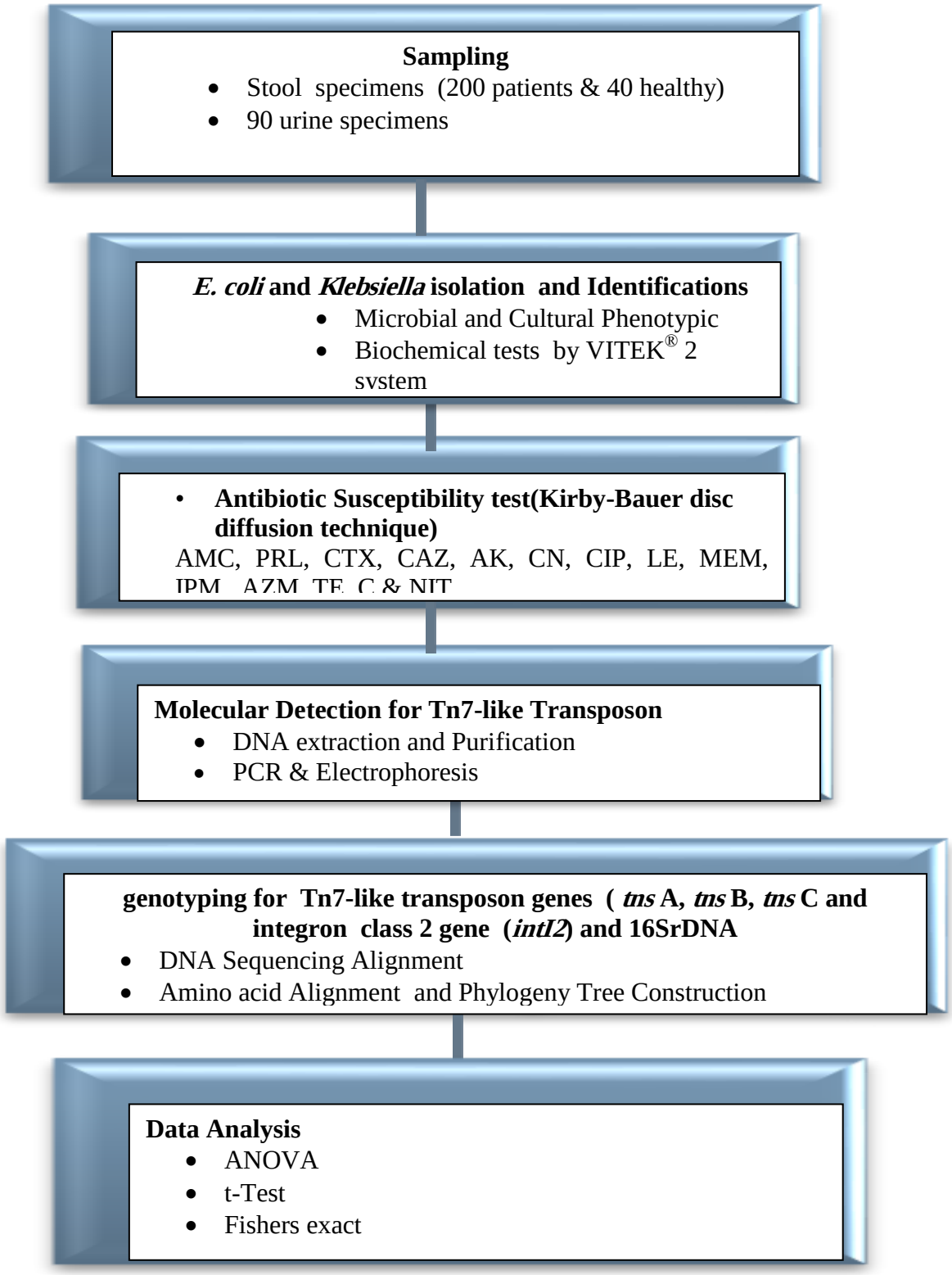


Figure (3-1): Flowchart of the study

3.3 Methods

3.3.1 Culture Media Preparations

The culture media used in the present study (Table 3-2) were prepared according to the Manufacturer's instructions. The media were sterilized by the autoclave at 121°C and 15 psi for 20 min.

3.3.2 Preparations of Solution and Buffers

The solutions and Buffers were sterilized at 121°C and 15 psi for 20 min by autoclave. or filtration (0.22 m Millipore filters). The base or acid solutions (1M NaOH or 1M HCl) were used to adjust the pH value of solutions before sterilization.

3.3.2.1 Normal Saline

Ready – use sterile normal saline (NS) was used for the preparation of bacterial suspension.

3.3.2.2 Turbidity Standard 0.5 McFarland's

The 0.5 McFarland standard tube (1.5×10^8 CFU/ml) was prepared by mixing 0.05 ml of 1.175% barium chloride ($BaCl_2.2H_2O$) with 9.95 ml of 1% sulfuric acid (H_2SO_4) in order to obtain precipitate of barium sulfate. The solution was used to compare the visible turbidity of bacterial suspension with the turbidity of the 0.5 McFarland's standard. The McFarland's standard tubes were tightly closed to prevent evaporation and stored in the dark at room temperature for 6 months. The accuracy of the prepared 0.5 McFarland's standard was verified using a spectrophotometer. The optical density (OD) should be between 0.08 and 0.1 when was measured at 625 nm (CLSI, 2019).

3.3.2.3 TBE Buffer

The Tris-Borate-EDTA (TBE) buffer was used in gel electrophoresis procedure for separating DNA molecules at the final concentration 1X TBE. Each 100ml of concentrated 10X TBE was diluted to 1X TBE by adding 900ml of sterile distal water (Green and Sambrook, 2012).

3.3.2.4 Loading Buffer

It was prepared by dissolving a Bromophenol blue (0.25%) and 40% Sucrose, then were stored at 4 °C (Sambrook and Rushell, 2001).

3.3.3 Specimens collection

The study included 200 stool specimens collected from the infants and children with gastroenteritis of both gender who attended the hospitals of the city of Hillah and the Public Health Laboratory in Babylon Province, during a period that extended from November 2021 to January 2022. Control group includes 40 stool specimens collected from healthy infants and children.

In addition to 90 urine specimens of patients with urinary tract infections of both genders who attended the hospitals of Hillah city.

3.3.4 Isolation and identification

Serial dilutions (10^{-3}) were performed for each stool specimen, and these dilutions were grown on MacConkey agar plates (a selective medium for isolation and identification of enteriobacterial isolates) and incubated at 37 °C for 24 h. The single pure pink (lactose-fermented) colonies were then selected to subculture on the same medium for further purification. Then pure fermented isolates were grown on Eosin methylene blue (EMB) plats and incubated at 37 °C for 24 h to identify *E. coli* and *Klebsiella* isolates. The urine specimens were precipitated by

centrifugation at 6000 rpm for 10 min. The precipitate was cultured by sterile swab on MacConkey agar plates and incubated at 37 °C for 24 h. After that the pure pink colonies cultured on EMB plats**.**

Bacterial isolates were examined microscopically after staining by Gram stain to observe the color, size, shape, and arrangement of bacterial cells under a light microscope (Tille, 2022).

3.3.5 Biochemical Characterization

3.3.5.1 Oxidase test

A filter paper was saturated with two or three drops of oxidase reagent and mixed with a fresh bacterial colony with a sterile woody stick. The positive result was indicated by formation of purple color within 10-15 sec (Tille, 2022).

3.3.5.2 Catalase Test

A single colony was transported by woody stick from a 18-24 h. old culture plate into a clean slide. A few drops of 3% hydrogen peroxide were dropped on colonies; the relief of oxygen bubbles designated the presence of catalase (Tille, 2022).

3.3.5.3 Indole test

This test was performed by growing bacterial inoculum in peptone water medium at 37°C for 24 h, then adding 1-2 drops of kovac's reagent to the broth. The presence of oily red layer (red-ring) at the top of broth indicates to a positive test (Tille, 2022).

3.3.5.4 Methyl red test

MR-VP broth media were inoculated with bacterial isolates and incubated at 37 °C for 48h. After that about 5 drops of methyl red reagent were added to bacterial broth, the change of color to red indicates a positive result, a negative result represent a yellow color (Tille, 2022).

3.3.5.5 Voges- Proskauer test

MR-VP broth media were inoculated with bacterial isolates and incubated at 37 °C for 48h. After that drops of alpha- naphthol and strong alkali solutions (40% KOH) were added to bacterial broth. The conversion of the broth to pinkish-red colure indicates to positive results, due to the ability of bacteria to produce acetyl methyl carbinol from glucose fermentation, a negative result was the yellow color (Tille, 2022).

3.3.5.6 Citrate utilization test

The Simmons citrate agar slants were inoculated with tested bacteria by stabbing in the bottom and streaking on the slant, and incubated in at 37 °C for 24h. The change of the medium colure from green to blue indicates a positive result (Tille, 2022).

3.3.5.7 Urease test

Tubes containing urea agar were inoculated by bacterial isolates and incubated at 37°C for 22 h, the medium color turns to Pink indicates a positive result due to the ability of bacteria to produce urease that split urea to ammonia and CO_2 (Tille, 2022).

3.3.6 Preservation of bacterial isolates

After the diagnosis conformation of *E.coli* and *Klebsiella* isolates by VITEK® 2 system, Bacterial isolates were preserved in three type of stocks includes daily -working stock for a short period on nutrient agar

plates and stored at 4 C° and used for few days. The second bacterial stock as medium-term preservation up to four weeks was carried out by growing the bacteria on tryptic soy agar slant and stored at 4 C°. Also, Bacterial isolates were stored in a brain heart infusion broth containing 15% glycerol in deep freezing at -20C° for long term preservation (Tille, 2022).

3.3.7 Antibiotic susceptibility test

The Kirby-Bauer method according to CLSI (2019) were used to test different antibiotic susceptibility (Table 3-3) as follows:

A few colonies from a pure and fresh culture of *E. coli* and *Klebsiella* were transferred to a sterile test tube containing 5 ml of normal saline and then it was compared with 0.5 McFarland standards (1.5×10^8 CFU/ml). A portion of bacterial suspension was transferred by a sterile cotton swab, a, carefully and consistently spread on Mueller-Hinton agar medium, then plates were left to dry. Antibiotic discs were positioned on the inoculated plate using a sterile forceps (duplicate was done for each antibiotic). Later the plates were incubated at 37ºC for 18-24 h . Depending on CLSI (2019), the inhibition zones around the disks were measured by millimeter (mm) using a metric ruler.

3.3.8 Genomic DNA extraction

The bacterial genomic DNA was extracted using FavorPrep™ Genomic DNA Extraction Mini Kit according to the manufacturer's protocol. As follow:

1. The bacterial culture of each isolate was activated in LB broth for 18 h until growth reached a density of 100 CFU/ml. Approximately 1 ml of the broth was transferred to a 1.5 ml centrifuge tube and bacterial cells were precipitated by centrifugation at 14,000 rpm for 2 min. The pellets were resuspended by vortexing or pipettes in 1 mL of normal saline to wash

bacterial cells and removing debris and then re-sedimented by centrifugation 14,000 rpm for 1–2 min.

2. The pellets were resuspended in 200 µl of FATG buffer by vortexing and incubating for 5 at room temperature and then in 70°C for 10 min. During incubation, the tube was inverted every 2- 3 min until the cell lysate became clear.
3. The elution buffer was pre-heated in water bath at 70°C which used in DNA elution step.
4. Up to 200 µl of 96-100% ethanol was added to the sample with vortexed for 10 sec, then the sample was mixed well by pipette to prevent any precipitate formed.
5. The FABG column was placed into the collection tube, then the sample mixture was carefully transferred to the FABG column, and centrifuged at 14,000 rpm for 5 min. The collection tube was discarded and the FABG column was placed in a new collection tube.
6. W1 buffer (400 µl) was added to the FABG column and centrifuged at 14,000 rpm for 30 sec. The flow-through was discarded and the FABG column returned to the collection tube.
7. The later step was repeated using 600 µl of ethanol-containing wash buffer instead of W1 buffer and centrifuged at 14,000 rpm for 30 sec. The flow-through was discarded and the FABG column returned to the collection tube.
8. The column was further centrifuged at 14,000 rpm for 3 min to discard any remaining liquid containing any inhibitors of subsequent enzymatic reactions.
9. The dry FABG column was placed into a new 1.5 ml microcentrifuge tube, and 100µl of preheated elution buffer or TE was added to the membrane center of FABG column to be completely absorbed.
10. The FAGB column was incubated at 37 °C for 10 min in an incubator .

Then it was centrifuged for 1 min at a maximum speed of 14,000 rpm to elute the DNA . If higher DNA yield were required, the DNA elution step was repeated for further DNA recovery and the total volume could be 200 µl.

11. Genomic DNA concentration and purity were measured by NanoDrop spectrophotometer , and then stored at −20 °C until use.

3.3.9 Detection of Tn7-like transposon by Polymerase Chain Reaction

3.3.9.1 Primer pairs preparation

Oligonucleotide primers *tnsA, tnsB, tnsC, intel2, 16S E.coli* (Table 3-4) were prepared by dissolving the lyophilized product by adding an appropriate amount of sterile ddH_2O depending on the instructions manufacturer to get 100 pmol/µl of the final concentration as a stock solution and stored in a deep freezer at -20 °C until use. The working primer solution was prepared by diluting the primer stock solution to 10 pmol /µl of the final concentration by adding 10 µl of the stock solution to 90 µl of ddH_2O and stored in a deep freeze(-20°C) until use.

3.3.9.2 Reaction mixture of PCR

DNA amplification was accomplished with a final volume of 25 µl of reaction mixture as listed in the table (3-5).

Table (3-5): Contents of the Reaction Mixture

Contents of reaction mixture	Volume (µl)
DNA template	4
Green master mix (Promega)	12.5
Downstream primer (10 pmol/µl)	2
Upstream primer (10 pmol/µl)	2
Nuclease free water	4.5
Total volume	25

3.3.9.3 Polymerase Chain Reaction (PCR)

Target DNA amplification was performed by conventional PCR using specific primer pairs for each studied gene previously mentioned in Table (3-4).The annealing temperature of used primer pairs were adjusted by using gradient PCR. The reaction was performed as three successive time steps including denaturation, annealing and extension as mentioned in Table (3-6). The amplified products were electrophoresed through an agarose gel at an appropriate time interval and were visualized and documented by the gel imaging system under UV light.

Table(3-6): PCR -Thermal Cycling conditions

Gene	Initial Denaturant. °C (min)	Denaturant. °C (sec)	Anneal. °C (sec)	Extension °C (sec)	Final Extension °C (min)	Cycles
tnsA	95 (2)	95 (30)	53.9 (30)	72(80)	72 (5)	35
tnsB	95(2)	95(30)	54.6(30)	72(90)	72(5)	35
tnsC	95(2)	95(30)	53.8(30)	72(80)	72(5)	35
intI2	95(2)	95(30)	58.1(30)	72(80)	72(5)	35
16s E.coli	95(2)	95(30)	60.3(30)	72(30)	72(5)	35

3.3.10 Agarose Gel Electrophoresis

The integrity of the DNA molecules was verified by migration in agarose gel electrophorese. Agarose gel (1-2%) was prepared by dissolving agarose powder in 1X TBE buffer solution in the microwave for 1-3 min until all agarose particles were dissolved and the gel became clear. The agarose solution was cooled to about 50°C. An safe red dye was added to a final concentration 0.5 µg/mL (usually 1–2 µl of laboratory stock solution per 100 ml gel) and mixed well. The agarose gel was cast as a horizontal plate. Plastic combs were used to create wells into which DNA would be loaded. Agarose was slowly poured into a gel tray with a fine comb in place. The gel is allowed to solidify and the gel tray is placed in a gel tank and filled with 1X TBE buffer. Prior to

loading, 5 µl of the DNA sample was mixed with 1 µl of loading dye weighting the sample into solution, to prevent the DNA sample from leaving the well, and also includes a visible marker (Bromophenol blue dye) to track the progress of the run. Unknown DNA samples were often laden with running along with the DNA ladder. The electrodes are properly connected and an electric field is applied along the gel (5 V/cm) or at 80-100 V for 40-60 min (Green and Sambrook, 2012).

3.3.11 DNA sequencing of Amplified product

The amplified DNA was re-extracted and purified by gel electrophoresis according to the protocol suggested by Macrogen sequencing corporation (Macrogen/Korea) and then submitted to this company for sequencing. The DNA sequence data of the studied genes were analyzed and aligned according to BioEdit and MEGA-X programs and compared with reference sequences available in the GenBank (NCBI) database for identification of polymorphisms and phylogenetic tree construction using neighbor-joining.

3.3.12 Statistical Analysis

Data for this study were statistically analyzed using SPSS 19 program by t-test, one-way ANOVA and Fishers exact test and the P value ($P \leq 0.05$) was statistically significant (Kirkpatrick and Brooke, 2015).

Chapter Four

Result and Discussion

4. Results and Discussion

4.1 Bacterial Isolation and Identification

Out of 200 stool specimens collected from infants and children with gastroenteritis were 18 (9%) positive for *E. coli* and 10 (5%) for *Klebsiella spp.* (Fig.4-1) and the rest of specimens were negative (86%) which includes other pathogens. The control group includes 24 (60%) *E. coli* and 6 (15%) *Klebsiella* spp. and excluded 25% of specimens. While the percentage of isolation of pathogenic *E. coli* from the patient urine with UTI was 35.56% (32 isolates) and 11.11% (10 isolates) of *Klebsiella spp*., and the rest specimens (53.33%) containing other pathogens which were excluded.

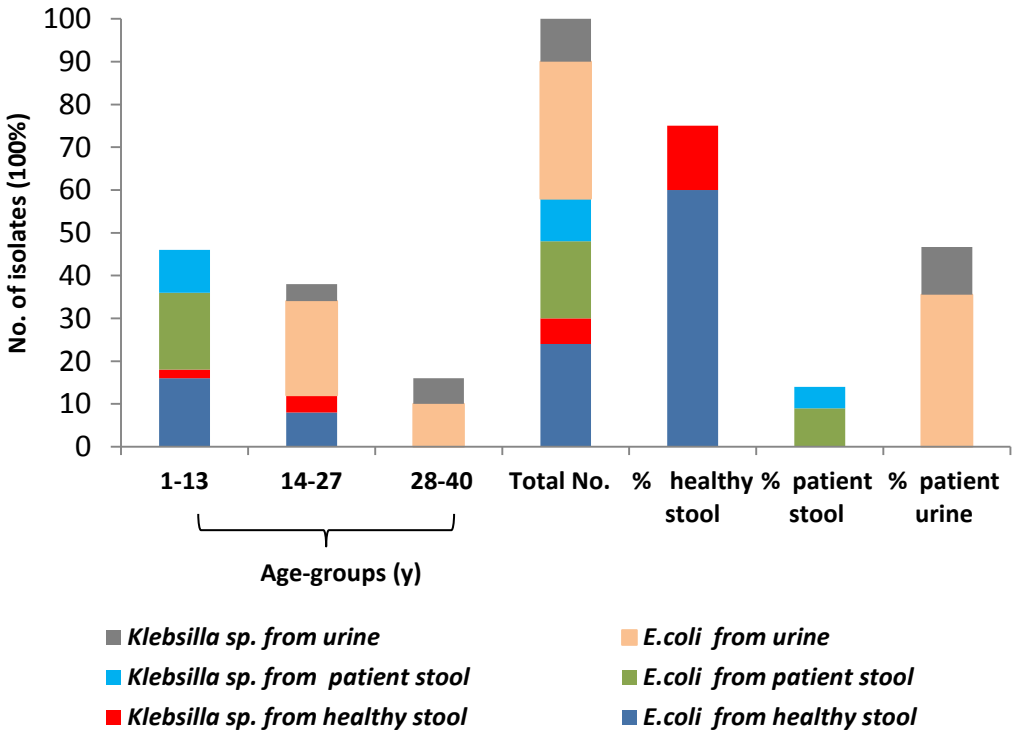


Figure (4-1): Percentage of total isolates number of *E. coli and Klebsiella spp.* isolated from different sources according age-groups.

The Enterobacterial colonies were isolated using Nutrient agar and MacConkey agar as a selective and differential medium for only cultivation Gram-negative bacterial species like Enterobacteriaceae members, which differentiated between lactose fermenter and non-lactose fermenter also containing bile salt and crystal violate which inhibits the growing of Gram-

positive bacterial species (Setia-Atmaja ,2009; Anderson, 2013). The single pure colonies of bacteria were primary identified according microbial, cultural and some biochemical characteristics as shown in the table (4-1) which appeared the main diagnosis features of *Escherichia coli* and *Klebsiella spp.*

The selected bacteria were Gram-negative coccobacilli or bacilli cell-shaped, non-spore forming and *Klebsiella* form polysaccharide capsules (encapsulated). When *E. coli* and *Klebsiella spp.* were cultured on MacConkey agar, they appeared variable features, including small moist red-pink colonies (2-3mm) due to strong lactose fermentation indicating to *E. coli* , but the mucoid to mucoviscus large colonies (3-10 mm) were *Klebsiella spp*. Whereas *E. coli* showed dark-blue-black colonies with green metallic sheening on EMB agar plates indicting strong lactose fermentation and acid production which precipitate the green metallic pigment (Fig. 4-2). Whereas *Klebsiella spp.* had brown with dark-centered, mucoid colonies on EMB agar plate, indicting fermentation of lactose and acid production (Collins *et al*., 2004; Madigan *et al*., 2006; Lal and Cheeptham, 2007). *E. coli* was motile but not *Klebsiella*. Urease production was positive for *Klebsiella* and negative for *E. coli* , in addition to other diagnosis characteristics as shown in the table (4-1).

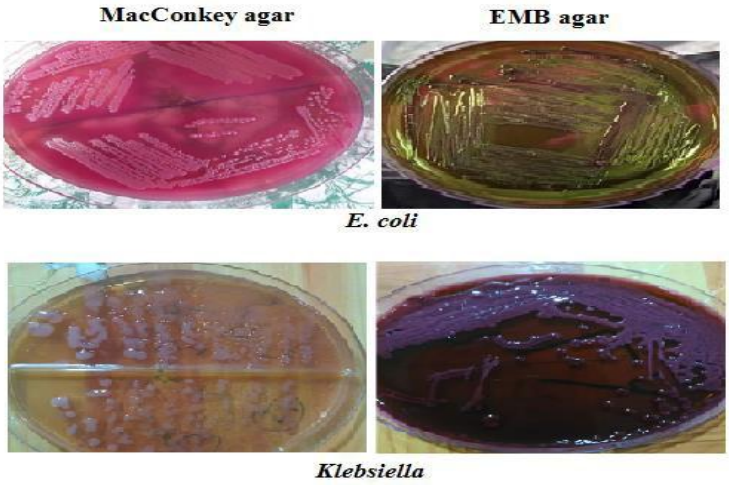


Figure (4-2): Morphological features of *E. coli* and *Klebsiella* colonies on MacConkey agar and EMB agar plates were grown at 37°C for 24 h.

Table (4-1): The main Microbial, cultural and biochemical characteristics of *E. coli* and *Klebsiella spp.* isolated from healthy and with Gastroenteritis and UTI.

Examination Tests	characteristics of isolated bacteria	
	E. coli	***Klebsiella***
Microscopic features	Gram negative short rod-shaped cells, non- spore forming, non- encapsulated under normal conditions and motile bacteria	Gram negative rod-shaped cells, non- spore forming, encapsulated and non-motile bacteria
Growth on MacConkey agar	Flat, moist, small red-pink colonies with a smooth edges.	Convex, mucoid - mucoviscus Large pink colonies with smooth edge.
Growth on EMB agar	dark-blue-black colonies with green metallic sheening color.	Brown with dark-centered mucoid colonies.
Lactose fermentation	Positive	Positive
Glucose fermentation	Positive	Positive
Acid production	Positive	Positive
Urease production	(Negative)	(Positive)
Indole test	Positive	Negative
Methyl red test	Positive	Negative
Voges-Proskaure test	Negative	Positive
Citrate utilization	Negative	Positive
Oxidase test	Negative	Negative
Catalase test	Positive	Positive
No. of isolates (%) from all samples	74 (74%)	26 (26%)

The most common facultative anaerobes of human coliform flora is *Escherichia coli*. Within hours of birth, the organism colonizes the infant's digestive tract, *E. coli* and other bacteria take over, and both host and guest benefit. *E. coli* is usually contained in a safe environment. However, in the case of weakened or immunosuppressed, this is not the case until typical hosts, or when the gastrointestinal barriers are breached. Non-pathogenic *E. coli* strains can cause infection, most feces diarrheal *Escherichia coli* should be cultured in cases of frequent diarrhea, especially in travelers and children conducted in cases of frequent diarrhea (Terlizzi *et al*., 2017).

The prevalence of urinary tract infections with *E. coli* was 76.19% (32/42) with a predominance of isolation in age group 14-27 years (Fig.4-1). *Klebsiella* was responsible for 23.81% (10/42) of UTIs. These results were consists with Mouanga *et al.* (2021) who found the prevalence of UTIs with *E. coli* was followed by *K. pneumoniae* was responsible of UTIs.

In the present study, a two-step procedure that combines phenotypic methods with molecular methods were used to identify the bacterial isolates. For isolates which give a reliable identification result by phenotypic identification procedures, sequence analyses are not required; and for isolates which are difficult to identify by conventional techniques, 16SrDNA sequencing is an effective means for identification. As the majority of isolates (around 90%) fall in the first category, costs will be kept to a minimum, allowing this technology to be within the reach of many microbiological laboratories.

Depending to phenotypic identification procedures, numbers of selected Enterobacterial species (74 isolates of *E. coli* and 26 isolates of *Klebsiella spp*.) were obtained from all studied samples , as shown in the figure (4-1). These isolates were confirmed their diagnosis using VITEK® 2 system.

The results of VITEK® 2 system revealed that 74 isolates of *E. coli* (98-99% probability), 24 isolates of *Klebsiella spp*. (97-99% probability) except two

isolates were confused to be *Enterobacter cloacae complex* (Appendix 1), including the isolates W4-19 (96% probability) and W1-21(88% probability) which isolated from healthy stool of adult persons. These isolates were later confirmed their identification using 16S rRNA gene sequencing and reconstructing phylogenetic trees.

4.2 Antimicrobial Susceptibility of *Escherichia coli* and *Klebsiella* Isolated from Different Sources

In the past decades, antimicrobial resistance has become a global threat to public health systems around the world. Among those bacteria that pose the greatest threat to human health due to their increasing resistance to antibiotics are members of the Enterobacteriaceae family, especially *E. coli* and *Klebsiella spp.* Among the different mechanisms of antibiotic resistance developed by bacteria, those in Enterobacteriaceae are more diverse than those in other families and include resistance to different antibiotic groups, advantages that partly explain why these microorganisms are among the most common causes of antibiotic- resistant bacteria in humans. In view of the ever-increasing number of infections caused by multidrug-resistant *E. coli* due to their ease of transmission via the fecal-oral route between humans and from environmental sources, understanding the epidemiology of these strains and their resistance mechanisms is an essential component in the fight against these infections. So the present study subjected to antimicrobial resistance of *E. coli* and *Klebsiella spp.* which were isolated from different sources like healthy and patient stool samples and urine of patients with UTI.

The results showed that both bacterial species (*E. coli* and *Klebsiella spp.*) have variable abilities to resist the studied antimicrobial drugs, including 14 antibiotics belonging to nine different classes (Table 4- 2) that have different patterns or mechanisms in stopping the growth or killing of microorganisms. All bacterial isolates revealed highly significant of antimicrobial resistance almost for all antibiotic except carbapenems, as shown the table (4- 2). About 72% of total

isolates were multidrug resistance (MDR) because they appeared resistant to at least three classes of antibiotics (Fig. 4-5). Only two *E. coli* isolate out of 24 isolates (8.3%) were recovered from healthy stool samples and 6.25% of *E. coli* isolates (2 isolates out of 32) which were obtained from urine samples were sensitive to all antibiotics. Although the *E. coli* and *Klebsiella* were isolated from stool samples of healthy people, they showed high resistance to antibiotics and one of them resist to eight antimicrobial classes, that indicates the ability of *E. coli* to acquire antibiotic resistance traits from other bacteria within its environment, and the gut microbiota of humans can contain over 1,000 different antibiotic resistance genes and the transmission of these traits between intestinal commensals is an ongoing phenomenon (Hu et al., 2013).

Out of the 100 isolates of *E. coli* and *Klebsiella* isolated from healthy persons and patients with gastroenteritis and UTI, the highest rates of antibiotic resistance were observed in *E. coli* isolates than *Klebsiella* with Amoxiclline-clavulanate (70.58%), Cefotaxime (58.96%) and Ceftazidime (57.81%) . while the lowest frequency of resistance was found with meropenem (4.86%), and all isolates were sensitive to imipenem (100%) as shown in the table (4-2). These results consist with previous study (Mouanga *et al.*, 2021) that found the resistance of *E. coli* higher than *K. pneumoniae* which isolated from patients with UTI were 44% for Amoxicillin-clavulanic acid and 33% for Cefotaxime and lowest resistance against Imipenem (2%).

Table (4-2): Proportion of *E. coli* and *Klebsiella* resistant to drugs which isolated from healthy and patients with Gastroenteritis and UTI

Antimicrobial categories	Drug	Breakpoints (mm)			% Resistance							*P*-value
					Healthy stool		Patient stool with Gastro-enteritis		Patient urine with UTI		Total	
		R ≤	I	S ≥	*E. coli* n=24	*Klebsiella a* n=6	*E. coli* n=18	*Klebsiella la* n=10	*E. coli* n=32	*Klebsiella a* n=10	n=100	
β-lactamase inhibitors/ Penicillin's	Amoxicillin-clavulanate (AMC, 30µg)	13	14-17	18	62.5	83.3	88.9	80	68.8	40	70.58	0.752
	Piperacilline (PRL,100µg)	13	14-16	17	50	IR	44.4	IR	12.5	IR	24	-
Extended Spectrum 3rd generation cephalosporins	Cefotaxime (CTX,30µg)	22	23-25	26	58.3	50	55.5	60	50	80	58.96	<0.001
	Ceftazidime (CAZ, 30µg)	17	18-20	21	58.3	33.3	77.8	80	37.5	60	57.81	<0.001
Aminoglycosides	Amikacin (AK, 30µg)	14	15-16	17	25	33.3	22.2	0	6.3	20	17.80	<0.001
	Gentamicin (CN, 10µg)	12	13-14	15	8.3	33.3	11.1	0	18.8	40	18.58	0.005
Quinolones/ Fluoroquinolones	Ciprofloxacin (CIP, 5µg)	21	22-25	26	41.7	0	11.1	40	37.5	20	25.05	0.846
	Levofloxacin (LE, 5µg)	16	17-20	21	16.7	0	22.2	40	18.8	60	26.28	0.025
Carbapenems	Meropenem (MEM, 10µg)	19	20-22	23	16.7	0	0	0	12.5	0	4.86	<0.001
	Imipenem (IPM, 10µg)	19	20-22	23	0	0	0	0	0	0	0.00	
Macrolids	Azithromycin (AZM, 15µg)	12	-	13	41.7	33.3	77.8	40	43.8	60	49.43	0.622
Tetracyclines	Tetracycline (TE, 30µg).	11	12-14	15	33.3	33.3	77.8	60	18.8	20	40.53	0.701
Chloramphenicol	Chloramphenicol icol (C, 30µg)	12	13-17	18	16.7	0	44.4	40	6.3	0	27.35	0.141
Nitrofurantoins	Nitrofurantoin n (NIT, 300µg)	14	15-16	17	33.3	33.3	11.1	0	25	0	17.11	0.057

* R, resistant; I, intermediate; S, susceptible; IR = inherently resistant; % = percentage. *P* value < 0.05 calculated for total *E. coli* and *Klebsiella* isolates

The results of the current study regarding to cephalosporins resistance were not agreed with the results of previous studies that found the resistance of *E. coli* against Ceftazidime and Cefotaxime about were variable according locations, time and source of isolations. At 2015, Lee *et al*. in North Korea found the resistance of *E coli* against Ceftazidime was 6.8% and 15.5% for Cefotaxime. While Kafilzadeh and Farsimadan (2016) found the resistance of Ceftazidime and Cefotaxime were 38.9 and 42.2% respectively. Whereas Maleki *et al.* (2016) in Iran reported the resistance of Ceftazidime and Cefotaxime were 26.1% and 30 % respectively. In Egypt, a study found the proportion of Cefotaxime and Ceftazidime resistances in *E. coli* strains reached 74.4% and 64.3 respectively (Hegazy *et al.*, 2018). And they thought the main reason of resistance of the two antibiotics may be due to the bacteria had efficient circulating pumps efflux systems which deliver antibiotics outside the bacterial cells. In addition to the most of pathogenic *E. coli* strains are characterized by their multiple resistance this is due to the transfer of resistance genes among species of the same or closely related genus from donor to the recipient cell (Doprint *et al.*, 2002).

The present study found the majority of bacterial strains were multidrug resistant (MDR) (72%) for at least three-classes, including 75.68% MDR-phenotype of *E. coli* and 61.53 % MDR- phenotype of *Klebsiella* and the rest isolates were non-MDR phenotype , in addition to two *E. coli* isolates were sensitive to all antibiotics under study as shown in the table (4-3). Overall, beta-lactam resistance was significantly more frequent in MDR strains compared to non-MDR strains for Amoxicillin- clavulanate ($p < 0.002$), Ceftazidime ($p < 0.014$), Piperacilline ($p < 0.028$) and other antibiotic classes such as macrolids (azithromycin: $p < 0.001$), quinolones/ fluoroquinolones (ciprofloxacin and levofloxacin), tetracyclines and chloramphenicol ($P < 0.005$).

Most studied isolates appeared multi drug resistant phenotype of *E. coli* and *Klebsiella* isolates, the percentage of MDR- phenotype in *E. coli* and *Klebsiella* which isolated from urine samples was higher than patient stools and healthy stool samples. That may be related to the type of isolation sources as indicated by the Fisher exact test <0.005 (Fig.4-3). These results consist with results of Abdu *et al*. (2018) and Mouanga *et al.* (2021).

The antibiotic resistance of *E. coli* and other bacteria due to bacterial cells had several mechanisms such as production of β-lactamase enzymes, including cephalosporins and penicillin's, which break down the β-lactam ring of penicillin's and cephalosporins, or change the target site of antibiotic by mutations, or decrease outer membrane permeability of bacterial cell, or by efflux pump systems or RND family efflux systems (AcrAB-ToIC, MdfA, YhiV), in addition to inhibiting DNA synthesis for tetracyclines, quinolones group and aminoglycosides, Nitrofurantoins resistances. (Platansing , 2015; Zaman *et al.* , 2017; Kapoor, 2017).

The development of antibacterial resistance in *E. coli* and other bacteria is multifactorial, but has accorded with the incorporation of these agents into the therapeutic resource of human and veterinary medicine. It is largely supposed that antibiotic resistance is the only consequence of human activity and chemotherapy with antibiotics. However, genomic studies of human microbiota and environmental bacteria have revealed large numbers of resistance determinants or genes within their genomes that were not obtained from horizontal transmission and preceded the clinical application of antibiotics in therapy (Sommer *et al.,* 2009). This type of antibiotic resistance is known as intrinsic resistance and provides selective benefit to the producing strains by inhibiting or eliminating other bacteria that compete for resources. Intrinsic resistance differs from the newly developed exogenous antibiotic resistance in that previously there is no contribution to human activities and the latter is mainly driven by antibiotic selection pressure (Cox and Wright, 2013).

Table (4-3): Resistance of *E. coli* and *Klebsiella* isolates to antibiotics

Antibiotics	*E. coli*			*Klebsiella*			*E. coli* & *Klebsiella*			*P** value	*P* value
	All isolates	MDR Status of Isolates		All isolates	MDR Status of Isolates		All isolates	MDR Status of Isolates			
	n=74	MDR* n=56 (75.68 %) n (%)	Non MDR n=18 (24.32 %) n (%)	n=26	MDR n=16 (61.53 %) n (%)	Non-MRD n=8 (30.77%) n (%)	n=100	MDR Isolates n=72 n (%)	Non-MDR n=26 n(%)		
Pencillins AMC	52 (70.27 %)	48 (85.71 %)	4 (22.22 %)	18 (69.23 %)	14 (87.50 %)	4 (50%)	70 (70 %)	62 (86.11 %)	8 (30.76 %)	**0.034**	**0.002**
PRL	34 (45.94 %)	34 (60.71 %)	0 (0%)	14 (77.78 %)	10 (62.50 %)	4 (50%)	48 (48 %)	44 (61.11 %)	4 (15.38 %)	0.140	**0.028**
Fluoroquinones LE	12 (16.21 %)	10 (17.85 %)	2 (11.11 %)	8 (30.76 %)	8 (50%)	0 (0%)	20 (20 %)	18 (25%)	2 (7.69%)	0.119	**0.037**
CIP	18 (24.32 %)	18 (32.14 %)	0(0%)	8 (30.76 %)	8(50%)	0 (0%)	26(26%)	26(36.11%)	0(0%)	**0.022**	**<0.001**
Cephalosporis CAZ	38 (51.35 %)	38 (67.85 %)	0(0%)	18 (69.23 %)	14 (87.50 %)	4 (50%)	56 (56 %)	52 (72.22 %)	4 (15.38 %)	0.094	**0.014**
CTX	36 (48.64 %)	36 (64.28 %)	0(0%)	20 (76.92 %)	14 (87.50 %)	6 (75%)	56 (56 %)	50 (69.44 %)	6 (23.07 %)	0.216	0.076
Tetracyclines TE	30 (40.54 %)	28 (50%)	2 (11.11 %)	10 (38.46 %)	8 (50%)	2 (25%)	40 (40 %)	36 (50%)	4 (15.38 %)	**0.022**	**<0.001**
Aminoglycosides AK	8 (10.81 %)	8 (14.28 %)	0 (0%)	8 (30.76 %)	6 (37.50 %)	2 (25%)	16 (16 %)	14 (19.44 %)	2 (7.69%)	0.257	**0.138**
CN	12 (16.21 %)	10 (17.85 %)	2 (11.11 %)	8 (30.76 %)	8 (50%)	0 (0%)	20 (20 %)	18 (25%)	2 (7.69%)	0.119	**0.037**
Macrolids AZM	40 (54.05 %)	36 (64.28 %)	4 (22.22 %)	12 (46.15 %)	12 (75%)	0 (0%)	52 (52 %	48 (66.67 %)	4 (15.38 %)	**0.021**	**<0.001**
Nitrofurantoins NIT	16 (21.62 %)	12 (21.42 %)	4 (22.22 %)	2 (7.69 %)	2 (12.50 %)	0 (0%)	18 (18 %)	14 (19.44 %)	4 (15.38 %)	0.337	0.250
Chloramphenicol C	12 (16.21 %)	12 (21.42 %)	0 (0%)	6 (23.07 %)	6 (37.50 %)	0 (0%)	18 (18 %)	18 (25%)	0 (0%)	**0.034**	**0.002**
Carbapenems MEM	4 (5.40%)	4 (7.14%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (4%)	4 (5.56 %)	0 (0%)	0.211	0.061

Notes: * MDR multidrug resistance at least 3 classes; Comparison of resistance rates between *E. coli* and *K. pneumoniae*. In bold: significant P-value (<0.05). Abbreviation: NS, not significant.

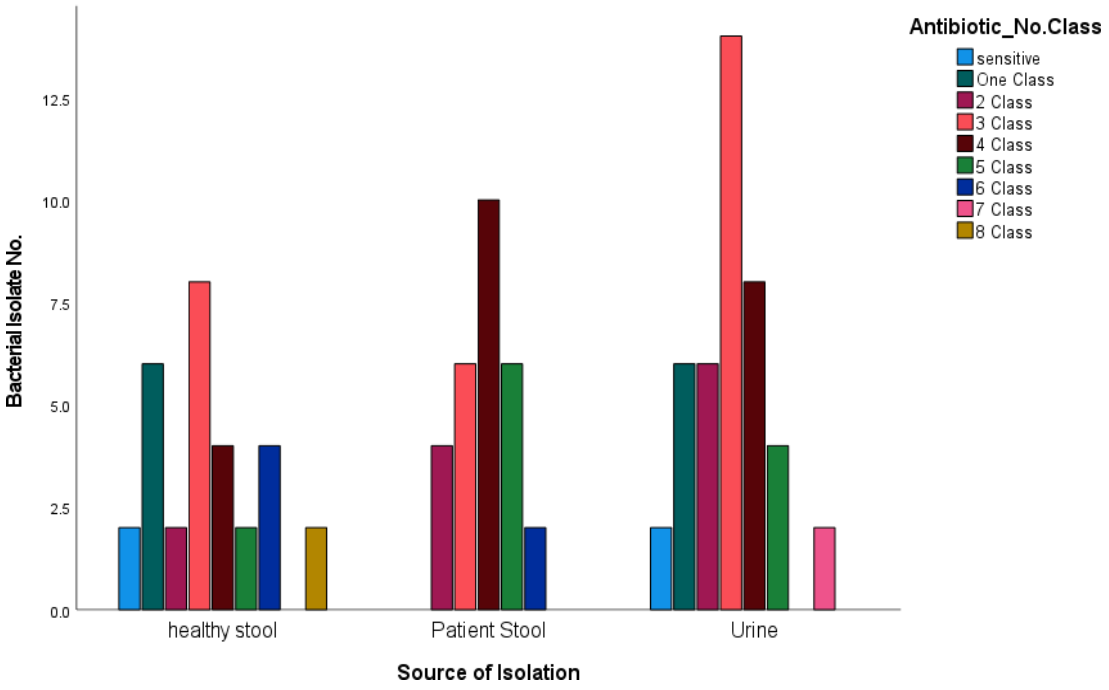


Figure (4-3): Total number of isolates resistant to drug classes according to isolation sources of health and patients with Gastroenteritis and UTI

Bacterial isolates are resistant to one or more up to 8 classes used in this study including, β-lactamase inhibitors/Penicillin's (AMC/PRL), Extended Spectrum 3rd generation cephalosporins (CTX, CAZ), Aminoglycosides (AK, CN), Quinolones/ Fluoroquinolones (CIP, LE), Carbapenems (MEM, IP), Macrolides (AZM), Tetracyclines (TE), Chloramphenicol's (C) and Nitrofurantoins (NIT).

Acquired or exogenous and ever-increasing resistance of *E. coli* to antibiotics is a major public health problem worldwide. In 2018, more than half of *E. coli* isolates reported to the European Center for Disease Prevention and Control were resistant to at least one group of antimicrobials under surveillance, and co-resistance to many antimicrobial groups was frequent (European Centre for Disease Prevention and Control, 2018); In the United States in 2017, the national prevalence of extended-release lactamases (ESBL)-producing strains of *E. coli* isolated from urinary tract infections (UTI) was 15.7%. In developing countries, the situation is getting worse, as reported by national surveillance data from Mexico, China and Turkey, where it has been

proven that resistant strains of *E. coli* spread more than 40% of the widely used cephalosporins and quinolones drugs all over the world for bacterial treatment infection, which agrees with the results of the present study. The present study revealed that the clinical isolates of *E. coli* and *Klebsiella* showed antibiotic resistance more than 57% for cephalosporins and 25% for quinolones. Also, resistance was high in the bacteria colonizing the intestines in healthy subjects as shown in the table (4-2).

Several human activities have been identified as major drivers of the current antibiotic resistance crisis, but the overuse of antibiotics has been shown to clearly influence the development of resistance (Read and Woods, 2014). The overuse of antibiotics are multifactorial and involve several aspects, including health, livestock, and pharmaceutical industries. Examples of these actions include unsuitable antibiotic prescribing by health care providers, extensive use of antibiotics in livestock and fish farming, patients not following antibiotic regimens, poor hygiene, bacterial mutations and lack of new antibiotics developed (WHO, 2020). In addition to global evidence indicates that elements in people's environment such as poor waste, non-potable drinking water, housing overcrowding and lack of hygiene, facilitate the development and transmission of resistant bacteria (Collignon *et al.*, 2018).

Unfortunately, overuse of these medications and inappropriate prescriptions are major contributors to this problem. In any antibiotic treatment against a bacterial infection, the sensitive bacteria will be killed; if pathogenic microorganisms are correctly targeted; However, along with infection with bacteria, the microbiota members of the individual, sensitive to the antibiotics used, will also be eliminated. In the case of resistant microorganisms, either belonging to the normal microorganisms or the target pathogenic microorganisms, these survivors will proliferate and become the dominant strain within the respective anatomical site.

Over prescribing of medicines is one of the most important factors contributing to the current antibacterial resistance crisis is the rapid evolution of bacteria under selective antibiotic pressure, as the continuous interaction between any given antibiotic and bacteria is an important aspect of increasing multidrug resistant strains (Kolar *et al.*,2001). An inappropriate treatment of acute respiratory infections with ciprofloxacin has led to development high rates of *E. coli* resistance worldwide (Cöplü, 2018; Shively et al., 2018; Garza-Gonzalez *et al*., 2019). Also, in Iraq the same circumstances, ciprofloxacin is prescribed to treat several infections such as urinary tract infection and acute respiratory infections that lead to a high rate of resistance against this agent in many pathogenic bacteria such as *E. coli* and *Klebsiella* (Table 4-2). In addition to the contribution of antibiotic abuse to resistance selection, Zhang *et al.* (2015) found epidemiological evidence that antibiotic resistance and virulence phenotypes of Diarrheagenic *E. coli* may be partially related. They found that people with diarrhea were using antibiotics more frequently before their symptoms appeared, an association that could be interpreted as antibiotics may disrupt the intestinal flora, allowing for the overgrowth of resistant pathogens.

Klein *et al.* (2018) found that worldwide antibiotic consumption increased significantly by about 39%, between 2000 and 2015. They also found that the average rate of antibiotic consumption was mainly by low and middle income earners in developing countries, that leads to highest rate of antibiotic resistance and prevalence of multidrug inflammatory strains. To make matters worse, consumption of antibiotics of last resort such as carbapenems and colistin is also on the rise, which is consistent with the emergence of strains of *E. coli* that are resistant to these agents.

Nordmann and Poirel, (2019) reported that resistance of *E. coli* to carbapenems is rare, with its prevalence depending on the region of the world under study, but does not exceed 3% and it was believed that in the future,

increased resistance to carbapenems. It can be observed in *E. coli*, since the enzymes responsible for its degradation, and thus its inactivation, carbapenemases, are primarily encoded on conjugative plasmids, and are highly transmissible (Nordmann and Poirel, 2013). This is consistent with the results of the current study, as it was found that the resistance against Meropenem had increased to more than 4.5% in *E. coli* which isolated from Iraqi people with UTI and healthy stool , as shown in Table (4-2).

Antibiotics are also used in livestock to treat clinical diseases and to prevent and control common diseases and to promote animal growth (McEwen and Fedorka-Cray, 2002). Unfortunately, this use of antibiotics has led to the spread and persistence of resistant bacteria in humans by two different mechanisms: (a) human ingestion of antibiotics via meat contaminated with antibiotics that enter the body and cause selective pressure on the host's microorganisms. and (b) resistant bacteria present in the intestines of food animals are transmitted to humans through contaminated meat. When livestock are treated or supplied with antibiotics, these agents exert the same selective pressure on their microorganisms as when humans ingest these drugs; Thus, overuse of antibiotics on food animals resulted in a high colonization rate of intestinal bacteria, including members of the Enterobacteriaceae family, such as *E. coli* and *Klebsiella spp.* , which have become resistant to various antimicrobials. Various studies around the world have shown that ready-to-eat animal products are contaminated with strains of *E. coli* that are resistant to different types of antibiotics, primarily β-lactam via bacterial production of extended-release β-lactamases (ESBL) (Kaesbohrer *et al.*,2019; Galindo-Méndez, 2019). Other studies found *E. coli* strains which isolated from meat have also shown resistance to other antibiotics, including species of last resort such as carbapenems (Köck *et al.*,2018) and colistin (Joshi *et al.*, 2019). If this undercooked contaminated meat was eaten by humans, it would likely colonize the gut, creating a reservoir for future antibiotic-resistant infections, such as

Ruppé *et al*(2013). showed that subjects with higher rates of intestinal colonization of ESBL-producing *E. coli* strains present a higher risk of urinary tract infection with these strains than patients without ESBL gut colonization (Ruppé *et al.*,2013). The human and animal intestines are a major reservoir of antibiotic-resistant *E. coli*, and elimination of these strains through the feces of colonized individuals, livestock, and pets allows access to humans via contaminated water and food. With the increase in the prevalence of fecal colonization by strains of *E. coli*, the number of human infections caused by them increases (Bourrel *et al.,* 2019; Merida-Vieyra *et al*., 2019).

The ability of *E. coli* to colonize various environments, including the intestines of humans and animals, has provided this organism with an evolutionary advantage to acquire antibiotic resistance traits from other bacteria within its environment, as well as their ease of transmission via feces-oral route. The gut microbiota of humans can contain over 1,000 different antibiotic resistance genes (Hu *et al.,* 2013). The transmission of these traits between intestinal commensals is an ongoing phenomenon. A major example of resistance gene transfer between environmental bacteria, including intestinal symbionts, and human pathogens is the *bla*CTX-M gene, which is the most prevalent ESBL gene in *E. coli* and *Klebsiella spp.* , and OXA-48-type. Carbapenems-hydrolyzing β- lactamase genes, which are increasingly reported in Enterobacteriaceae worldwide. The possible origin of the blaCTX-M genes has been identified in the chromosomal DNA of different environmental *Kluyvera* species (Canton and Coque, 2006), while the OXA-48 origin was found from the environmental waterborne *Shewanella* species (Poirel *et al.*, 2012).

Since many antibiotic resistance genes are associated with elements such as plasmids or transposons, and while the transfer of these elements may also occur through transformation or transduction, conjugation is often considered the mechanism most likely responsible for the transmission of these traits (von

Wintersdorff *et al.,* 2016). The above- mentioned ESBL and carbapenemases genes are prime examples of resistance genes with significant impact on human health that have spread among bacteria via plasmid conjugation. A study in China (Ye *et al.,* 2018) showed that transmission by conjugation of ESBL genes in *E. coli* occurs even in the food chain, a situation that partially explains the high fecal prevalence of ESBL-producing *E. coli* around the world.

However, due to the current globalization, resistant strains can easily be transmitted from one country to another. In a large cohort study of Dutch travelers to areas of the world with a high prevalence of ESBL- producing bacteria, 34.3% of people who were ESBL-negative prior to travel acquired these clones during their time abroad, and the largest number of acquisitions was among those who traveled to South Asia, and remained colonized for 12 months after his return (Arcilla *et al.,*2017). In addition, the same study showed that the estimated probability of transmission within families was 12%. Similar results were reported in a study in Spain, where up to 66% of isolates from patients with ESBL- producing *E. coli* infection were indistinguishable from those isolates from stool samples of their family members (Valverde *et al*., 2008). These results indicate that the acquisition of *E. coli*-resistant strains during travel is high and that transmission between family members can maintain these strains in the community for extended periods of time.

4.3 Molecular Detection of Tn7-Like Transposon

Mobile genetic elements facilitate horizontal transfer of genetic information between diverse organisms as a driving force in evolution. Transposons are motile genetic elements found as discrete DNA sequences capable of packing between loci in the genome. Bacterial Tn7 transposons and related elements in the Tn7-like family have a high degree of control over the selection of target sites for the transposition. To detect the presence of Tn7

transposition genes or related family using PCR technique must be extracted genomic DNA. The genomic DNA (Fig.4-4) was extracted from the bacterial samples as a first step to amplify the target region of studied genes such as Tn7-like transposon genes *tns*A, *tns*B, *tns*C, *tns*D & *tns*E which encoded to Tn7 transposase, integron class 2 (*intI*2) gene. and 16S rRNA gene of Enterobacterial isolates (taxid:543) such as *E. coli* and *Klebsiella*.

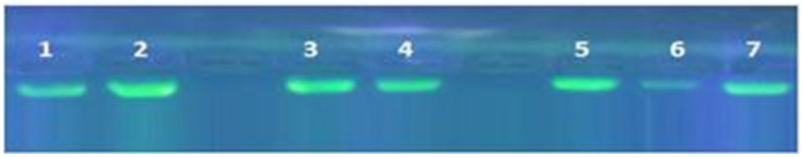


Fig. (4- 4): Agarose gel electrophoresis pattern of genomic DNA extracted from *E. coli* and *Klebsiella* isolated from stool and urine samples.

Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h (5 µl in each well), Staining method; precast RED Safe stain.(1-4 *E.coli*, 5-7 *Klebsiella*).

PCR and genetic sequencing methods were used to recognize some selected genes of Tn7-like transposon, relying on the partial sequences of *tns*A, *tns*B, *tns* C and *intI*2 genes, this is based on specific primes of these genes**.** After that reconstructing phylogenetic trees.

The genomic DNA of bacterial isolates were amplified and accomplished by the Thermo-cycler device under the optimal conditions as mentioned in the table (3-3). The results revealed that the presence a single band of each tested gene in agarose gel as shown in figure (4-5), that appeared the PCR-products of the Tn7 transposition genes including *tns*A gene (713bp), *tns*B gene (845bp) and *tns*C (893bp).

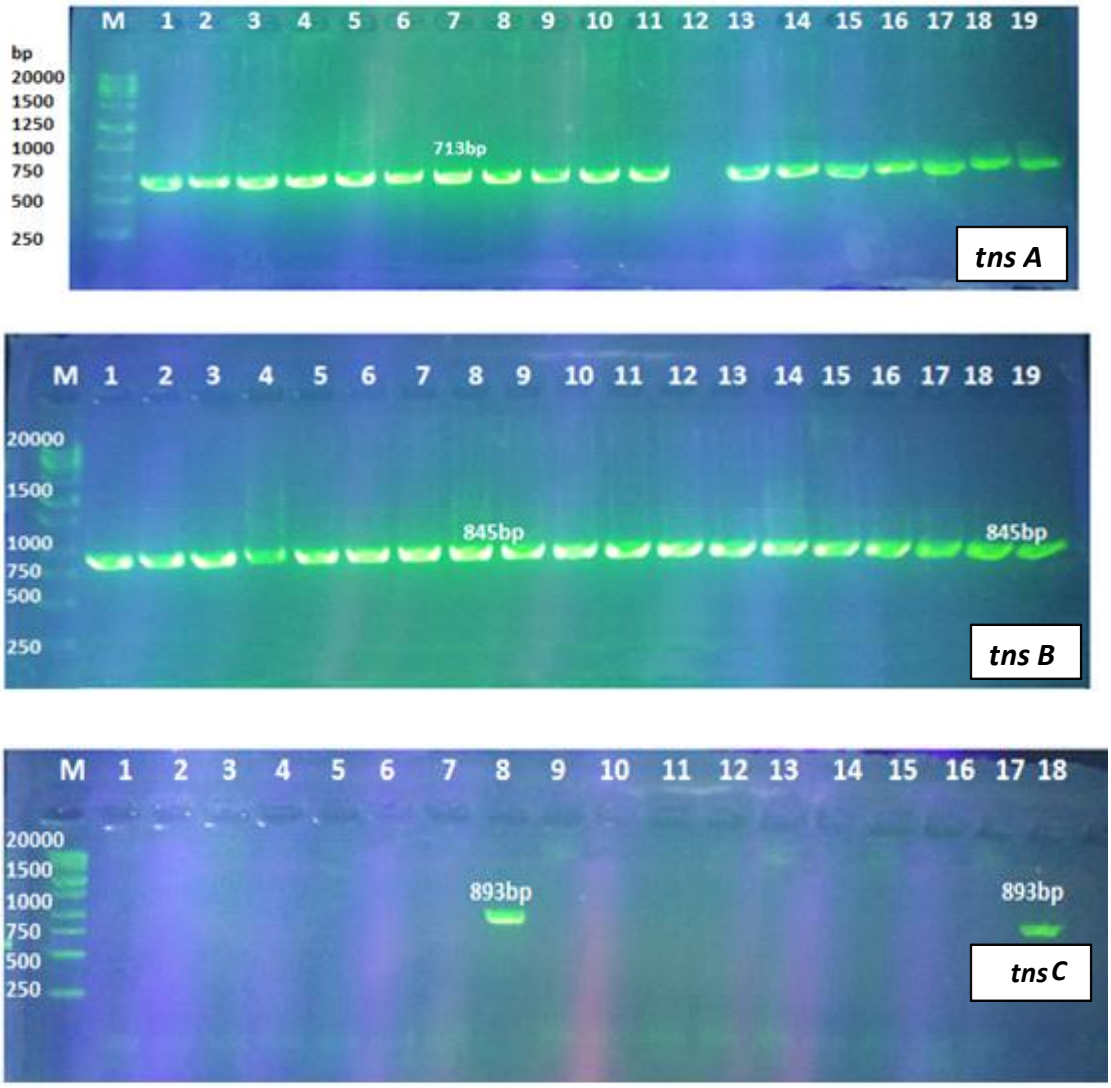


Figure (4-5): Agarose gel electrophoresis of *tns A, tns B and tns C* amplified product patterns of *E. coli* and *Klebsiella* isolates.

M: refers to DNA size marker 250-20000 bp (IntronBio /Korea); lanes (1 – 10 *E.coli*, 11-19 *Klebsiella*) refer to PCR products of *tns A, tns B and tns C* genes (713bp, 845bp & 893bp respectively) of *E. coli* and *Klebsiella* isolated from stool samples of healthy and patient with gastroenteritis and urine samples of patients with UTI. Electrophoresis conditions: 1.5% agarose concentration; 100 V, 20 mA for 60 min. Staining method; precast RED Safe stain.

The figure (4-6) appeared the PCR-product of integron class 2 (*intI2*) at size 789bp. Some *E. coli* and *Klebsiella* isolates were harboring integron gene in addition to Tn7-transposition genes. These isolates appeared variable patterns of transposon genes. There were eight patterns, three of them associated with the presence of *intI2* gene, including *tns* A, *tns*B, *tns* C & *intI2*; *tns* A, *tns*B & *intI2* and *tns*B & *intI2* as shown in the figure (4-7) and table (4-4). About 14% of total isolates harbor the one of these patterns. The other five patterns of Tn7 like transposon, including *tns*A, *tns*B & *tns*C; *tns* A & *tns*B; *tns* A & *tns* C; *tns* A only; *tns*B only and distributed among 62% of total isolates, whereas 24% of total isolates were negative which were not detected the presence of Tn7 like transposon nor *intI2* gene. These patterns distributed among *E. coli* and *Klebsiella* isolates which recovered from three isolation sources (healthy stool, patient stool of gastroenteritis and urine of patient with UTI). Generally, *E. coli* and *Klebsiella* isolates harbored different Tn7 like transposons (complete or truncated) were associated with source of isolation (p 0.029; Fishers exact test) and they related with pathogenic bacteria in spite of some normal flora harboring the mobile genetic elements. *E. coli* and *Klebsiella* isolates.

Figure (4-6): Agarose gel electrophoresis of *intI2* amplified product patterns of *E. coli* and *Klebsiella* isolates.

M: refers to DNA size marker (250-20000 bp); lanes 2 and 12 refer to PCR product of *intI2* gene (789bp) of *E. coli* and *Klebsiella* isolated from stool samples of healthy and patient with gastroenteritis. Electrophoresis conditions: 1.5% agarose concentration; 100 V, 20 mA for 60 min. Staining method; precast RED Safe stain.

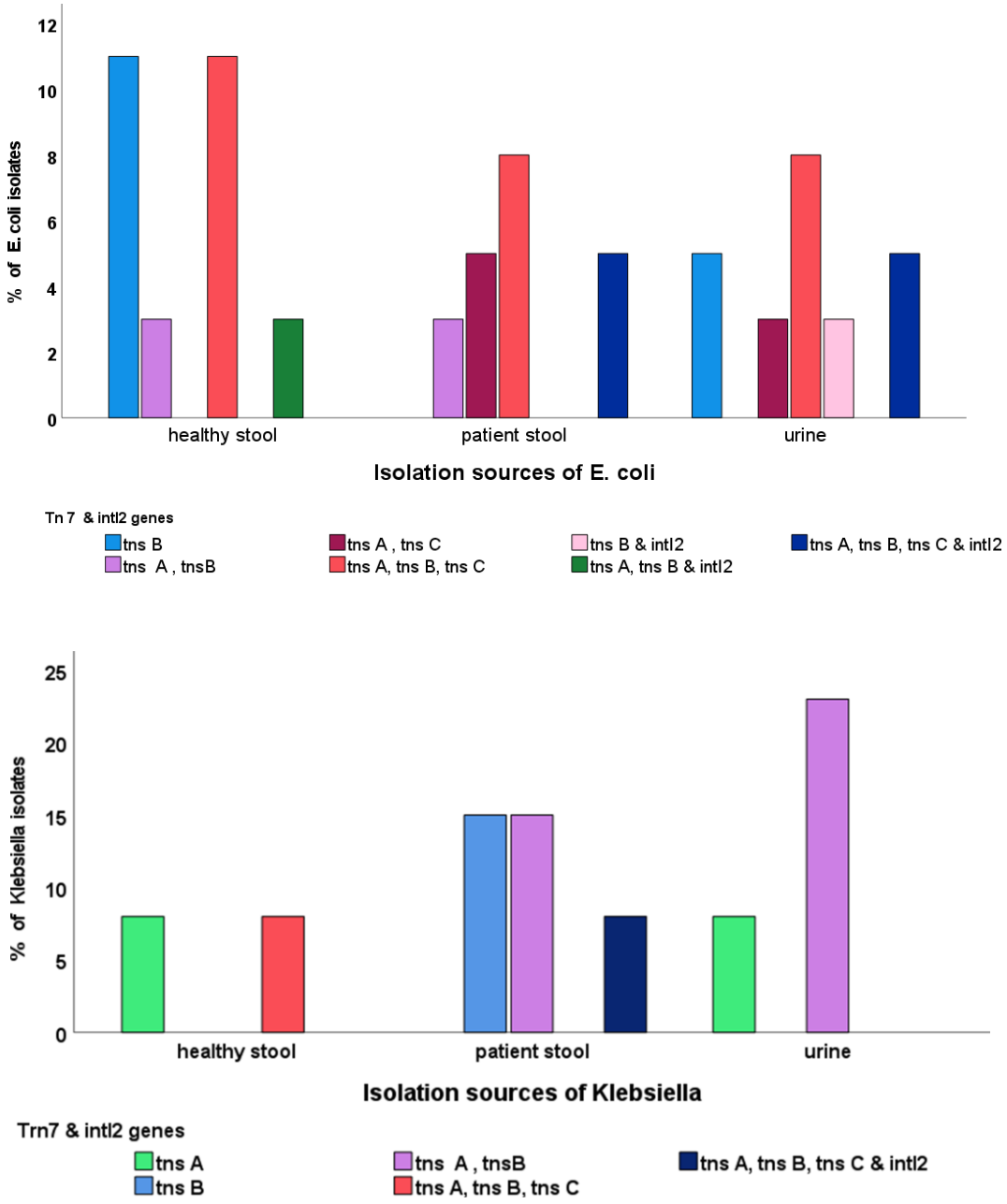


Figure (4-7): Distribution of Tn7 transposition genes and integron class 2 gene (*intI2*) among *E. coli* and *Klebsiella* isolated from different sources

Fisher exact test was used to analyze the data. Data labeled with entirely different letters were significantly different ($p < 0.05$).

So, one isolate from each Tn7-group patterns was selected to represent the eight groups for further analysis of the Tn7 transposition genes and the *intI2* gene (Table 4-4). The present study appeared there are correlation between the presence Tn7 like transposon and class 2 integron with the studied antibiotic resistances ($P < 0.029$; Chi Square test) as shown in the figure (4-8-A). In the other hand, The presence of Tn7 is associated with multidrug resistance of the studied bacteria ($P < 0.001$; Chi Square test). as shown the figure (4-8-B) , which revealed most MDR *E. coli* and *Klebsiella* (resist to more than 3 classes of antimicrobial drugs) were harbor different patterns of Tn 7 like transposons and intI2 gene.

The Tn*7*-like transposons are essential mobile elements to transfer bacterial antimicrobial resistance. Compared with information obtained from studies about the transposition mechanism of the Tn*7*-like transposons, available data on the comprehensive analysis of Tn*7*-like transposons in *Enterobacterales* isolates are still inadequate. Also MDR intestinal bacteria such as *E. coli* is known to be associated with integrons (Kargar *et al.*, 2014).

The various percentages of resistance in different parts of the world are due to differences in the prevalence of antibiotic consumption in each country (WHO, 2012). In this study the frequency of present integron class2 was estimated as 14% that associated with Tn7 like transposon for total studied isolates and as 18.42% for positive isolates that carrying Tn7 genes. The integron prevalence was relatively higher than in other investigations; for example, Rezaee *et al* .(2012) reported 5.08% of class 2 integron. In other research by Ranjbaran *et al* . (2013) a class 2 integron prevalence of 8% was reported. Whereas, Jones *et al*. (2003) reported that 47% of MDR isolates carried class 1 and 2 integrons, whereas no integron class 3 was detected. Furthermore, research by Farshad *et al* . (2008) found frequency of 41.10% for class 2 integron.

As previously noted, the presence of integrons is closely related to resistance to quinolones, aminoglycoside compounds, Beta-lactam, trimethoprim and chloramphenicol antibiotics (Chang *et al*., 2000; Singh *et al*., 2005; Su *et al*., 2006). This study also detected a considerable correlation between class 2 integron and resistance to Amoxicillin- clavulanate (AMC), Piperacilline (PRL), Cefotaxime (CTX), Gentamicin (CN), Azithromycin (AZM), Nitrofurantoin (NIT) ($P < 0.05$: Fisher exact test). This study consist with previous studies, for example Kargar *et al.* (2014) reported there were a substantial correlation between class 2 integron and resistance aminoglycosides, co-trimoxazole, cephalexin, ampicillin, and chloramphenicol and it correspondent with class 1 integron and resistance to Amikacin, gentamicin, chloramphenicol, ampicillin, tetracycline, nalidixic acid, and co-trimoxazole among a total of 69 MDR *E. coli* strains .

Table (4-4): Groups of Tn7 transposition gene patterns and integron class 2 gene and the characteristics of selected local isolates for each group.

Group No.	Tn7 gene Patterns	*Integron*	Total *E. coli* n=74 No.(%)	Total *Klebsiella a a* n=26 No. (%)	Total isolate No. (%)	Selected isolates	Isolation source	No. of antibiotic resistance (class)
1	*tns* A, *tns* B, *tns* C	*IntI* 2	8 (10.81)	2 (7.69%)	10 (10%)	*E. coli* R1 -23	Patient stool	4 (4) AMC,CAZ, C, AZM
2	*tns* A, *tns* B	*IntI* 2	2 (2.7%)	0 (0%)	2 (2%)	*E. coli* R2-5	Healthy stool	Sensitive
3	*tns* B	*IntI* 2	2 (2.7%)	0 (0%)	2 (2%)	*E. coli* R3-27	Urine	4 (4) AMC,CTX,NIT, AZM
4	*tns* A, *tns* B, *tns* C	-	20 (27.03%)	2 (7.69%)	22 (22%)	*E. coli* 10	Healthy stool	8 (6) PRL,AMC,CAZ, CTX,TE, NIT,AZM,MEM
5	*tns* A, *tns* B	-	4 (5.41%)	10 (38.46 %)	14 (14%)	*Klebsiella* W4-19	Patient stool	2 (2) CTX, AK
6	*tns* A, *tns* C	-	6 (8.11%)	0 (0%)	6 (6%)	*E. coli* W3-17	Urine	4 (3) PRL,AMC,CIP, CAZ,CTX
7	*tns* A	-	0 (0%)	4 (15.38 %)	4 (4%)	*Klebsiella* W1-21	Healthy stool	5 (3) PRL,AMC,CAZ, CTX,C
8	*tns* B	-	12 (16.22%	4 (15.38 %)	16 (16%)	*E. coli* W2-4	Healthy stool	9 (5) PRL,AMC,LE,CIP,CAZ,CTX,CN, AK,AZM
Total Positive isolates for Tn7 & intI2 genes			12 (16.21%)	2 (7.69%)	14 (14%)			
Total Positive isolates for Tn7 genes			42 (56.76%)	20 (76.92 %)	62 (62%)			
Total Positive isolates			54 [b] (72.97%)	22 [c] (84.61 %)	76 [a] (76%)			*P* <0.001
Total Negative			20 (27.03%)	4 (15.39 %)	24 (24%)			

Tn7 transposition genes **:** *tns* A, *tns* B, *tns* C ; *IntI*2: integron class 2

Fishers exact test was used to analyze the data. Data labeled with entirely different letters were significantly different ($p < 0.05$).

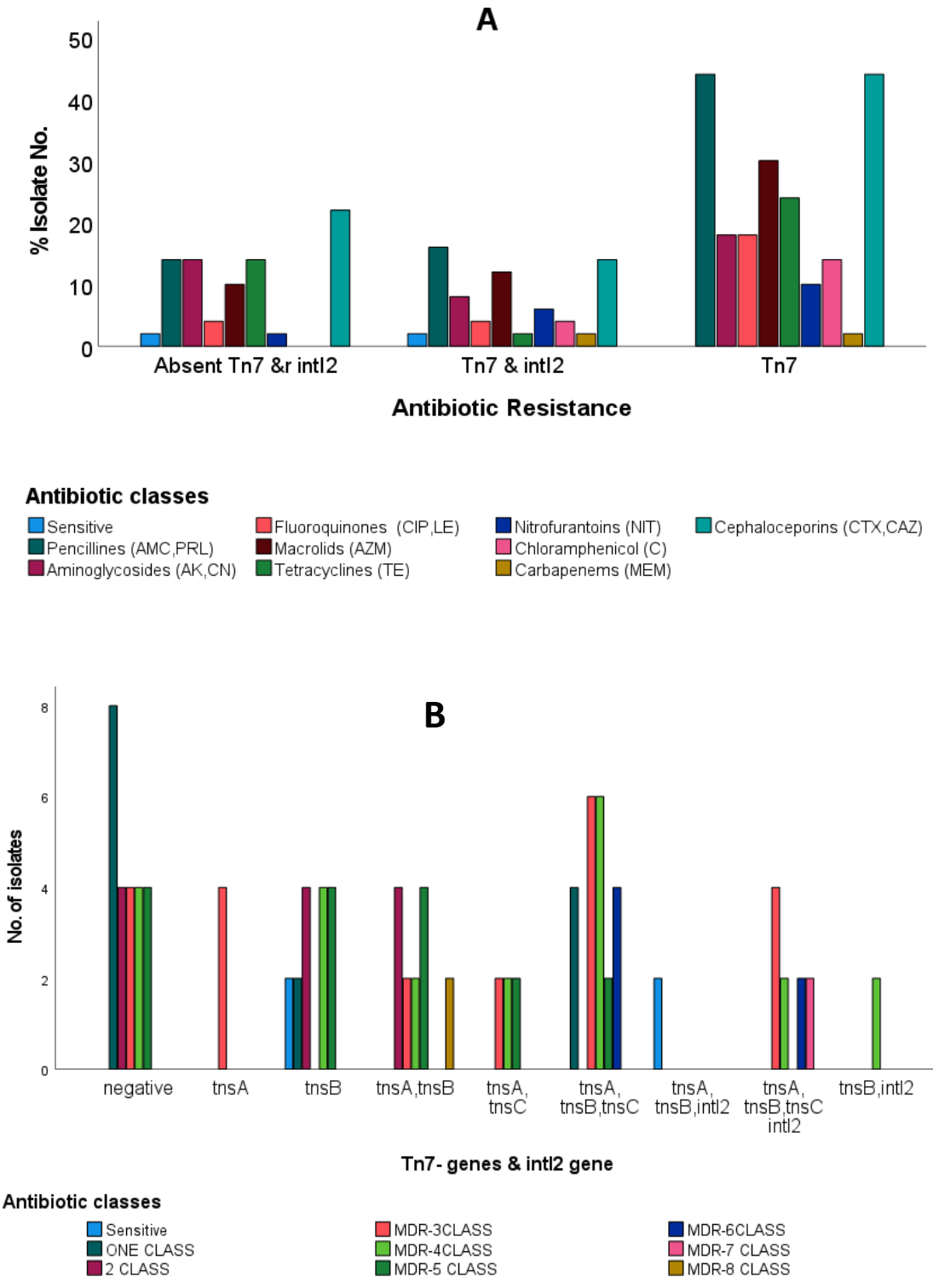


Figure (4-8): Association of MDR-bacterial isolates with the presence Tn7-transposition genes and *intI2* gene

4.4 Molecular analysis of Tn7 transposition genes

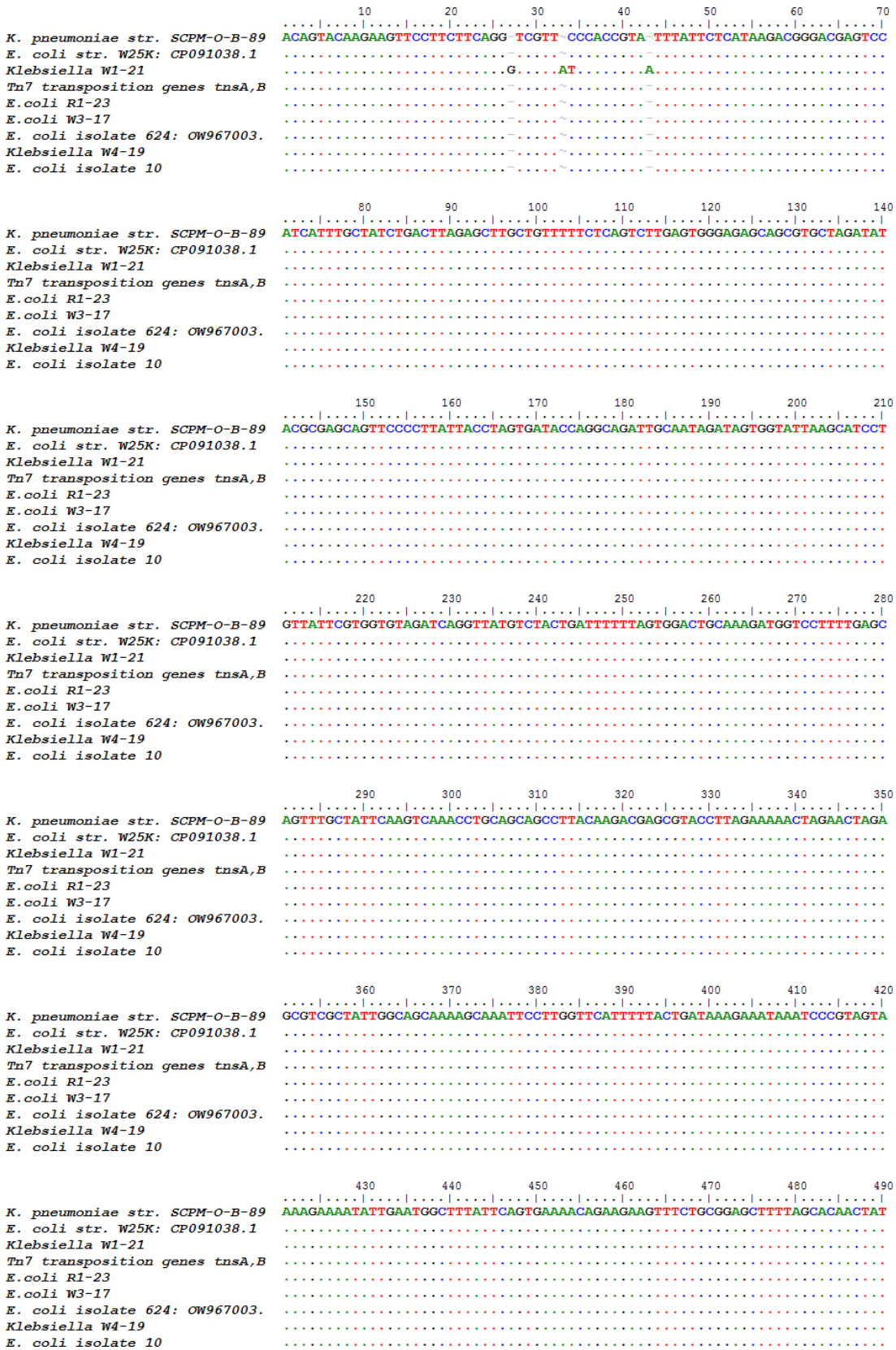
The prototypic Tn7 utilizes five core genes tnsABCDE encodes five transposition proteins: TnsA, TnsB, TnsC, TnsD and TnsE to accomplish the dual-pathway lifestyle. TnsABC form the core machinery that carries out the transposition reaction into positions selected by TnsD or TnsE. TnsA and TnsB together form the transposase that specifically recognizes the ends of the transposon. TnsAB excises Tn7 from the donor site by introducing double-stranded DNA breaks (DSBs) at each end of the transposable element, and then joins the exposed transposon ends to the target (Li *et al*., 2013).

4.4.1 Genetic analysis of *tns*A

PCR-DNA sequencing results of *tns*A of the selected local isolates revealed that the alignment sequence of the four local isolates (Fig. 4-9), including *E. coli* isolate 10, *E. coli* R1-23, *E. coli* W3-17 and *Klebsiella* W4-19, harbor intact *tns*A gene that leads to translated to active protein as indicated by the predicated proteins in comparison with those available in databank as shown in the figures (4-10) which appeared pair amino acid alignment and Tns A protein. When TnsA amino acids of *Klebsiella* W4-19 for example to other local isolates except *Klebsiella* W1-21 was modeling, the results appeared that the 228 residues (100% of TnsA sequence) have been modeled according to the site http://www.sbg.bio.ic.ac.uk/phyre2/html/flibview.cgi?pdb=c1f1zB compared with the template c1f1zB. The results showed 100% confidence by the single highest scoring template c1f1zB as shown in the figure(4-11)The studied amino acid sequence include 228 residues is represent the protein region 34-261aa out of 273aa of complete TnsA protein. There is no amino acid change of the three essential active site residues of TnsA including E63, D114 and K132 of the TnsA native protein (Hickman *et al*., 2000) that represent the 30 E, D81, K 99 residues at the studied region (34-261) as shown in figure (4-12), suggesting TnsA maintain transposase activity in all

local selected isolates. Whereas the *Klebsiella* W1-21 had four mutations in coding region of *tns*A gene, three of them insertion G, A and A nucleotides at the sequences, 27 (126 of coding region), the sequence 33 (132 of coding region) and 43 (142 of coding region) respectively (Fig. 4-9) and institution mutation C > T at the sequence 34 (133 of coding region of *tns*A) as shown the figure (4-10). These mutations leads to substitution of three amino acids and insertion one acid in sequence of targeted protein, including Arginine to Leucine at site 11 (R44→L44) , Histidine to Threonine (H46→T46), Arginine to Valine (A47→V47) at positions 13 & 14 respectively and insertion Serine (S43) at position 10 (Fig.4-10). These change may be not effect or slightly effect on catalytic activity of TnsA because the no institution of the three essential active site residues of TnsA including E63, D114 and K132 of the TnsA native protein (Hickman *et al*., 2000).

When this protein modeled the results appeared the secondary structure model of *Klebsiella* W1-21 TnsA protein had coverage about 97% (234 residues) and 99% identity to TnsA a catalytic component of Tn7 transposition system with 100% confidence by the single highest scoring template c1f1zB and 98% identity of endonuclease tnsA endonuclease (catalytic site),N-terminal domain of d1t0fa2 template model (http://www.sbg.bio.ic.ac.uk/phyre2/html/flibview.cgi?pdb=d1t0fa2) as shown in the figure (4-11),which appeared the secondary structure of this protein composed of 39% alpha helix, 22% Beta strand and 14% disordered amino acids, in compression with the Tns A native protein composed of 39% alpha helix, 21% Beta strand and 9% disordered amino acids. Depending these results may be the hypothetical *Klebsiella* W1-21 TnsA protein is normal active or less/highly active to confirm this hypothesis it is required further analysis by Tns A protein extracting, purification to homogenize and amino acid sequencing in addition to determine the endonuclease activity of Tns A protein.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E. coli str. W25K: CP091038.1
Klebsiella W1-21 G AT A
Tn7 transposition genes tnsA,B
E.coli R1-23
E.coli W3-17
E. coli isolate 624: OW967003.
Klebsiella W4-19
E. coli isolate 10
80 90 100 110 120 130 140
K. pneumoniae str. SCPM-O-B-89 ATCATTTGCTATCTGACTTAGAGCTTGCTGTTTTTCTCAGTCTTGAGTGGGAGAGCAGCGTGCTAGATAT
E. coli str. W25K: CP091038.1
Klebsiella W1-21
Tn7 transposition genes tnsA,B
E.coli R1-23
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E. coli isolate 624: OW967003.
Klebsiella W4-19
E. coli isolate 10
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K. pneumoniae str. SCPM-O-B-89 ACGCGAGCAGTTCCCCTTATTACCTAGTGATACCAGGCAGATTGCAATAGATAGTGGTATTAAGCATCCT
E. coli str. W25K: CP091038.1
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Tn7 transposition genes tnsA,B
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Klebsiella W4-19
E. coli isolate 10
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K. pneumoniae str. SCPM-O-B-89 GTTATTCGTGGTGTAGATCAGGTTATGTCTACTGATTTTTTAGTGGACTGCAAAGATGGTCCTTTTGAGC
E. coli str. W25K: CP091038.1
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Klebsiella W4-19
E. coli isolate 10
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Klebsiella W4-19
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E.coli R1-23
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Klebsiella W4-19
E. coli isolate 10
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K. pneumoniae str. SCPM-O-B-89 AAAGAAAATATTGAATGGCTTTATTCAGTGAAAACAGAAGAAGTTTCTGCGGAGCTTTTAGCACAACTAT
E. coli str. W25K: CP091038.1
Klebsiella W1-21
Tn7 transposition genes tnsA,B
E.coli R1-23
E.coli W3-17
E. coli isolate 624: OW967003.
Klebsiella W4-19
E. coli isolate 10

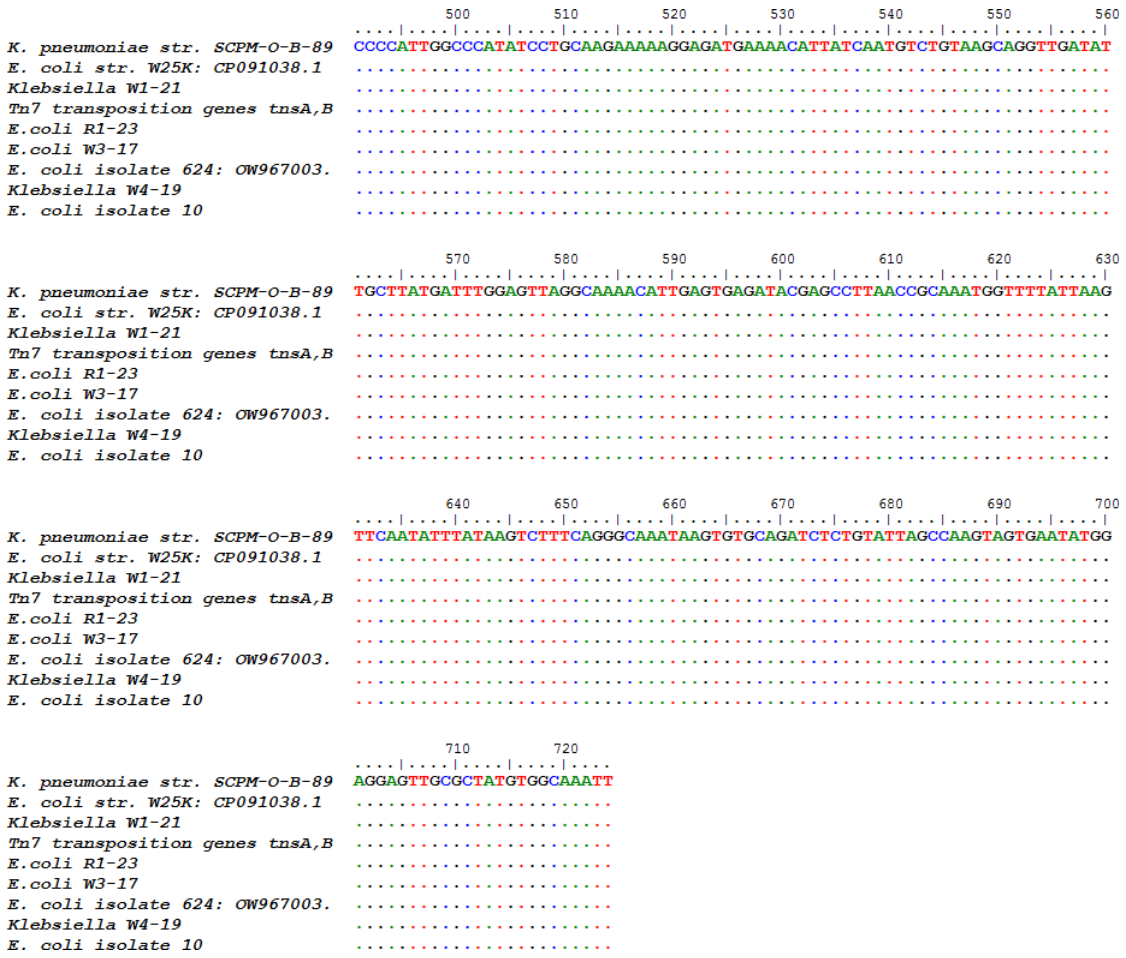


Figure (4-9): Nucleotides sequence alignment of the coding region of *tns*A gene among the local isolates and nucleotides sequence of those available in databanks.

The local isolates including *E. coli* isolate 10, R1-23, W3-17, *Klebsiella* W1-21 and isolate W4-19 Data indicated that the position of nucleotides is different among isolates and identical data for all isolates are not shown by Bio Edit program version 7.2.5.

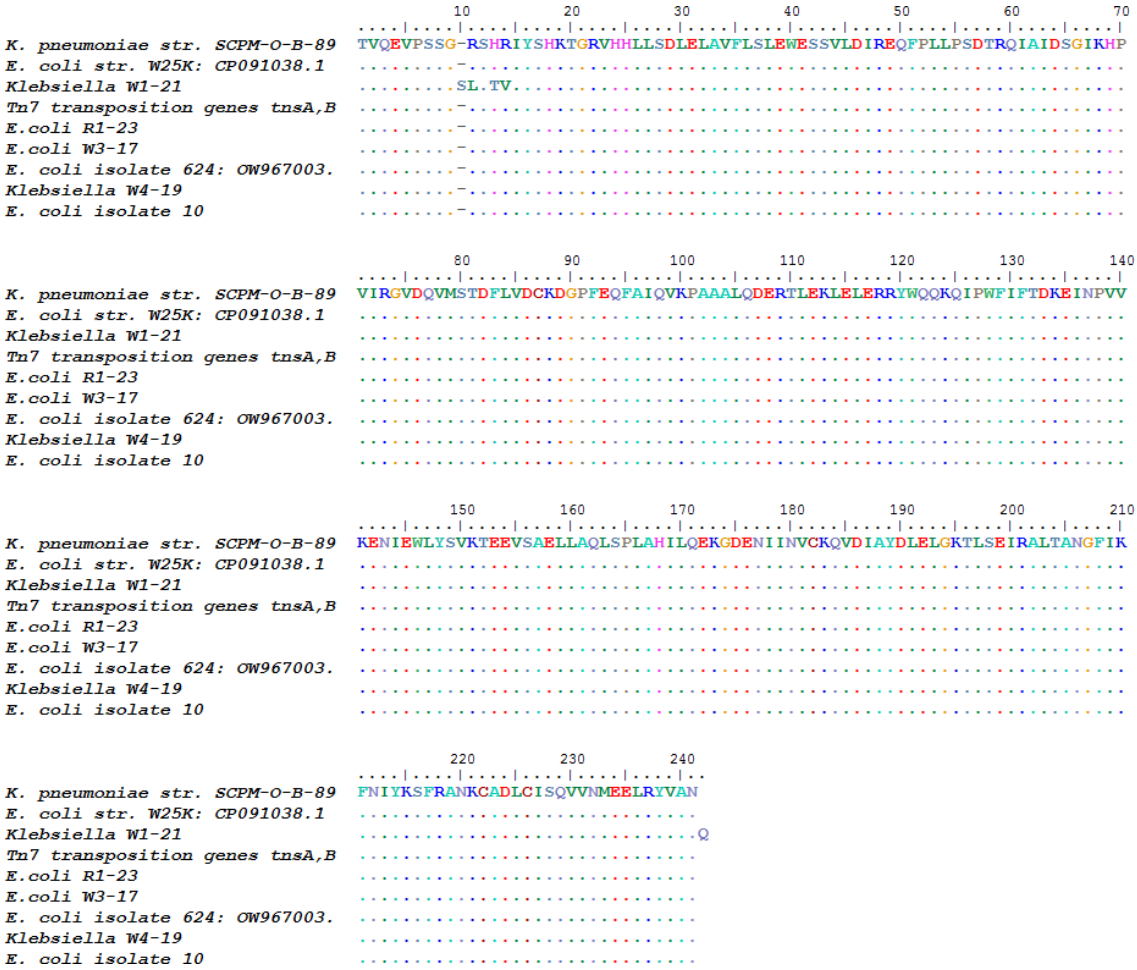


Figure (4-10): Pair sequence alignment of the amino acid of the *tns*A gene among the isolates *E. coli* isolate 10, R1-23, W3-17, *Klebsiella* W1-21 and isolate W4-19 and amino acid sequences of those available in databanks. draw based on the alignment of Bio Edit program version 7.2.5

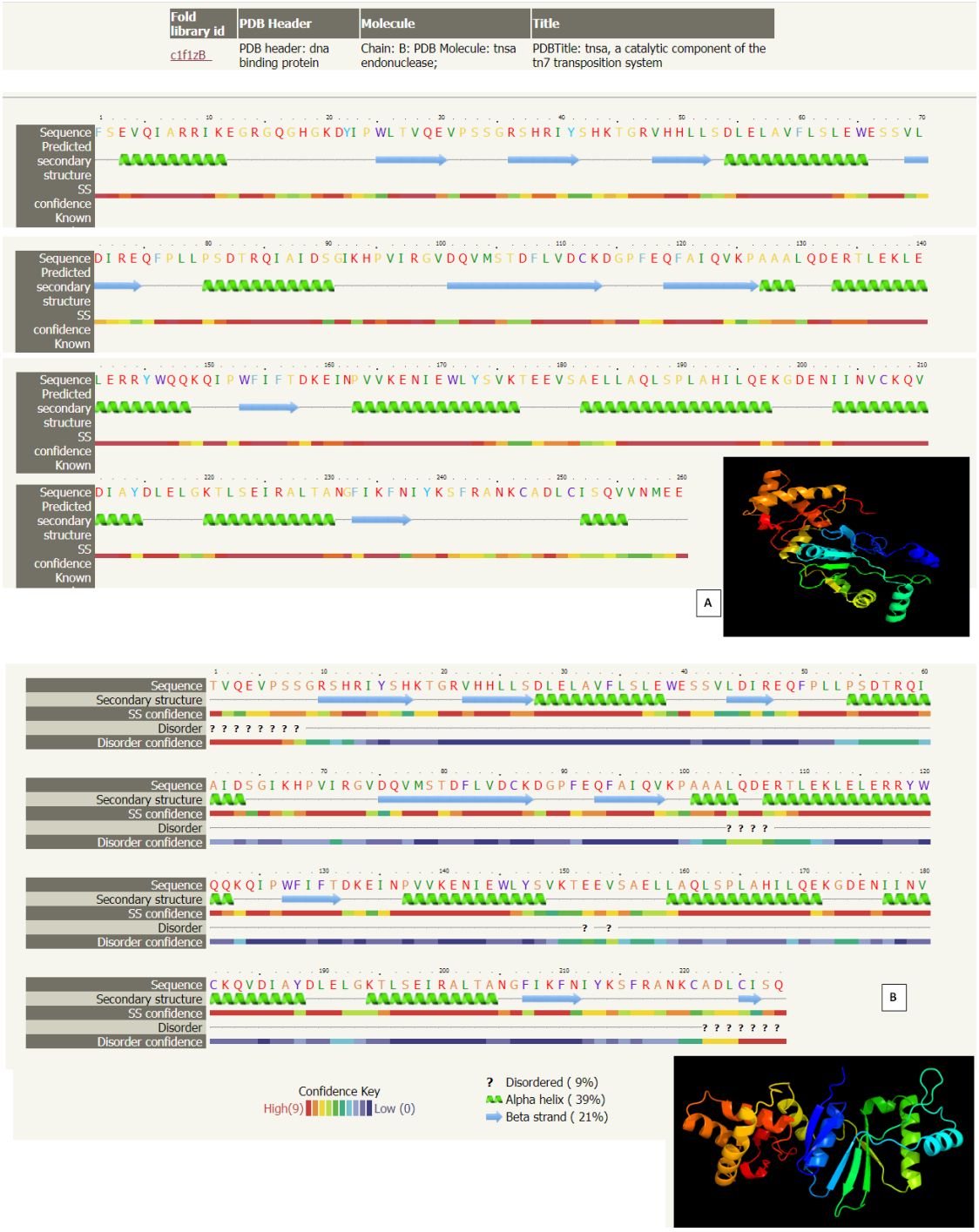


Figure (4-11): Secondary structure prediction of the TnsA protein of *Klebsiella* W4-19 and modeled with template c1f1zB (A)

(A) c1f1zB template; (B):228 residues (coverage 100% of TnsA sequence of *Klebsiella* W4-19) have been modeled with 100% confidence and 100% identity by the single highest scoring template c1f1zB. http://www.sbg.bio.ic.ac.uk/phyre2/html/flibview.cgi?pdb=c1f1zB_

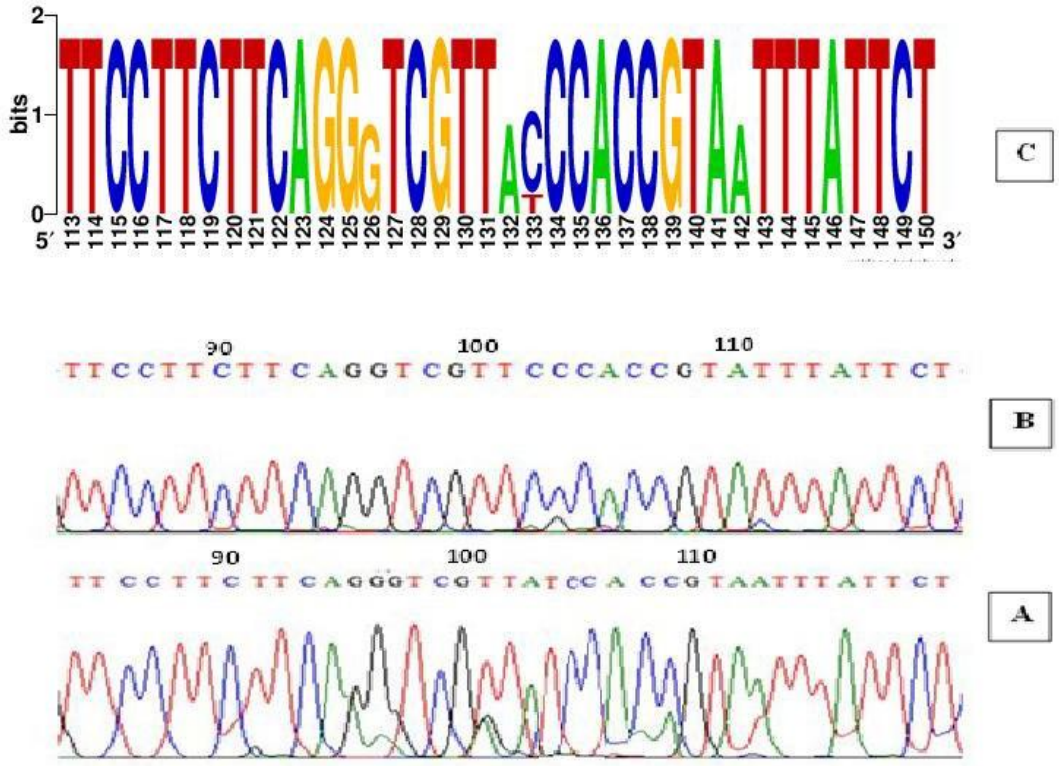


Figure (4-12): The Weblogo of repeats of coding region of *tnsA* gene. The sequences were each mutation site of the *tns* A, a catalytic component of the Tn7 transposition system (C); B: DNA sequence of *Klebsiella* W4-19; A: DNA sequence of *Klebsiella* W1-21.

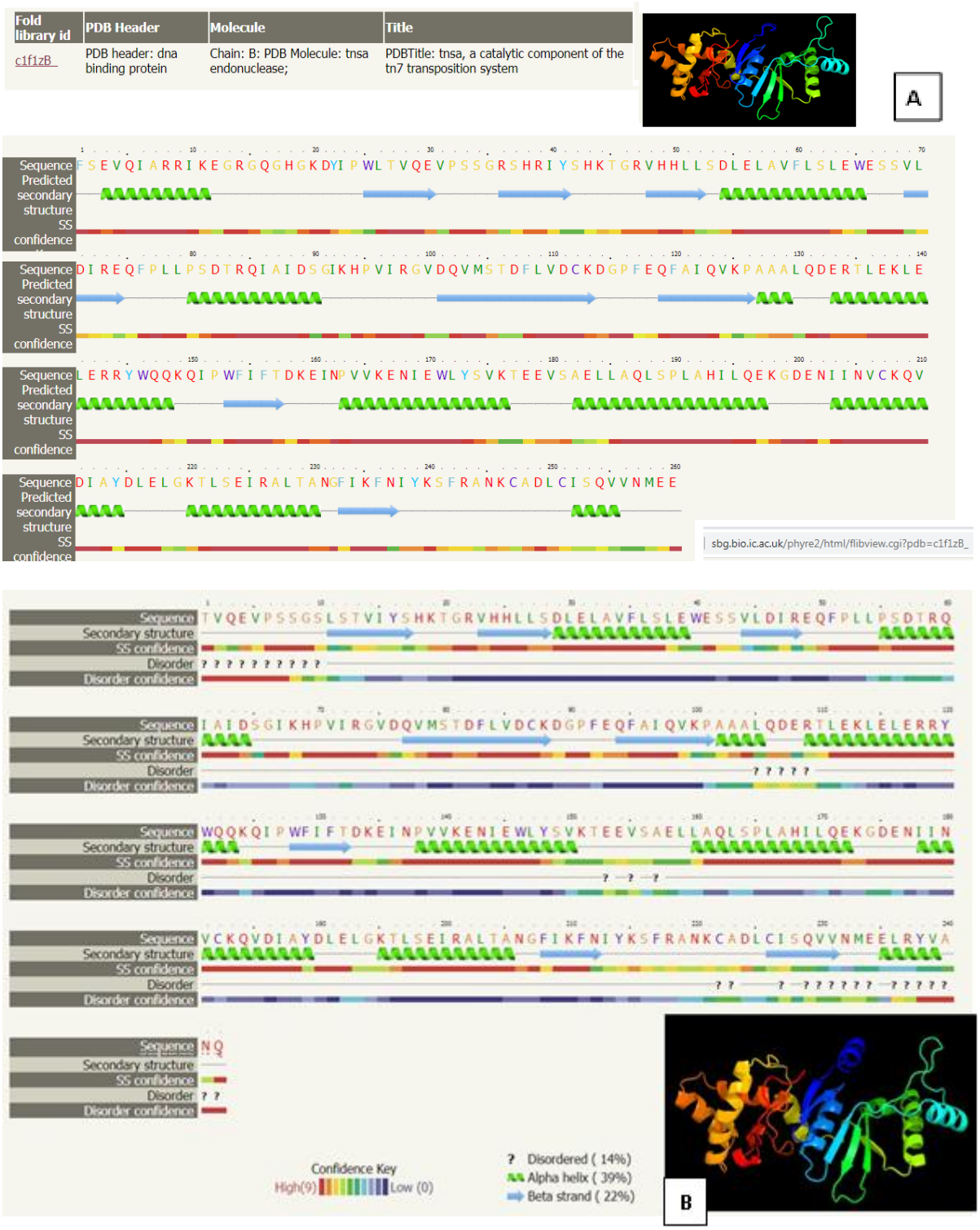


Figure (4-13): Secondary structure prediction of the TnsA protein of *Klebsiella* W1-21 and modeled with template c1f1zB.

(A) c1f1zB template; (B):234 residues (coverage 97% of TnsA sequence of *Klebsiella* W1-21) have been modeled with 99% identity and 100% confidence by the single highest scoring template 1f1zB. http://www.sbg.bio.ic.ac.uk/phyre2/phyre2_output/d3da721e11335db4/summary.html

Tns A is homology with other endonuclease of some mobile genetic elements like Tn*6230* (*Salmonella enterica* subsp. *enterica* ser. Senftenberg), and Tn*6022* (*Acinetobacter baumannii* D36) and the differences not greater than 10% which identified using BLAST searches (Altschul *et al*., 1997; Peters *et al*., 2014). Therefore the predicted Tns A protein in this study was alignment with other an endonuclease proteins of those available in databank as shown in the figure (4-11). The phylogenic tree of TnsA protein refer to the TnsA proteins of the most local isolates except *Klebsiella* W1-21 were homologous the other strains in databank in the N-terminal of endonuclease though it in the clade of *Citrobacter freundii* WP130009279. Whereas, *Klebsiella* W1-21 in the clade of *Citrobacter freundii* 22 HCB2664981. These results consist with previous studies that reported TnsA proteins can be identified in genome databases by a conserved motif found in the protein (*Tn7_Tnp_TnsA_N*) (pfam08722) (Hickman *et al*.,2000; Punta *et al*., 2012). The number of transposons identified using this conserved motif suggests that a large and diverse group of heteromeric transposase elements exists. To gather a manageable collection of putative elements for analysis, BLAST searches was performed with TnsA from the canonical Tn*7* from *Citrobacter freundii*, and candidate heteromeric elements that appeared highly diverged from Tn*7* in *Escherichia coli* and *Klebsiella* (Rose, 2010; Moreno Switt *et al*., 2012).

Twenty eight candidates from each of these five local isolates were chosen for further analyses where the amino acid sequence of TnsA was used to infer a phylogeny of the elements (Fig. 4-14). In previous study found that all of the bacterial examples had the three catalytic residues found in TnsA from Tn*7* (Hickman *et al*.,2000), but two other TnsA residues were also found conserved, S60 and Q82. S60 was previously identified as liganded to a Cl− in the TnsA crystal structure. The role of Q82 is unknown, but it is likely important for transposition given that it is so highly conserved. These TnsA-encoding elements also all had homologues of TnsB and TnsC supporting the

idea that bona fide transposons were identified. Elements in the clade with Tn*7* all encoded homologues of the five Tns proteins encoded in Tn*7* and were inserted downstream of *glmS* gene (Peters *et al*., 2014).

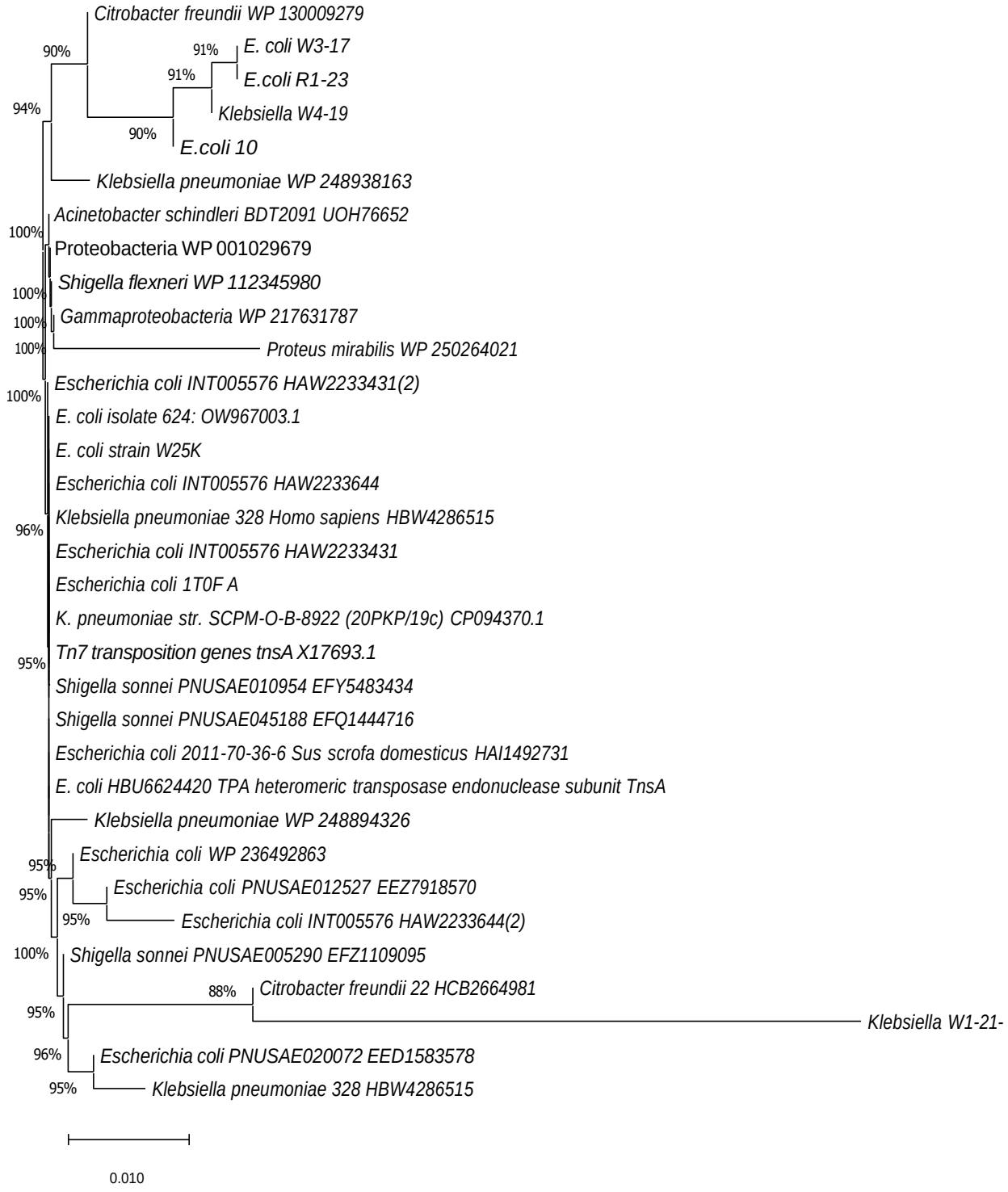


Figure (4-14): Phylogenetic tree with selected TnsA protein homologues from Tn7 that available in NCBI database.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). Homologues to TnsA from Tn*7* of *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Shigella sonnei*, *Shigella flexneri* and *Acinetobacter schindleri* which identified using BLAST searches (Altschul *et al*., 1997)

4.4.2Genetic analysis of *tns*B

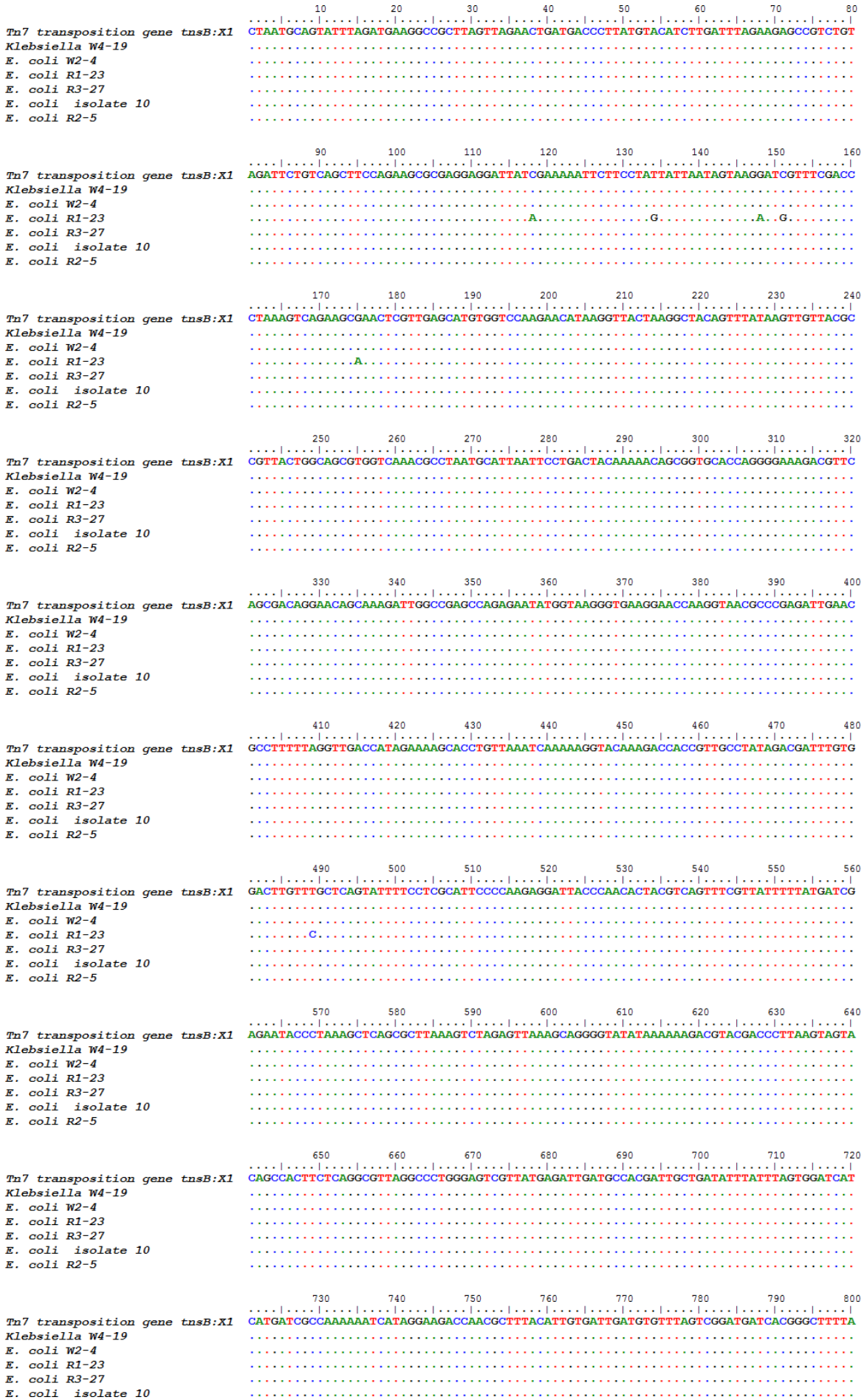
PCR-DNA sequencing results of *tns*B of the selected local isolates revealed that the alignment sequence of the five local isolates (Fig. 4-15), including *E. coli* isolate 10, *E. coli* R2-5, *E. coli* R3-27, *E. coli* W2-4 and *Klebsiella* W4-18, harbor intact *tns*B gene that leads to translated to active protein as indicated by the predicated proteins in comparison with those available in databank as shown in the figures (4-16) which appeared pair amino acid alignment and Tns B protein. When TnsB amino acids of local isolates except *E. coli* R1-23 were modeling, the results appeared that the 228 residues (100% identity sequence of TnsB) have been modeled with 100% confidence and coverage 84% by the single highest scoring template c7pikC as shown in the figure (4-17)B. That indicates all the *tns*B genes in all local selected isolates were functional genes.

Whereas the *E. coli* R1-23 had six mutations in coding region of *tns*B gene, they were institution mutations, including C > A at the sequence 118, T > G at the sequence 134, G > A at the sequences 148 and 175 , C > G at the sequence 151and T > C at the sequence 489 as shown the figure (4-15). These mutations (in the region 118-175) leads to change four amino acids in polypeptide sequence (in the region 45-59) of the targeted protein (Fig. 4-16), including the sequences 45, 50, 51 and 59 which the amino acids changed from I>S (isoleucine to serine) , D>N (aspartate to asparagine), R > G (arginine to glycine) and E > K (glutamate to lysine) respectively. Whereas the mutation at the site 489 is not effect on amino acid phenotype (F> F; Phenylalanine).

A missense mutation occurs when there is a mistake in the DNA code and one of the DNA base pairs is changed. The single change means that the DNA now encodes for a different amino acid, known as a substitution. Sometimes a change in the amino acid has no effect on the resulting protein's function at all. On some occasions, the change in amino acid actually enhances

the protein's function, but in other cases it can ultimately render the protein as faulty. So TnsB amino acids of local isolate *E. coli* R1-23 was modelled, the results appeared that the 228 residues (99% identity sequence of TnsB) have been modeled with 100% confidence and coverage 84% by the single highest scoring template c7pikC as shown in the figure (4-17) C which revealed the mutations in the region 45-59 of protein was slightly effected on secondary structure with the percentage of alpha helix and disordered amino acid sequence (41%, and 24% respectively) in compression with non-mutated TnsB protein which appeared 43% alpha helix and 23% disordered amino acid sequence.

Seventeen TnsB protein of candidates from each of these six local isolates were chosen for further analyses where the amino acid sequence of TnsB was used to infer a phylogeny of the elements (Fig. 4-18). The phylogeny tree of TnsB protein revealed that most local isolates, including *E. coli* isolate 10, *E. coli* R2-5, *E. coli* R3-27, *E. coli* W2-4 and *Klebsiella* W4-19, produce DNA binding Protein Chain C (Tn7 transposition protein Tns B) were homologues (98.7% identity) to TnsB protein from Tn*7* of *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Shigella sonnei*, *Shigella flexneri* and *S. dysenteriae* which identified using BLAST searches and located in the same clade. Whereas *E. coli* R1-23 was located in different clade.


10 20 30 40 50 60 70 80
Tn7 transposition gene tnsB:X1 CTAATGCAGTATTTAGATGAAGGCCGCTTAGTTAGAACTGATGACCCTTATGTACATCTTGATTTAGAAGAGCCGTCTGT
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
90 100 110 120 130 140 150 160
Tn7 transposition gene tnsB:X1 AGATTCTGTCAGCTTCCAGAAGCGCGAGGAGGATTATCGAAAAATTCTTCCTATTATTAATAGTAAGGATCGTTTCGACC
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
170 180 190 200 210 220 230 240
Tn7 transposition gene tnsB:X1 CTAAAGTCAGAAGCGAACTCGTTGAGCATGTGGTCCAAGAACATAAGGTTACTAAGGCTACAGTTTATAAGTTGTTACGC
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
250 260 270 280 290 300 310 320
Tn7 transposition gene tnsB:X1 CGTTACTGGCAGCGTGGTCAAACGCCTAATGCATTAATTCCTGACTACAAAAACAGCGGTGCACCAGGGGAAAGACGTTC
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
330 340 350 360 370 380 390 400
Tn7 transposition gene tnsB:X1 AGCGACAGGAACAGCAAAGATTGGCCGAGCCAGAGAATATGGTAAGGGTGAAGGAACCAAGGTAACGCCCGAGATTGAAC
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
410 420 430 440 450 460 470 480
Tn7 transposition gene tnsB:X1 GCCTTTTTAGGTTGACCATAGAAAAGCACCTGTTAAATCAAAAAGGTACAAAGACCACCGTTGCCTATAGACGATTTGTG
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
490 500 510 520 530 540 550 560
Tn7 transposition gene tnsB:X1 GACTTGTTTGCTCAGTATTTTCCTCGCATTCCCCAAGAGGATTACCCAACACTACGTCAGTTTCGTTATTTTTATGATCG
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
570 580 590 600 610 620 630 640
Tn7 transposition gene tnsB:X1 AGAATACCCTAAAGCTCAGCGCTTAAAGTCTAGAGTTAAAGCAGGGGTATATAAAAAAGACGTACGACCCTTAAGTAGTA
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
650 660 670 680 690 700 710 720
Tn7 transposition gene tnsB:X1 CAGCCACTTCTCAGGCGTTAGGCCCTGGGAGTCGTTATGAGATTGATGCCACGATTGCTGATATTTATTTAGTGGATCAT
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10
E. coli R2-5
730 740 750 760 770 780 790 800
Tn7 transposition gene tnsB:X1 CATGATCGCCAAAAAATCATAGGAAGACCAACGCTTTACATTGTGATTGATGTGTTTAGTCGGATGATCACGGGCTTTTA
Klebsiella W4-19
E. coli W2-4
E. coli R1-23
E. coli R3-27
E. coli isolate 10

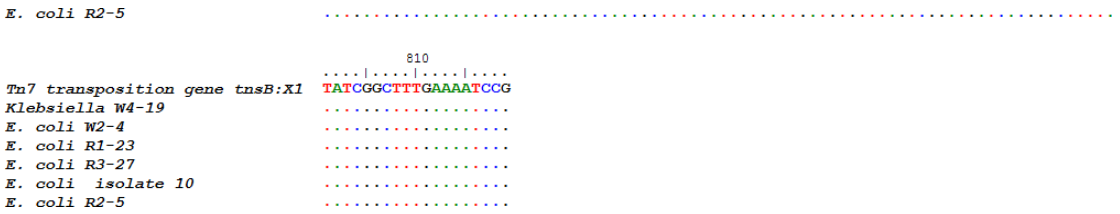


Figure (4-15): Nucleotides sequence alignment of the coding region of *tns*B gene among the local isolates and nucleotides sequence of those available in databanks.

The local isolates including *E. coli* isolate 10, *E. coli* R1-23, *E. coli* R2-5, *E. coli R3-27 E. coli* W2-4 and *Klebsiella* W4-19. Data indicated that the position of nucleotides is different among isolates and identical data for all isolates are not shown by Bio Edit program version 7.2.5.

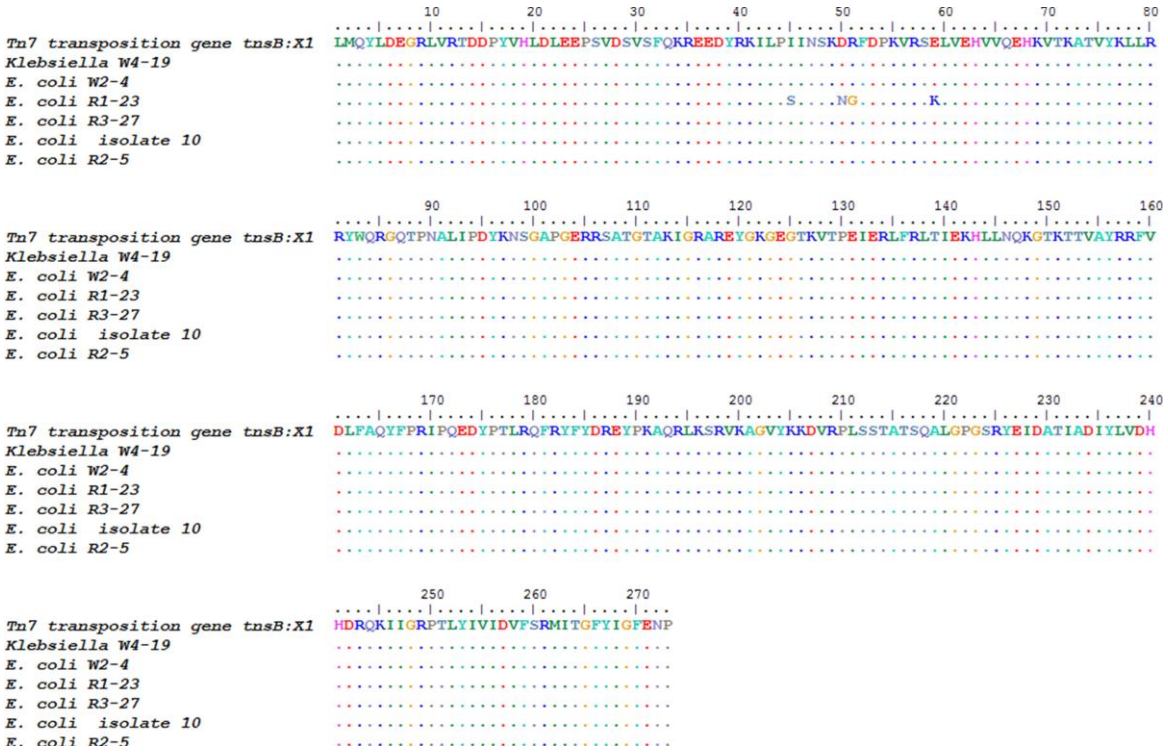


Figure (4-16): Pair sequence alignment of the amino acid of the TnsB protein among the local isolates including *E. coli* isolate 10, *E. coli* R1-23, *E. coli* R2-5, *E. coli R3-27 E. coli* W2-4 and *Klebsiella* W4-19 and Tns B of Tn7 transition of that available in databanks. draw based on the alignment of Bio Edit program version 7.2.5

Fold library id	PDB Header	Molecule	Title
c7pikC_	2.68	PDB header: dna binding protein	Chain: C: PDB Molecule: transposon tn7 transposition protein tnsb;

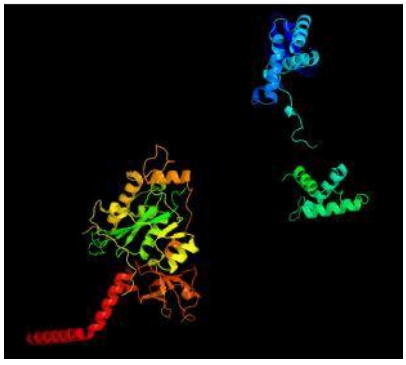
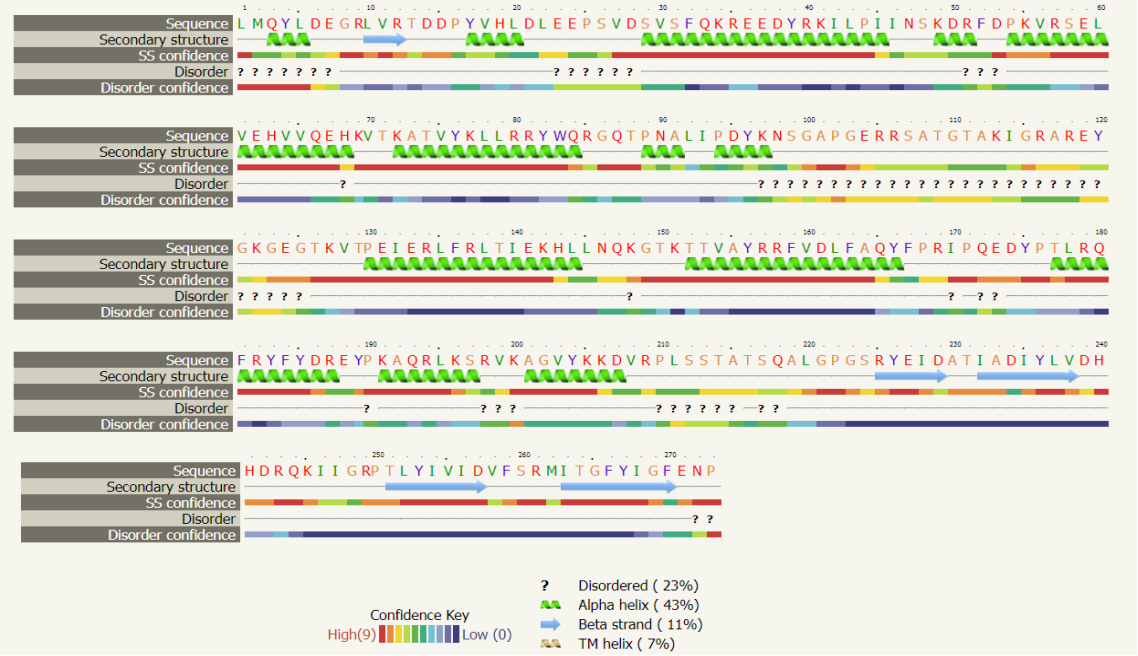
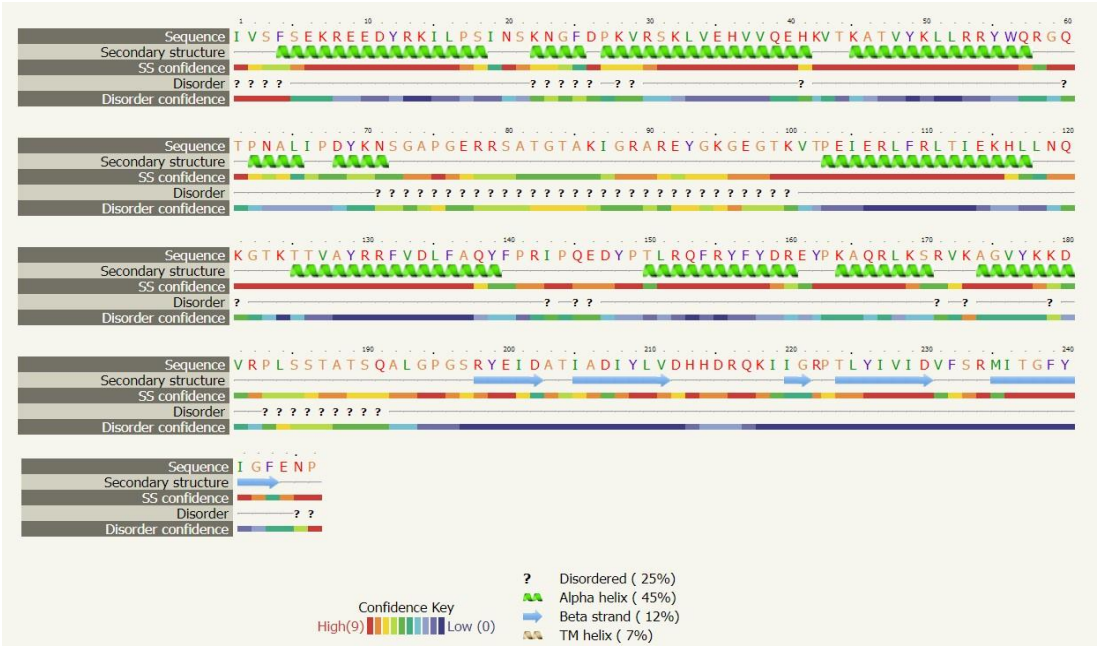


Image coloured by rainbow N → C terminus
http://www.sbg.bio.ic.ac.uk/phyre2/html/flibview.cgi?pdb=c7pikC_

A : c7pikC_ model; Chain: C: PDB Molecule: transposon tn7 transposition protein TnsB



B: Tn7: Tns B protein of Standard selected Databank strains and all of the local isolates except *E. coli* R1-23; The **c7pikC_** model had 228 residues that coverage 84% of the selected sequences, they have been modelled with 100.0% confidence by the single highest scoring template with 100% identity.



C: Tn7: Tns B protein of *E. coli* R1-23; The **c7pikC_** model had 228 residues that coverage 84% of the selected sequence. It have been modelled with 100.0% confidence by the single highest scoring template with 99% identity.

Figure (4-17): Secondary structure prediction of the TnsB protein of selected local isolates (B & C) and modeled with template c1pikC (A) .

http://www.sbg.bio.ic.ac.uk/phyre2/phyre2_output/615b107141c3c7a1/summary.html

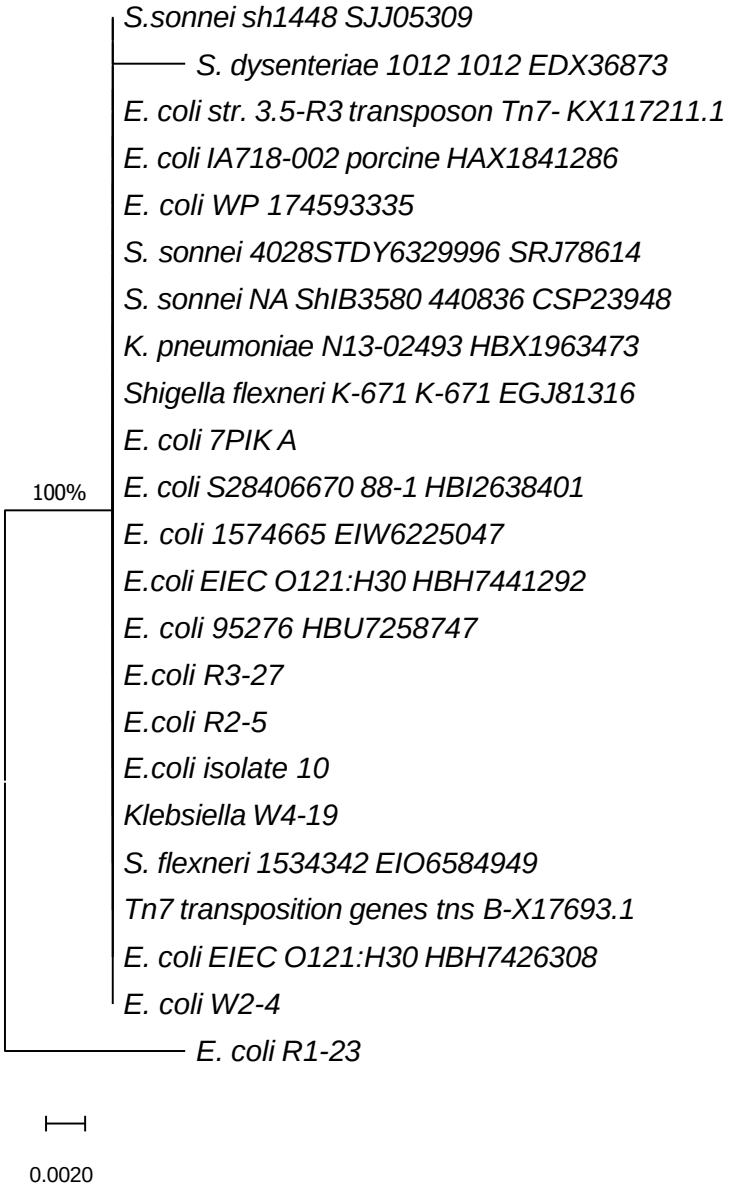


Figure (4-18): Phylogenetic tree with selected TnsB protein homologues from Tn7 that available in NCBI database.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). Homologues to TnsB protein from Tn*7* of *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Shigella sonnei*, *Shigella flexneri* and *S. dysenteriae* which identified using BLAST searches (Altschul *et al*., 1997)

Tn*7*-like elements are the only elements characterized to date where the transposase is comprised of two proteins, TnsA and TnsB, which function together to carry out the chemistry that underlies transposition (May and Craig, 1996; Sarnovsky *et al*., 1996). It has been shown recently that the interaction between these two proteins is important for regulating transposition (Choi *et al*., 2013). TnsB belongs to a large family of proteins utilized by many bacterial transposons and retroviruses sometimes referred to as the DDE recombinase (*rve*) (pfam00665) (Dyda *et al*., 1994; Punta *et al*., 2012). TnsB recognizes DNA sequences found in the left and right ends of the element and catalyses breaking events at the 3′ ends of the element that are joined directly to target DNA (McKown *et al*., 1987; Arciszewska and Craig, 1991).

The ability to liberate both the 3′ and 5′ ends and join the 3′ ends of the element directly to the target DNA allows Tn*7* (and presumably other heteromeric transposase elements) to carry out cut-and-paste transposition in a distinct way from elements with a single transposase that form hairpin structures during excision (Roth *et al*., 1992; Kennedy *et al*., 1998; Bhasin *et al*., 1999). Staggered joining events with the target DNA during transposition results in gaps at the ends of the element that are filled by DNA polymerase forming a target site duplication that is characteristic of each element (5bp in the case of Tn7 (Bainton *etal*., 1991).

4.4.3 Genetic analysis of *tnsC*

The results of PCR-DNA sequencing of tnsC in selected local isolates including *E. coli isolate10, E. coli R1-23 and E. coli* W3-17 which alignment with databank strains revealed that the presence substitution mutations in all of the local isolates as shown in the figure (4-19), including A>T at position 100 of the sequences of *E. coli isolate10, E. coli W3-17* , A>C at the position 379 of the sequence *E. coli R1-23,* and four mutations at the region 773-780 of the *E. coli* W3-17 sequence , A>C, CC>TT, and A>T at the 773, 776,777 and 780 . These mutations leads to change amino acid Tns C protein as shown in the figure (4-20) and figure (4-21), the first mutation convert the amino acid threonine (T) to serine (S) at the sequence 34 (the amino acid 211of TnsC protein) of polypeptide for *E. coli isolate10, E. coli W3-17*. The second mutation converted asparagine (N) to histidine (H) at position 127 (the amino acid 305 of Tns C protein) in *E. coli R1-23* and the mutations at the region 773-780 leads to change three amino acids DTE>AID (aspartic acid, threonine, glutamic acid to alanine, isoleucine and aspartic acid) at the amino acid sequence 436-438 of polypeptide in the tns C of *E. coli* W3-17 (Fig. 4-20). To further analysis of Tns C protein of the local isolates *E. coli W3-17* and *E. coli R1-23* , these proteins were secondary structure modelled using phyre2 online tool for observing the effect of these mutations on the protein folding (http://www.sbg.bio.ic.ac.uk/phyre2/html/flibview.cgi?pdb=c7mbwB_).

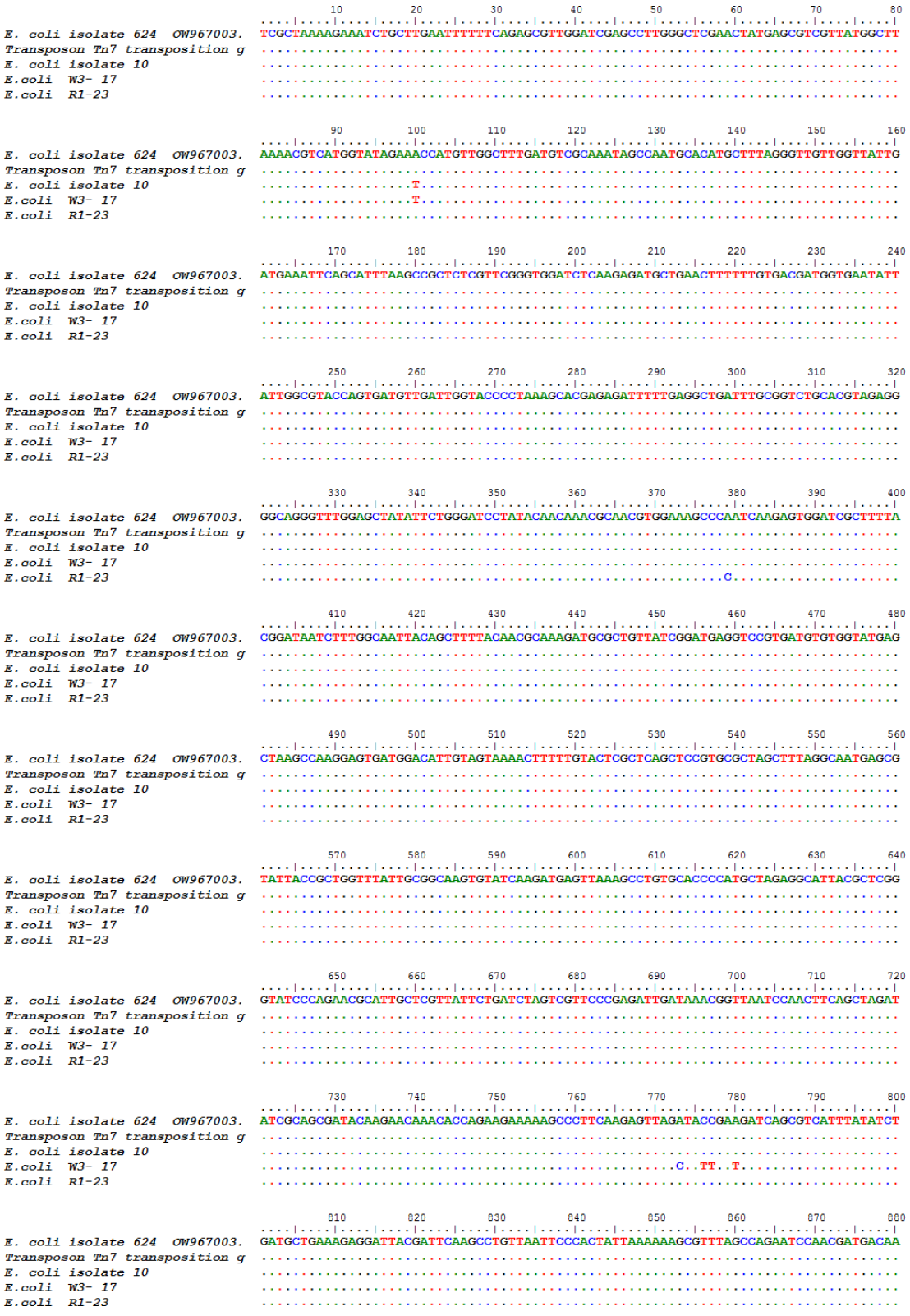


Figure (4-19) : Nucleotides sequence alignment of the coding region of *tns*C gene among the local isolates and nucleotides sequence of those available in databanks.

The local isolates including *E. coli* isolate 10, *E. coli* R1-23 and *E. coli* W3-17. Data indicated that the position of nucleotides is different among isolates and identical data for all isolates are not shown by Bio Edit program version 7.2.5.

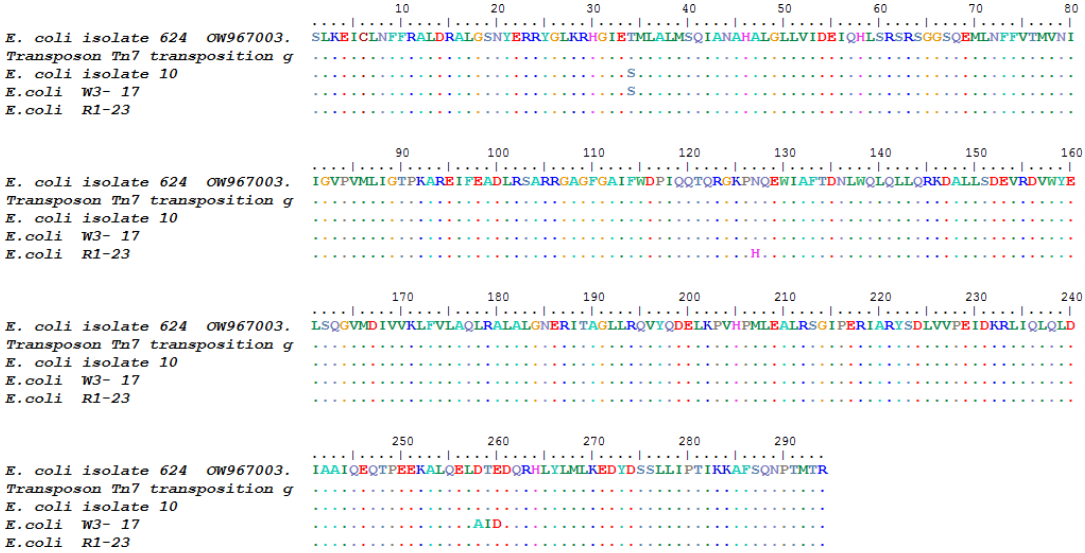


Figure (4-20): Pair sequence alignment of the amino acid of the TnsC protein among the local isolates including *E. coli* isolate 10, *E. coli* R1-23, *E. coli* W3-17 and Tns C of Tn7 transition of that available in databanks. draw based on the alignment of Bio Edit program version 7.2.5

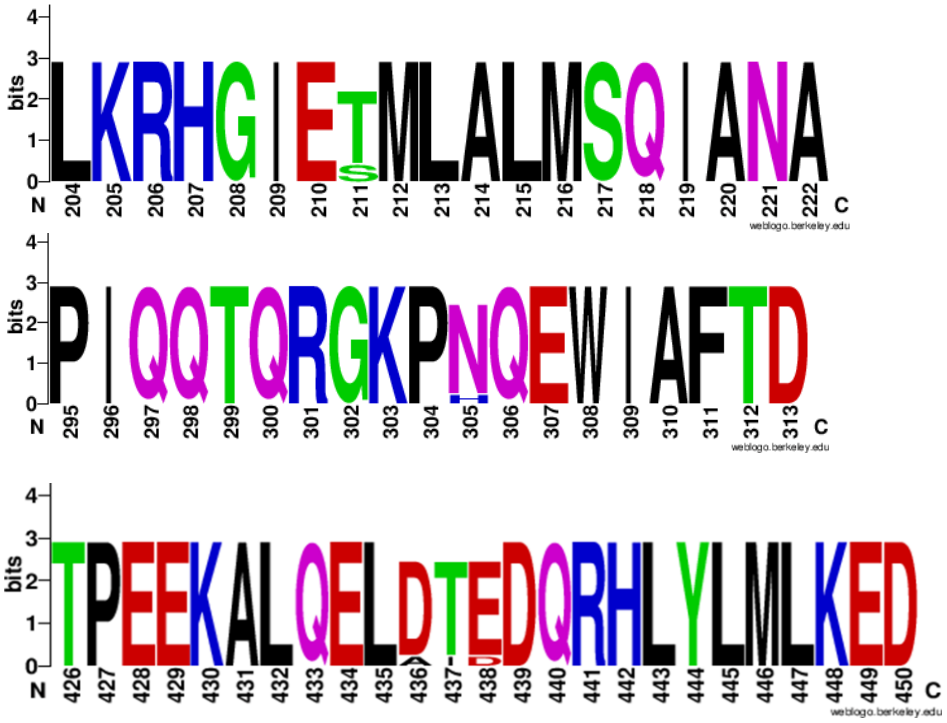
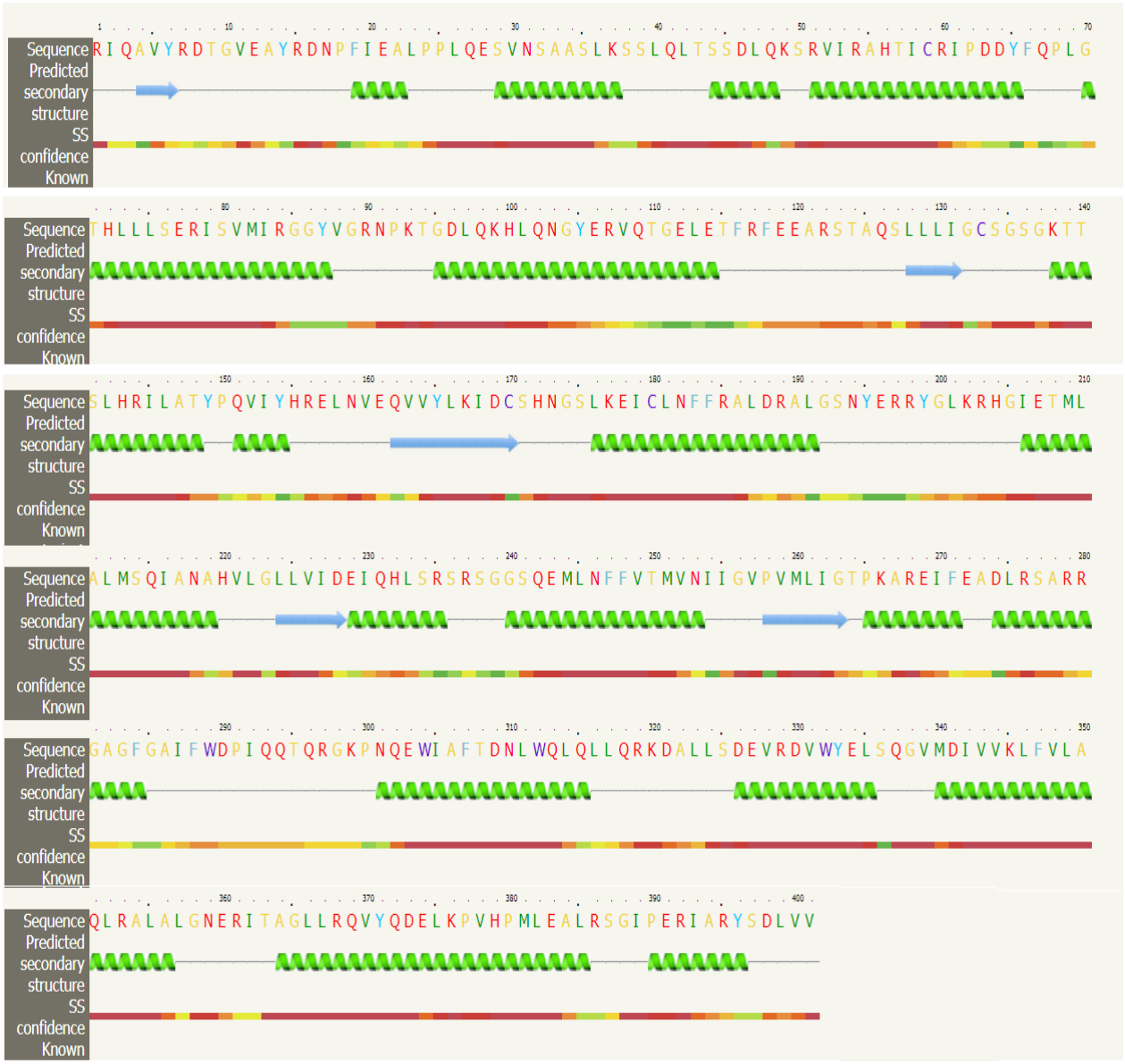


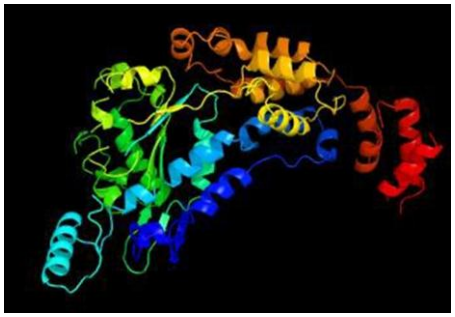
Figure (4-21): The Weblogo of repeats of TnsC protein. The sequences were each mutation site of the TnsC, a DNA binding protein as component of the Tn7 transposition system in *E. coli isolate10, E. coli R1-23 and E. coli* W3-17

The TnsC secondary structure of Tn7 databank strain and the local isolates *E. coli* W3-17 and *E. coli* R1-23. The local isolate *E. coli* W3-17 was modeled figure (4-22), the results appeared that the 285 residues (90% identity sequence of TnsC) have been modeled with 100% confidence and coverage 97% by the single highest scoring template c7mbwB and its 99% identity with c7mcsF model (1-503) residues of DNA –binding protein motif as shown in the figure (4-22-C) which revealed the mutations in the region 58-60 of protein was slightly effected on secondary structure with the percentage of disordered amino acid sequence (24% respectively) in compression with non-mutated TnsC protein (Tn7 of databank strains) which appeared 25% disordered amino acid sequence and the same percentage of Alpha helix and Beta strand (68% and 3% respectively). Also there was no effect on secondary structure of the other local isolates *E. coli* R1-23 figure (4-22-D) .

Thirteen TnsC protein of candidates from each of these three local isolates were chosen for further analyses where the amino acid sequence of TnsC was used to infer a phylogeny of the elements (Fig. 4-23). The phylogeny tree of TnsC protein revealed that all local isolates, including *E. coli* isolate 10, *E. coli* R1-23, and *E. coli* W3- produce DNA binding Protein Chain B (Tn7 transposition protein Tns C) were homologues (96.5% identity) to TnsC protein from Tn*7* of *Escherichia coli*, *Shigella sonnei* and *Shigella* which identified using BLAST searches and located in the same clade for TnsC of *E. coli* R1-23. Whereas *E. coli* W3-17 and *E. coli* isolate 10 appeared highly diverged from Tn*7* of candidate heteromeric elements that was located in different clade.

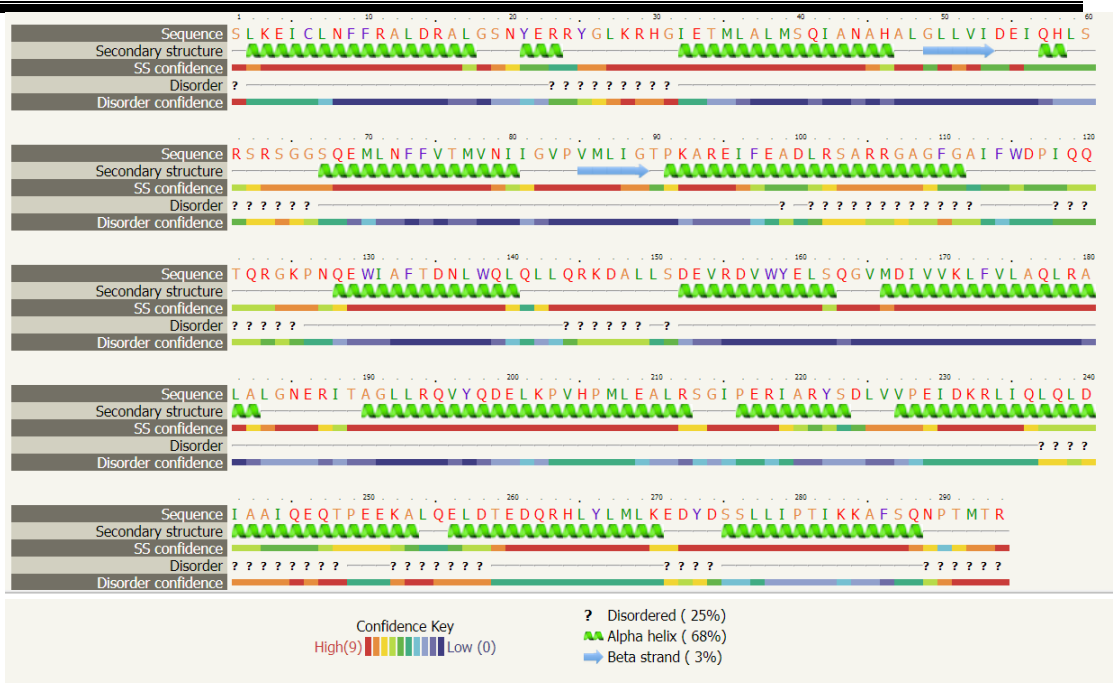
Fold library id	PDB Header	Molecule	Title
c7mcsF	3.56	PDB header: dna binding protein/dna	Chain: F: PDB Molecule: transposon tn7 transposition protein tnsc;



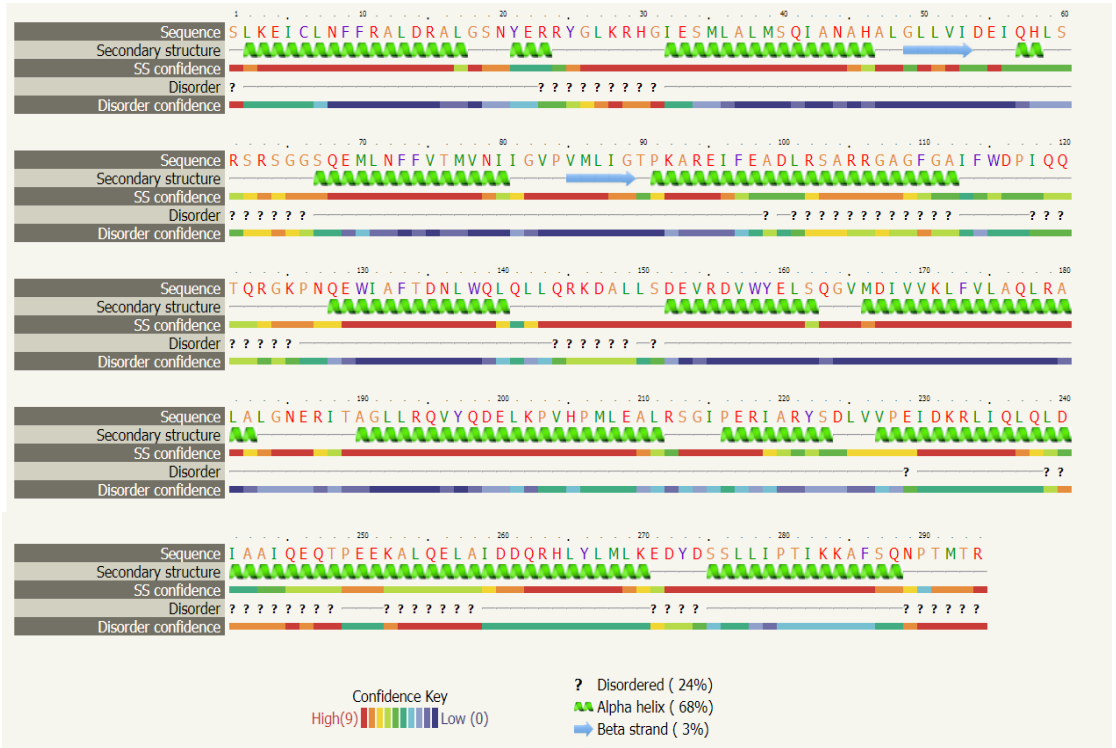


Cryo-electron microscopy structure of TnsC (1-503) A225V bound to DNA of c7mcsF model Source : *E. coli* **DOI:** 10.2210/pdb7mcs/pdb https://www.ebi.ac.uk/pdbe/entry/pdb/7mcs

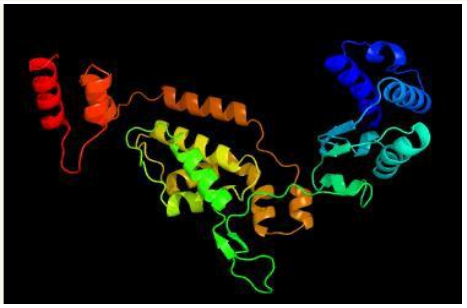
A: C7mbwB: Chain: B: PDB Molecule: transposon tn7 transposition protein tnsc;

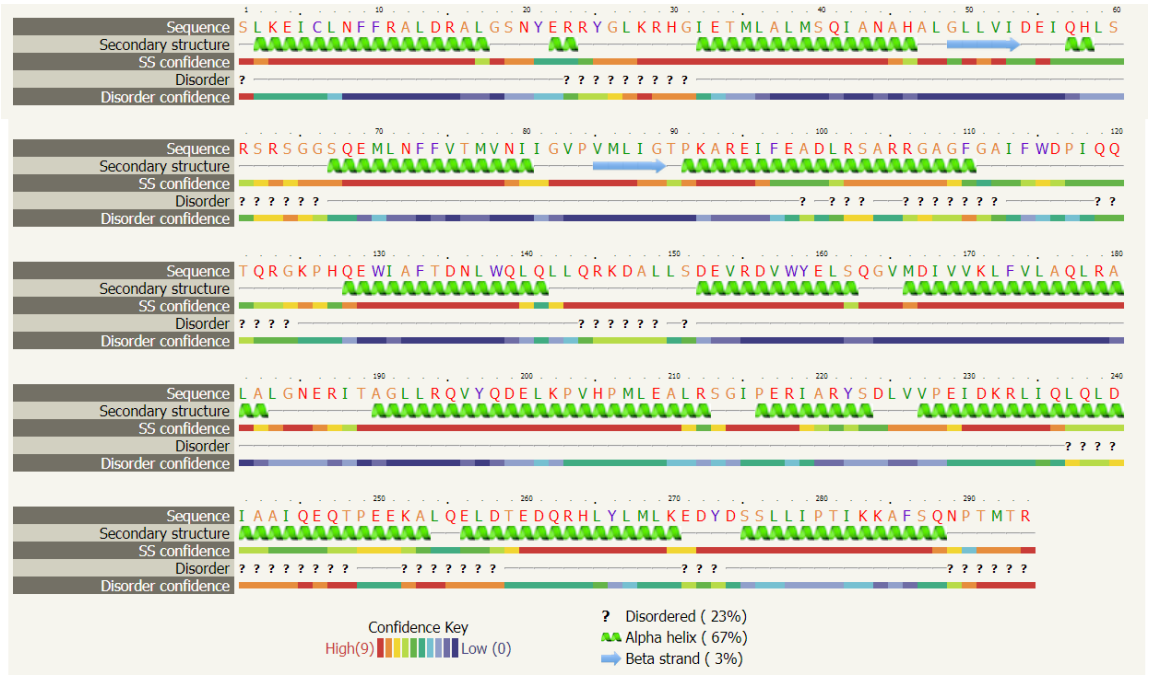


B: Tn7: Tns C protein of selected Databank strains. The **c7mbwB_** model had 284 residues that coverage 97% of the selected sequences, they have been modelled with 100.0% confidence by the single highest scoring template with 92% identity and 100% identity with **c7mcsF** model (1-503)a 225v bound to dna ,motif of DNA –binding



C: Tn7: Tns C protein of *E. coli* W3-17. The **c7mbwB_** model had 285 residues that coverage 97% of the selected sequences, they have been modelled with 100.0% confidence by the single highest scoring template with 90% identity and 99% identity with **c7mcsF** model (1-503) bound to dna (DNA –binding protein motif).





D: Tn7: Tns C protein of *E. coli* R1-23. The **c7mbwB** model had 284 residues that coverage 97% of the selected sequences, they have been modelled with 100.0% confidence by the single highest scoring template with 91% identity and 99% identity with **c7mcsF** model (1-503) bound to dna (DNA –binding protein motif).

Figure (4-22): Secondary structure prediction of the TnsC protein of selected reference standard Tn7 –TnsC protein (B) and the local isolates (C & D) and modeled with template c7mbwB and c7mcsF (A) .

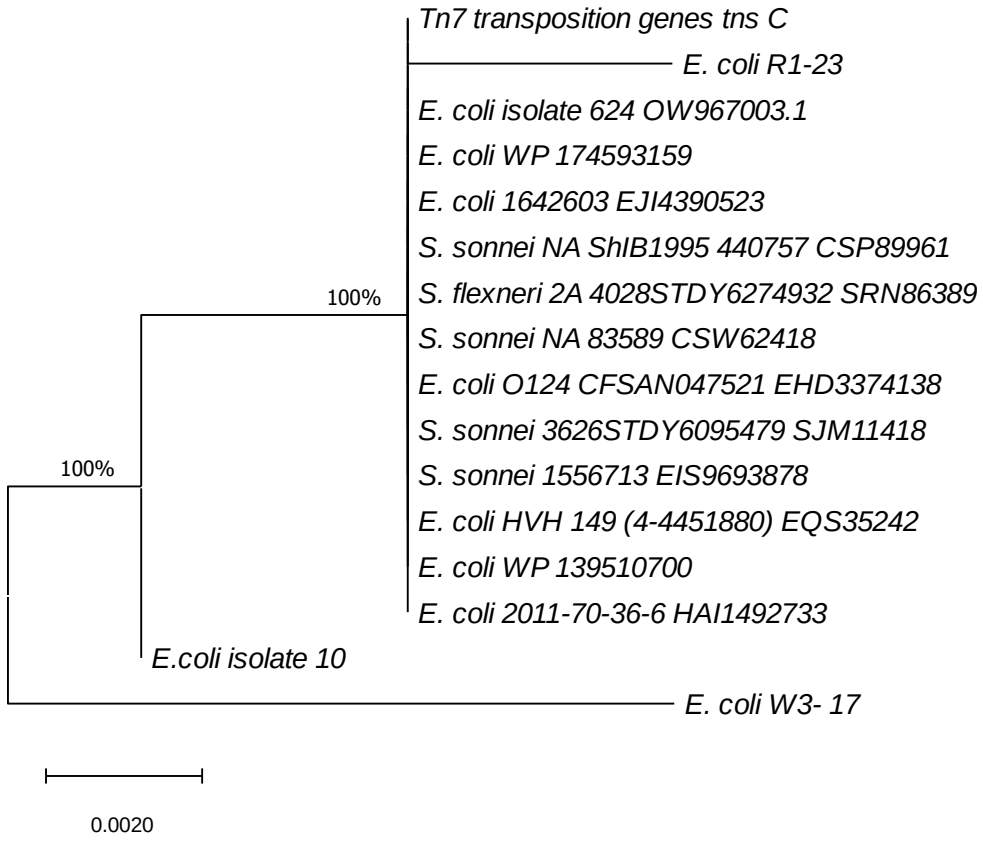


Figure (4-23): Phylogenetic tree of TnsC protein with selected TnsC protein homologues from Tn7 that available in NCBI database.

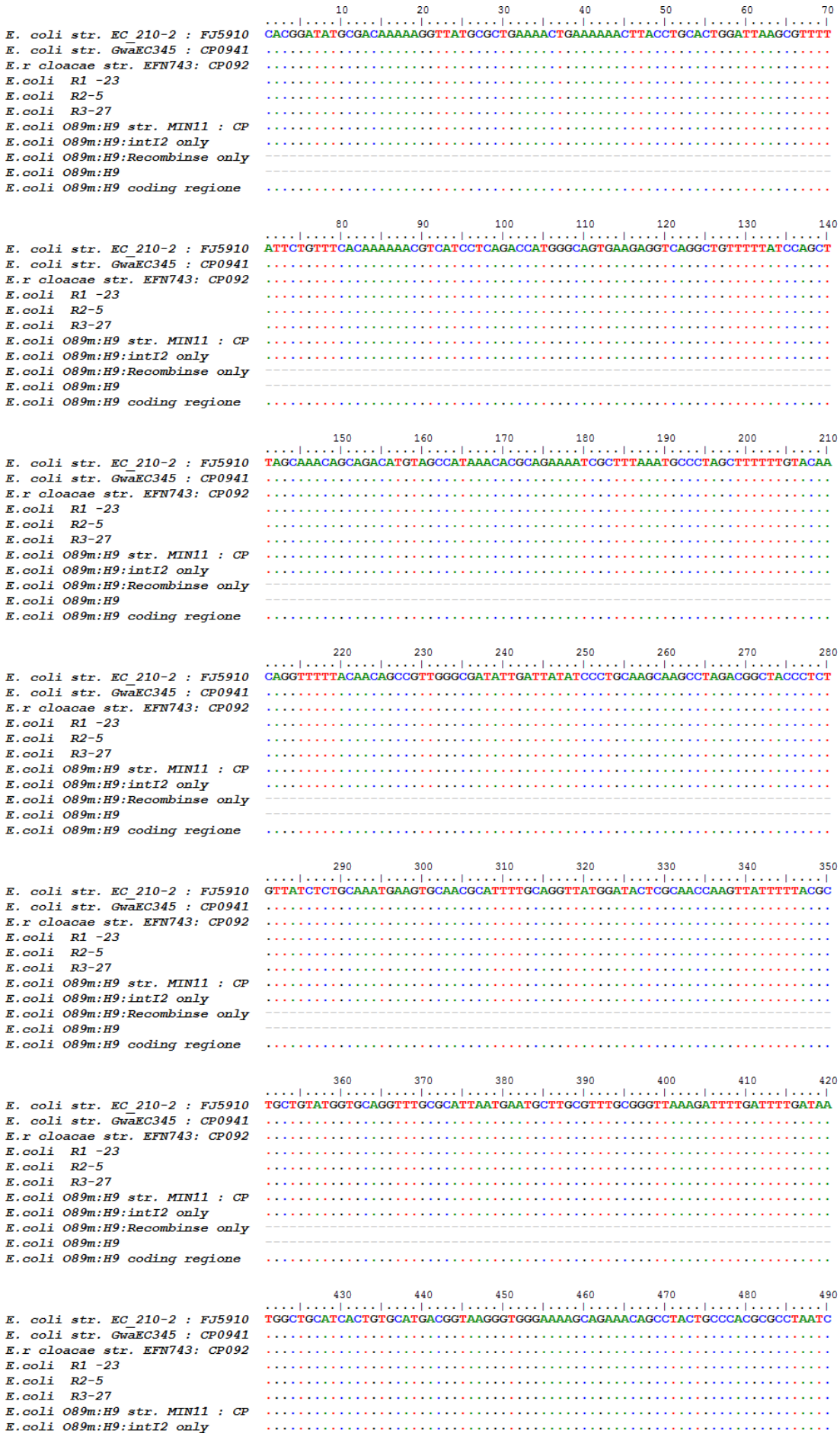
The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). Homologues to TnsC protein from Tn*7* of *Escherichia coli*, *Shigella sonnei* and *Shigella flexneri* which identified using BLAST searches (Altschul *et al*., 1997)

There are a number of elements that utilize AAA proteins for regulating transposition; one that is particularly well- characterized is MuB from bacteriophage Mu (Baker *et al*., 1991; Yamauchi and Baker, 1998). Work with MuB highlights an additional important role for these proteins in helping to engage the target DNA for transposition (Chaconas et al., 1985; Maxwell *et al*., 1987). Transposon Tn5090/Tn5053 also encodes a protein with ATP-binding motifs, TniB, which likely helps regulate transposition (Radstrom *et al*., 1994; Kholodii *et al*., 1995). Work with Tn7 indicates that TnsC is recruited to the attTn7 site by TnsD (Bainton *et al*., 1993; Kuduvalli *et al*., 2001).

4.5 Genetic analysis of integrase class 2 integron

The results of PCR-DNA sequencing of *intI2* gene in selected local isolates including *E. coli R1-23, E. coli R2-5 and E. coli R3-27* which alignment with databank strains revealed that the amplified fragment including two genes *intI2* gene (505bp encoding to 167aa) , non-coding region (114bp) and Tyrosine-type recombinase/ integrase gene (218bp encoding to 32aa) of the reference strains *E. coli* O89m:H9 strain MIN11 GenBank: CP069666.1 (integrase class 2;*intI2*: QTF31073.1 , Tyrosine-type recombinase-integrase QTF31074.1) as shown in the figures (4-24 and 4-25). The amplified non-coding region at the site 513 contain SNP T>A in Genbank strains and the local isolates *E. coli* R1-23, *E. coli* R2-5 & *E. coli* R3-27 contain SNP A>G at the site 693 of recombinase/integrase gene. That convert D aspartate >G glycine (Fig. 4- 24). whereas the *intI2* gene of local isolates was identical to the gene of the selected reference databank strains. The figure (4-25) showed the Evolutionary relationships of local isolates harboring *intI2* gene with available NCBI databank strains.

Integrons were DNA elements that function as gene-capture and expression systems. This element contains three necessary components located within the 50 conserved segment (the 5' and 3') include: an integrase gene (*IntI*), which encodes a site specific recombinase enzyme; an attI site, which is recognized by the integrase and acts as an acceptor for gene cassettes; and a promoter region (PC. Gene cassettes become a part of the integron when integrated, in addition to part of the family of tyrosine recombinase (Mazel , 2006; Wei *et al.*, 2014). This elements associated with the Tn7 family of transposons (Tn7 and its derivatives such as Tn1825, Tn1826, and Tn4132) and resistance to aminoglycosides, co-trimoxazole, cefalexin, ampicillin, and chloramphenicol . It is prevalence in clinical isolates in Gram-negative bacteria such as *Acinetobacter*, *Shigella*, *Salmonella*, *Pseudomonas* (Hansson *et al.*, 2002; Wu *et al*., 2016; Akrami *et al*., 2019).


10 20 30 40 50 60 70
E. coli str. EC_210-2 : FJ5910 CACGGATATGCGACAAAAAGGTTATGCGCTGAAAACTGAAAAAACTTACCTGCACTGGATTAAGCGTTTT
E. coli str. GwaEC345 : CP0941
E.r cloacae str. EFN743: CP092
E.coli R1 -23
E.coli R2-5
E.coli R3-27
E.coli O89m:H9 str. MIN11 : CP
E.coli O89m:H9:intI2 only
E.coli O89m:H9:Recombinse only
E.coli O89m:H9
E.coli O89m:H9 coding regione
80 90 100 110 120 130 140
E. coli str. EC_210-2 : FJ5910 ATTCTGTTTCACAAAAAACGTCATCCTCAGACCATGGGCAGTGAAGAGGTCAGGCTGTTTTTATCCAGCT
E. coli str. GwaEC345 : CP0941
E.r cloacae str. EFN743: CP092
E.coli R1 -23
E.coli R2-5
E.coli R3-27
E.coli O89m:H9 str. MIN11 : CP
E.coli O89m:H9:intI2 only
E.coli O89m:H9:Recombinse only
E.coli O89m:H9
E.coli O89m:H9 coding regione
150 160 170 180 190 200 210
E. coli str. EC_210-2 : FJ5910 TAGCAAACAGCAGACATGTAGCCATAAACACGCAGAAAATCGCTTTAAATGCCCTAGCTTTTTTGTACAA
E. coli str. GwaEC345 : CP0941
E.r cloacae str. EFN743: CP092
E.coli R1 -23
E.coli R2-5
E.coli R3-27
E.coli O89m:H9 str. MIN11 : CP
E.coli O89m:H9:intI2 only
E.coli O89m:H9:Recombinse only
E.coli O89m:H9
E.coli O89m:H9 coding regione
220 230 240 250 260 270 280
E. coli str. EC_210-2 : FJ5910 CAGGTTTTTACAACAGCCGTTGGGCGATATTGATTATATCCCTGCAAGCAAGCCTAGACGGCTACCCTCT
E. coli str. GwaEC345 : CP0941
E.r cloacae str. EFN743: CP092
E.coli R1 -23
E.coli R2-5
E.coli R3-27
E.coli O89m:H9 str. MIN11 : CP
E.coli O89m:H9:intI2 only
E.coli O89m:H9:Recombinse only
E.coli O89m:H9
E.coli O89m:H9 coding regione
290 300 310 320 330 340 350
E. coli str. EC_210-2 : FJ5910 GTTATCTCTGCAAATGAAGTGCAACGCATTTTGCAGGTTATGGATACTCGCAACCAAGTTATTTTTACGC
E. coli str. GwaEC345 : CP0941
E.r cloacae str. EFN743: CP092
E.coli R1 -23
E.coli R2-5
E.coli R3-27
E.coli O89m:H9 str. MIN11 : CP
E.coli O89m:H9:intI2 only
E.coli O89m:H9:Recombinse only
E.coli O89m:H9
E.coli O89m:H9 coding regione
360 370 380 390 400 410 420
E. coli str. EC_210-2 : FJ5910 TGCTGTATGGTGCAGGTTTGCGCATTAATGAATGCTTGCGTTTGCGGGTTAAAGATTTTGATTTTGATAA
E. coli str. GwaEC345 : CP0941
E.r cloacae str. EFN743: CP092
E.coli R1 -23
E.coli R2-5
E.coli R3-27
E.coli O89m:H9 str. MIN11 : CP
E.coli O89m:H9:intI2 only
E.coli O89m:H9:Recombinse only
E.coli O89m:H9
E.coli O89m:H9 coding regione
430 440 450 460 470 480 490
E. coli str. EC_210-2 : FJ5910 TGGCTGCATCACTGTGCATGACGGTAAGGGTGGGAAAAGCAGAAACAGCCTACTGCCCACGCGCCTAATC
E. coli str. GwaEC345 : CP0941
E.r cloacae str. EFN743: CP092
E.coli R1 -23
E.coli R2-5
E.coli R3-27
E.coli O89m:H9 str. MIN11 : CP
E.coli O89m:H9:intI2 only

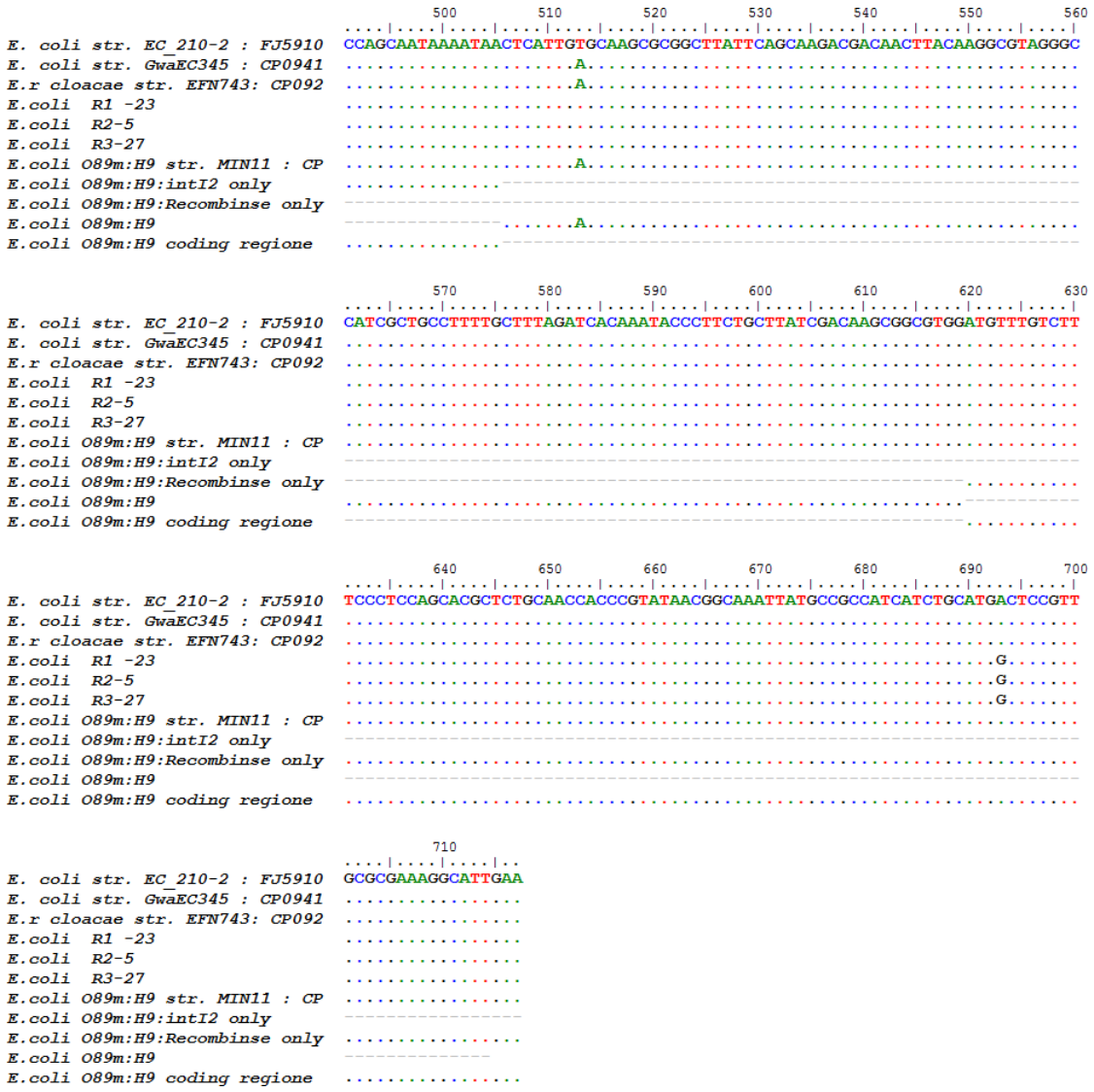


Figure (4-24): DNA alignment sequences of integrase class 2 integron gene with NCBI databank strains harboring *intI2* gene.

The amplified fragment including two genes *intI2* gene (505bp bp, 167aa) , non-coding region (114bp) and Tyrosine-type recombinase/ integrase gene (218bp and 32aa), Reference: *E. coli* O89m:H9 strain MIN11 GenBank: CP069666.1 integrase class 2;intI2: QTF31073.1 , Tyrosine-type recombinase-integrase QTF31074.1


10 20 30 40
E. coli str. EC_210-2 : FJ5910 TDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEV
Enterobacter cloacae str. EFN7 TDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEV
E.coli O89m:H9str.MIN11:CP0696 TDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEV
E.coli O89m:H9 non-coding
E.coli O89m:H9- coding region TDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEV
E. coli str. GwaEC345 plasmid TDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEV
E.coli R1-23 TDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEV
E.coli R2-5 TDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEV
E.coli R3-27 TDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEV
50 60 70 80
E. coli str. EC_210-2 : FJ5910 RLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGDI
Enterobacter cloacae str. EFN7 RLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGDI
E.coli O89m:H9str.MIN11:CP0696 RLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGDI
E.coli O89m:H9 non-coding
E.coli O89m:H9- coding region RLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGDI
E. coli str. GwaEC345 plasmid RLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGDI
E.coli R1-23 RLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGDI
E.coli R2-5 RLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGDI
E.coli R3-27 RLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGDI
90 100 110 120
E. coli str. EC_210-2 : FJ5910 DYIPASKPRRLPSVISANEVQRILQVMDTRNQVIFTLLYG
Enterobacter cloacae str. EFN7 DYIPASKPRRLPSVISANEVQRILQVMDTRNQVIFTLLYG
E.coli O89m:H9str.MIN11:CP0696 DYIPASKPRRLPSVISANEVQRILQVMDTRNQVIFTLLYG
E.coli O89m:H9 non-coding
E.coli O89m:H9- coding region DYIPASKPRRLPSVISANEVQRILQVMDTRNQVIFTLLYG
E. coli str. GwaEC345 plasmid DYIPASKPRRLPSVISANEVQRILQVMDTRNQVIFTLLYG
E.coli R1-23 DYIPASKPRRLPSVISANEVQRILQVMDTRNQVIFTLLYG
E.coli R2-5 DYIPASKPRRLPSVISANEVQRILQVMDTRNQVIFTLLYG
E.coli R3-27 DYIPASKPRRLPSVISANEVQRILQVMDTRNQVIFTLLYG
130 140 150 160
E. coli str. EC_210-2 : FJ5910 AGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPT
Enterobacter cloacae str. EFN7 AGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPT
E.coli O89m:H9str.MIN11:CP0696 AGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPT
E.coli O89m:H9 non-coding
E.coli O89m:H9- coding region AGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPT
E. coli str. GwaEC345 plasmid AGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPT
E.coli R1-23 AGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPT
E.coli R2-5 AGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPT
E.coli R3-27 AGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPT
170 180 190 200
E. coli str. EC_210-2 : FJ5910 RLIPAIK-CTCATTGTGCAAGCGCGGCTTATTCAGCAAGA
Enterobacter cloacae str. EFN7 RLIPAIK-CTCATTGAGCAAGCGCGGCTTATTCAGCAAGA
E.coli O89m:H9str.MIN11:CP0696 RLIPAIK-CTCATTGAGCAAGCGCGGCTTATTCAGCAAGA
E.coli O89m:H9 non-coding --------CTCATTGAGCAAGCGCGGCTTATTCAGCAAGA
E.coli O89m:H9- coding region RLIPAIK
E. coli str. GwaEC345 plasmid RLIPAIK-CTCATTGAGCAAGCGCGGCTTATTCAGCAAGA
E.coli R1-23 RLIPAIK-CTCATTGTGCAAGCGCGGCTTATTCAGCAAGA
E.coli R2-5 RLIPAIK-CTCATTGTGCAAGCGCGGCTTATTCAGCAAGA
E.coli R3-27 RLIPAIK-CTCATTGTGCAAGCGCGGCTTATTCAGCAAGA
210 220 230 240
E. coli str. EC_210-2 : FJ5910 CGACAACTTACAAGGCGTAGGGCCATCGCTGCCTTTTGCT
Enterobacter cloacae str. EFN7 CGACAACTTACAAGGCGTAGGGCCATCGCTGCCTTTTGCT
E.coli O89m:H9str.MIN11:CP0696 CGACAACTTACAAGGCGTAGGGCCATCGCTGCCTTTTGCT
E.coli O89m:H9 non-coding CGACAACTTACAAGGCGTAGGGCCATCGCTGCCTTTTGCT
E.coli O89m:H9- coding region

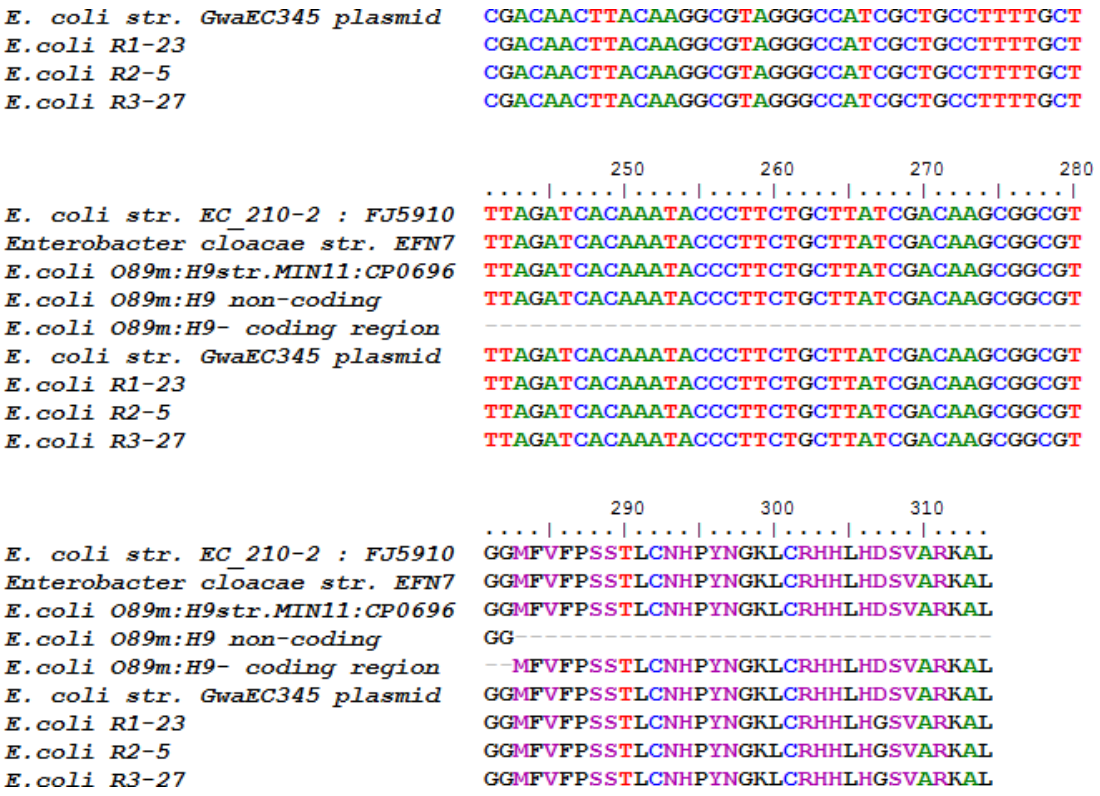


Figure (4-25): Pair sequence alignment of amino acid of integrase class 2 integron protein with additional sequence of tyrosine type-recombinase/integrase protein which separated by non-coding region among local isolates and databank strains.

intI2 gene (505bp out of 537bp encoded 167aa out of 178aa, non-coding region 114bp) and Tyrosine-type recombinase/ integrase gene (218bp out 327bp encoded to 32aa out of 108aa), Reference: Escherichia coli O89m:H9 strain MIN11 GenBank: CP069666.1 integrase class 2;intI2: QTF31073.1 , Tyrosine-type recombinase- integrase QTF31074.1 draw based on the alignment of Bio Edit program version 7.2.5.

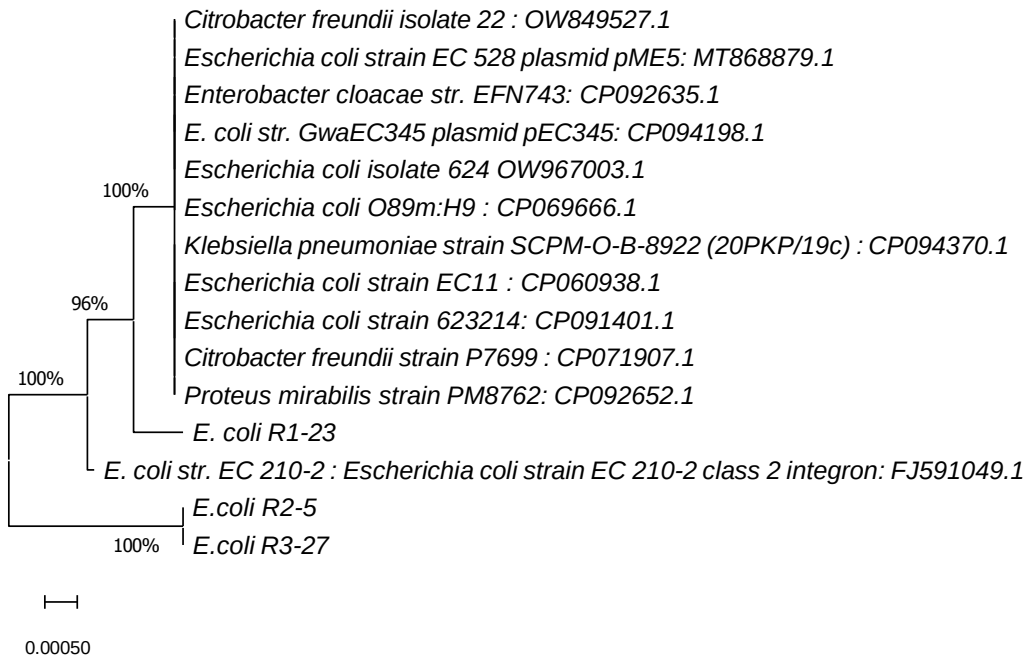


Figure (4-26): Phylogenetic tree of local isolates harboring *intI2* gene with available NCBI databank strains

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). This analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 747 positions in the final dataset.

The overall results of this study indicate The Tn7-like transposons are major mobile elements to transfer antimicrobial multidrug resistance among enteriobacterial species. The information obtained from previous studies about the transposition mechanism of the Tn7-like transposons and its derivative in comparison with available data of this study on the comprehensive analysis of Tn7-like transposons in *E. coli* and *Klebsiella* local isolates are still insufficient. So this study was determined the occurrence of Tn7-like transposons in *E. coli* and *Klebsiella* isolates obtained from three sources including human healthy stool, patient stool with enterogastritis and human urine with UTI. Among the one hundred.

E. coli and *Klebsiella* isolates, 76 strains that carry Tn7- like transposons genes were identified, including 32% harbored complete core of transposition genes (*tns* ABC) and 44% had one or two genes (*tns*AB or *tnsAC*)

that indicated may be derivatives or types of Tn7- like transposons and three of them associated with the presences of integron class 2 integrase (*intI2* gene). In both genus of bacteria, the separation rate of Tn7-like transposons was different according source of isolations (healthy human could be asymptomatic carriers of the bacteria and may cause an impact on human health under appropriate conditions like weak immunity. These results provide evidence of the Enterobacteriaceae strains as a possible reservoir of Tn7-like transposons, a risk that deserves the attention. Antimicrobial susceptibility testing showed that *E. coli* and *Klebsiella* local strains harboring Tn7-like transposons displayed high resistance to a variety of antibiotics, with a 62% multidrug resistance rate (more than three class of antibiotics). Comparatively high rates of MDR in *Klebsiella* (61.53%) and *E. coli* (75.68%) strains may be due to the occurrence of multiple mobile elements in both bacteria, which was confirmed in previous studies (Beutlich *et al.*, 2011; Murgia *et al.*, 2015; Schultz *et al.*, 2015; Lei *et al.*, 2018; He *et al.*, 2020). This also indirect that Tn7-like transposons were likely to occur at the same time with other mobile elements. High resistance to pencillins , cephalosporins and Macrolids can be attributed to the intI2-associated resistance gene cassette carried by Tn7-like transposons

Relatively low resistance rates to gentamicin, kanamycin and ciprofloxacin were detected in the Tn7-like transposons positive strains. Furthermore, there was a significant association ($P < 0.05$) between the presence of Tn7 and intI2 genes and the resistance to kanamycin and gentamicin may be attributed to the intI2-associated resistance gene cassette carried by Tn7-like transposons. These results consist with the Doosti *et al.* (2016) study that reported there was a significant association between the presence of aadB gene and resistance to kanamycin and gentamicin, The aadB gene related with gene cassettes of *intI2*.

The diversity of Tn7-like transposons increased by various IS inserted sequences in these transposons which prompted by the ISs-mediated homologous recombination. For example, Tn6765 was highly homologous with Tn6450, the similarity of nucleic acid sequence was more than 99%, and the coverage was 96%, which was located on the chromosome detected by Chen *et al*. (2018). A study of He *et al.* (2020) was identified six Tn7-like transposons genetic structures by WGS analysis, which revealed that three of them (Tn6763, Tn6764, and Tn6817) were a typical Tn7 transposons structure and three novel Tn7- like transposons including Tn6813, Tn6814, and Tn6765 which were integrated into plasmids or chromosomes from *E. coli* and *P. mirabilis*. Also found the Tn6814 contains IS26 segment that showed high level homology to the segment characterized in *E. coli* plasmid pV408 and it carrying different mobile genetic elements (complete or truncated) and two resistance genes including blaCTX-M-65 and fosA3. The gene blaCTX-M-65 comprise of fragment (IS26-fipA-IS1380-blaCTX-M65- IS903B) in Tn6814 also indicated nucleotide identity to the consistent genetic structure that enclosed blaCTX-M-65 gene in Tn6813, with the exception of one IS26 sequence substituted by IS903B sequence in Tn6814.

Obviously, for Enterobacterales, carriage of Tn7-like transposons, each containing a set of resistance genes, may increase the chances of horizontal transfer of multiple resistance determinants to susceptible strains and may in turn bring unique advantages to the host and enable them to survive a strong antimicrobial selection pressure especially in commensal bacteria. Tn7-like transposons have been so successful at spreading into diverse relevant taxa that they could be used as a proxy for antibiotics pollution (Flores et al., 1992; Cleaver and Wickstrom, 2000). The carrier difference between Tn6765 and Tn6450 indicated that Tn7- like can be transmitted alternately on the chromosome and plasmid by cutting and inserting. To make matters worse, Tn6450 comes from a chicken source (Chen et al., 2018) and Tn6765 from a

swine source suggested that Tn7-like transposons can be transmitted from one animal to another with bacterial hosts (He et al., 2020).

The "core transposition machinery" of Tn7-like transposon consists of the transposase proteins TnsA and TnsB along with a regulator protein, TnsC (Choi *et al*., 2013). This core machinery is directed by one of two target selecting proteins, TnsD or TnsE. Transposition with TnsABC+TnsD has evolved to maximize the efficiency of vertical transmission of the element by directing transposition into the chromosome. Transposition with TnsABC+TnsE occurs preferentially into mobile plasmids through the ability of the TnsE protein to recognize features found enriched during DNA replication on the lagging-strand template (Wolkow et al., 1996; Peters and Craig, 2000, 2001a). Therefore, it cannot rule out the possibility that these Tn7-like transposons located on the chromosome will target transposition into mobile plasmids, facilitating the spread of Tn7-like transposons between bacteria during the propagation of the hosts. By comparing the genetic structure of different Tn7-like transposons, we can speculate that the multidrug-resistant Tn7- like transposons have a certain evolutionary relationship, which has contributed to Tn7-like transposons playing a vital role in the field of storing resistance genes. Although the assembly was original, each of these Tn7-like transposons, or parts thereof, was identical to those found in other plasmids or chromosomes. This was related to the fact that Tn7-like transposons can transfer between strains and accumulate genetic material via mobile genetic elements (He *et al.*, 2020).

An evolutionary direction of the Tn7 like transposons carrying strains by conjugation which the Tn7-carrying plasmid was successfully transferred to another strains. Although Tn7 located on the chromosomes could not be transferred by conjugation, that they could stably exist in the hosts. The comprehensively analyze study reported that the antibiotic resistance characteristics of Tn7-like transposons in Enterobacterales isolates from

livestock and poultry in China can be transferred from isolate to other by conjugation when Tn7-like transposons were integrated into plasmids in *E. coli* and *P. mirabilis*. Also they considered the multiple antibiotic resistance genes in Tn7-like transposons, that were found in chicken and swine, are highly troublesome and may become a serious risk by spreading in other nearby animals, humans, and the environment. Therefore, they recommended to vigorous measure should be taken to control the spread and emergence of mobile genetic resistance determinants in animals used for food production in China and the world (He *et al.*, 2020).

4.6 Molecular Bacterial Diagnosis

PCR and genetic sequencing methods were used to identify the confused bacterial isolates and some selected isolates which harbor Tn7- like transposon, relying on the partial sequences of 16S ribosomal RNA gene, this is based on specific primes of Enterobacteriaceae (taxid:543)**.** After that reconstructing phylogenetic trees. The results revealed that the presence a single band (250 bp) of the target sequence of *16S rRNA* gene in agarose gel (Fig. 4-27).

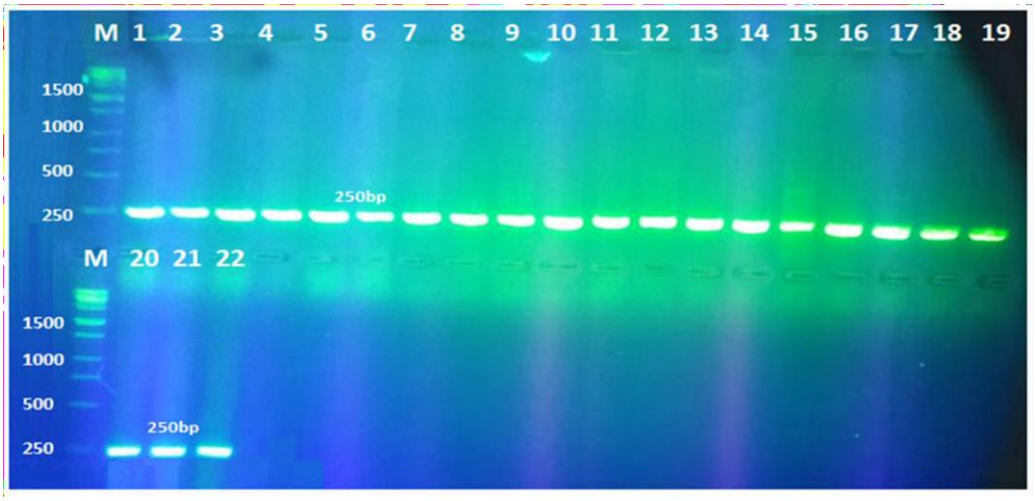


Figure (4-27) Agarose gel electrophoresis of *16SrRNA* amplified product patterns of *E. coli* and *Klebsiella spp.*

M: refers to DNA size marker 250-2000bp. Lanes 1 - 15 refer to PCR products of *16S rRNA* gene (250bp) of *E. coli* and *lanes 16-22 refer to Klebsiella spp.* isolates. Electrophoresis conditions: 1.5% agarose concentration; 100 V, 20 mA for 60 min. Staining method; precast RED Safe stain.

Pathogenic strains of *E. coli* are critical causes of diarrhea. However, they cannot be distinguished from *E. coli* of the intestinal microbiota by conventional microbiological tests. So the molecular analysis depending on PCR-16SrRNA gene sequencing is one of the vital method for bacterial identifications. The sequencing of 16S rRNA gene was conducted using genetic analyzer (ABIPrism 3130 and 3130 xlGeneticAnalyzer) at Macrogen sequencing company (Macrogen - Korea). The sequencing used both Primers forward and revers of 16SrRNA gene. The sequences were edited to exclude the PCR primer binding sites and manually were corrected using MEGA X version software. The partial gene sequences of *E. coli* and *Klebsiella* strains were compared automatically using the BLAST against the sequences of bacteria available in databanks (http://www.ncbi.nlm.nih.gov/). The phylogenetic analysis was performed using ClustalW Multiple alignment programed in BioEdit Sequence Alignment Editor software, and ClustalW programed in the MEGA .X software. The phylogenetic tree was constructed using the neighbor-joining algorithm (Saitou and Nei,1987 ; Tamura et al., 2004; Kumar et al., 2018).

The analysis of 16S rRNA gene of *E. coli* and *Klebsiella spp*. which harbor Tn7-like transposon as one an objective of this study to be confirmed their diagnosis by partial sequences (250 bp) of the 16S rRNA gene of both species. These species were isolated from different sources.

Alignment of the 16S rRNA gene of *E. coli* isolates W3-17, R1-23, R2-5 and R3-27 and *E. coli* W2-4,in addition to *Klebsiella sp.* W4-19, and W1-21 isolates against some of bacterial species. those available in databanks are shown in Figures (4-29) , (4-31) for *E. coli* and Figures (4- 33) and (4-35) for *Klebsiella spp*. According to the figures (4-28), they showed some similarity or difference between the aligned nucleotide

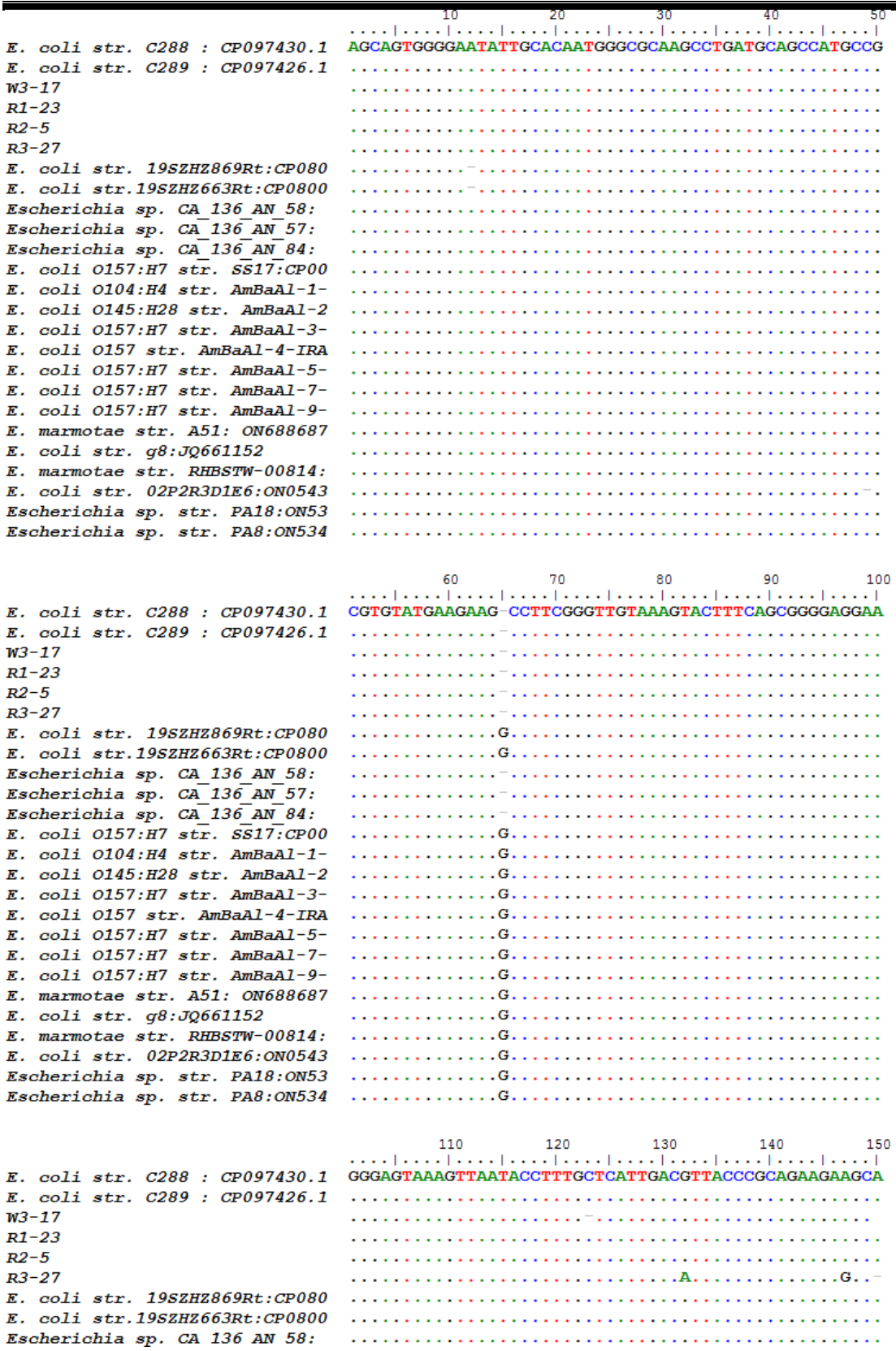
sequences. *E. coli* W3-17, R1-23, R2-5 and R3-27strains have a tendency to show similarity of nucleotide sequences about 98-100% with isolates originating from the same pathogenic species. These results of 16SrRNA gene sequencing are an extra method to identify bacteria, that are consistent with the previous study. The successfully using of 16S rRNA gene sequencing by Patel (2001) and Woo *et al.* (2008) to identify bacterial pathogen and discovery of novel bacteria in clinical microbiology laboratories; Also, Fattahi *et al.* (2013) established new method depending to 16S rRNA sequencing as a target for detection and classification of *Escherichia coli* in rainbow trout. That because of some causes, including the 16S rRNA gene is present in all bacteria as multigene family or operons and the function of 16S rRNA gene has not changed over time, making it a random sequence changes are more accurate measure of time (evolution); As well as the large size of 16S rRNA gene (1500 bp) became enough for bioinformatics analysis (Patel, 2001; Woo *et al.*, 2008; Fattahi *et al.*, 2013).

The analysis of similarity or nucleotides differences of the local *E. coli* strains (W3-17, R1-23, R2-5 and R3-27) that are summarized in Table (4-5) (Appendix2), which contain the percentage identity of the nucleotide sequence of databank bacterial strains as reference species with the local *E. coli* strains.

For sequence analysis, previous studies suggested the threshold values of sequence similarities of 99% and 95% for species and genus assignment, respectively (Bosshard *et al*., 2003; Fattahi *et al.*, 2013).

The summary of the 16S rRNA similarity analysis in Table (4-5) showed that *E. coli* R1-23 and *E. coli* sp. R2- 5 that originated from human feces with diarrhea had nucleotide similarity of 16S rRNA gene closely against some strains. These strains such as *E. coli* C288, *E. coli* C289, *Escherichia sp.* CA_136_AN_84, 57 & 58 strains and the Iraqi

strains belong to *E. coli* O157:H7 which originated from human feces and cattle feces and in addition to other databank strains mentioned in the Table(4-5) (Appendix 2) , are as high as 99.46-100%. Furthermore, and *E. coli* W3- 17 and *E. coli* sp. R3- 27 that originated from human urine with UTI also had high nucleotides similarity to the data of 16S rRNA that are available in GenBank also. It has nucleotides similarity to the same *E. coli* strains as high as 99.32-97.95%. Referred to the concept of similarity or nucleotides different between the query nucleotides and those compared, It is recommended when the sequences similarity is more than 90% when the query sequence more than 500 base , the query should be categorized as the same species (Bosshard *et al*., 2003; Janda and. Abbott, 2007). It may be that the high nucleotide similarity of 16S rRNA genes for isolates originated from cattle and humans and the result of the potential strain originated from cattle feces as a major reservoir and then passed on to humans as an apparent new host. The transmission of this bacterium from animals (cattle) to human can be enabled by the ingesting of meat that is less cooked or drinking water contaminated with feces or unpasteurized dairy products (Nataro and Kaper, 1998; Suardana, 2014)


10 20 30 40 50
E. coli str. C288 : CP097430.1 AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG
E. coli str. C289 : CP097426.1
W3-17
R1-23
R2-5
R3-27
E. coli str. 19SZHZ869Rt:CP080
E. coli str.19SZHZ663Rt:CP0800
Escherichia sp. CA_136_AN_58:
Escherichia sp. CA_136_AN_57:
Escherichia sp. CA_136_AN_84:
E. coli O157:H7 str. SS17:CP00
E. coli O104:H4 str. AmBaAl-1-
E. coli O145:H28 str. AmBaAl-2
E. coli O157:H7 str. AmBaAl-3-
E. coli O157 str. AmBaAl-4-IRA
E. coli O157:H7 str. AmBaAl-5-
E. coli O157:H7 str. AmBaAl-7-
E. coli O157:H7 str. AmBaAl-9-
E. marmotae str. A51: ON688687
E. coli str. g8:JQ661152
E. marmotae str. RHBSTW-00814:
E. coli str. 02P2R3D1E6:ON0543
Escherichia sp. str. PA18:ON53
Escherichia sp. str. PA8:ON534
60 70 80 90 100
E. coli str. C288 : CP097430.1 CGTGTATGAAGAAG-CCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA
E. coli str. C289 : CP097426.1
W3-17
R1-23
R2-5
R3-27
E. coli str. 19SZHZ869Rt:CP080
E. coli str.19SZHZ663Rt:CP0800
Escherichia sp. CA_136_AN_58:
Escherichia sp. CA_136_AN_57:
Escherichia sp. CA_136_AN_84:
E. coli O157:H7 str. SS17:CP00
E. coli O104:H4 str. AmBaAl-1-
E. coli O145:H28 str. AmBaAl-2
E. coli O157:H7 str. AmBaAl-3-
E. coli O157 str. AmBaAl-4-IRA
E. coli O157:H7 str. AmBaAl-5-
E. coli O157:H7 str. AmBaAl-7-
E. coli O157:H7 str. AmBaAl-9-
E. marmotae str. A51: ON688687
E. coli str. g8:JQ661152
E. marmotae str. RHBSTW-00814:
E. coli str. 02P2R3D1E6:ON0543
Escherichia sp. str. PA18:ON53
Escherichia sp. str. PA8:ON534
110 120 130 140 150
E. coli str. C288 : CP097430.1 GGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCA
E. coli str. C289 : CP097426.1
W3-17
R1-23
R2-5
R3-27
E. coli str. 19SZHZ869Rt:CP080
E. coli str.19SZHZ663Rt:CP0800
Escherichia sp. CA 136 AN 58:

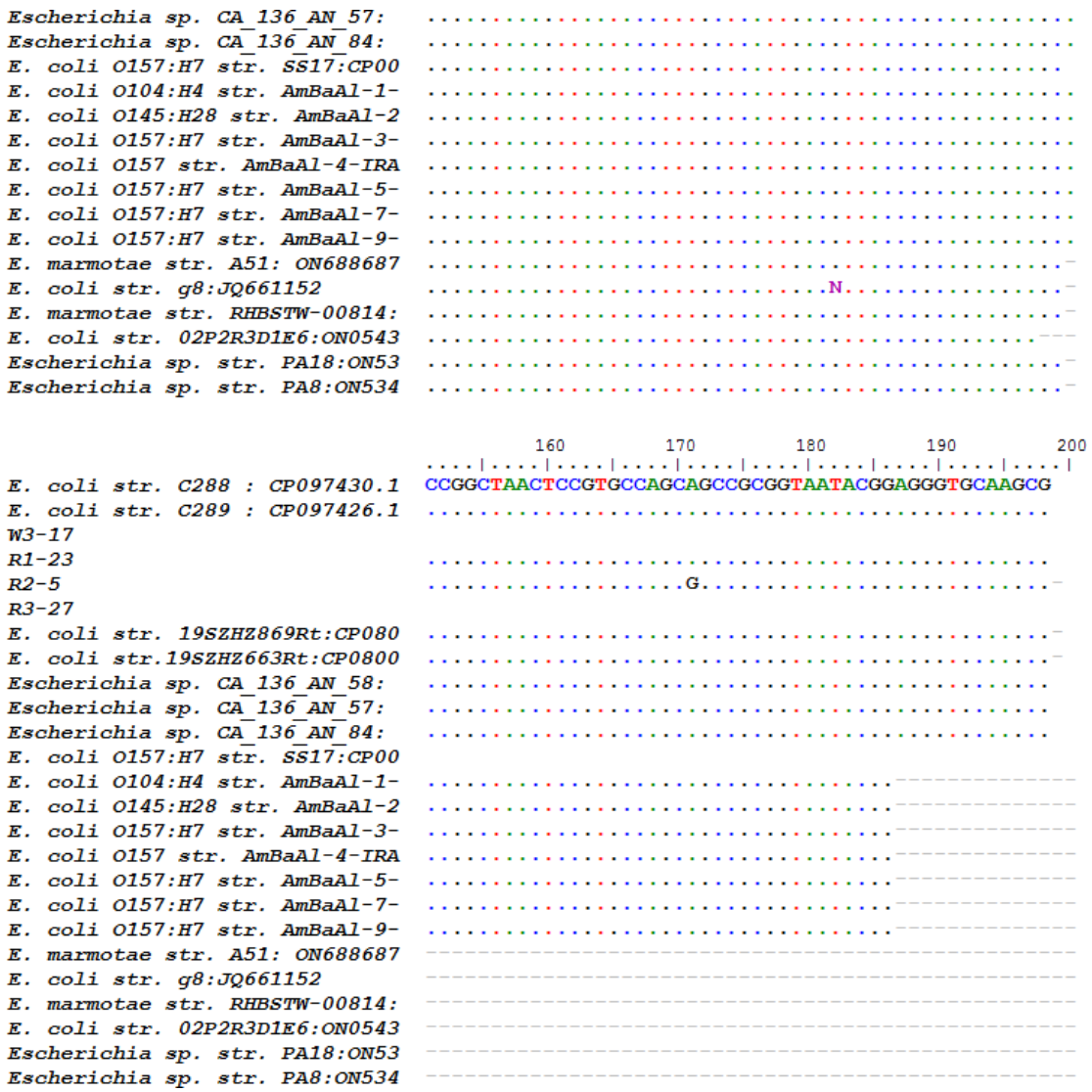


Figure (4-28): Nucleotides sequence of the 16S rRNA gene of the isolates *E. coli* strain R1-23, strain R2-5, strain R3-27 and strain W3-17 among nucleotides sequence of those available in databanks. Data indicated that the position of nucleotides is different among isolates and identical data for all isolates are not shown.

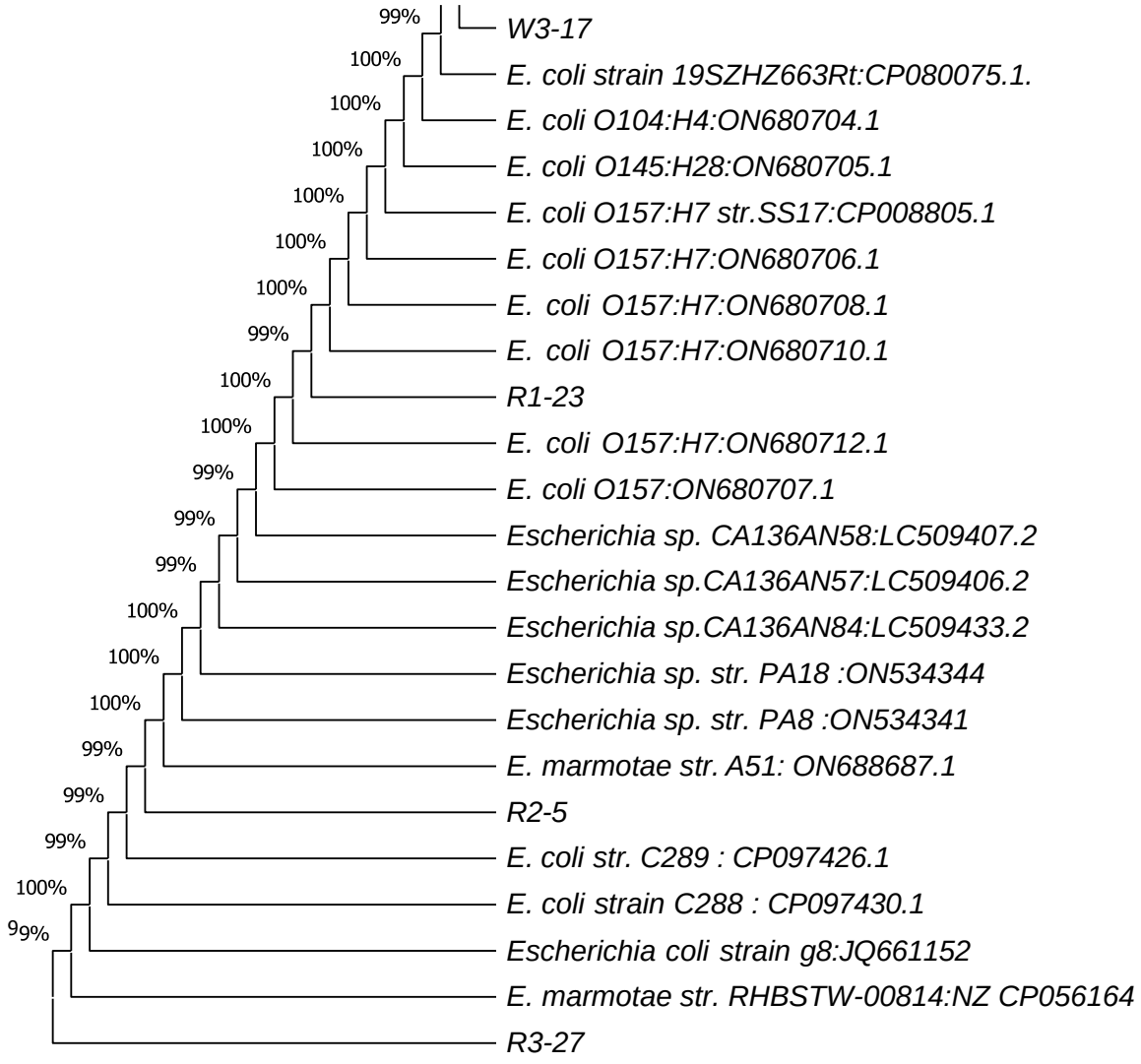


Figure (4-29): Phylogenetic tree of *E. coli* strain R1- 23,strain R2-5, strain R3-27 and strain W3-17 with other NCBI databank strains.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). This analysis involved 25 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 149 positions in the final dataset.

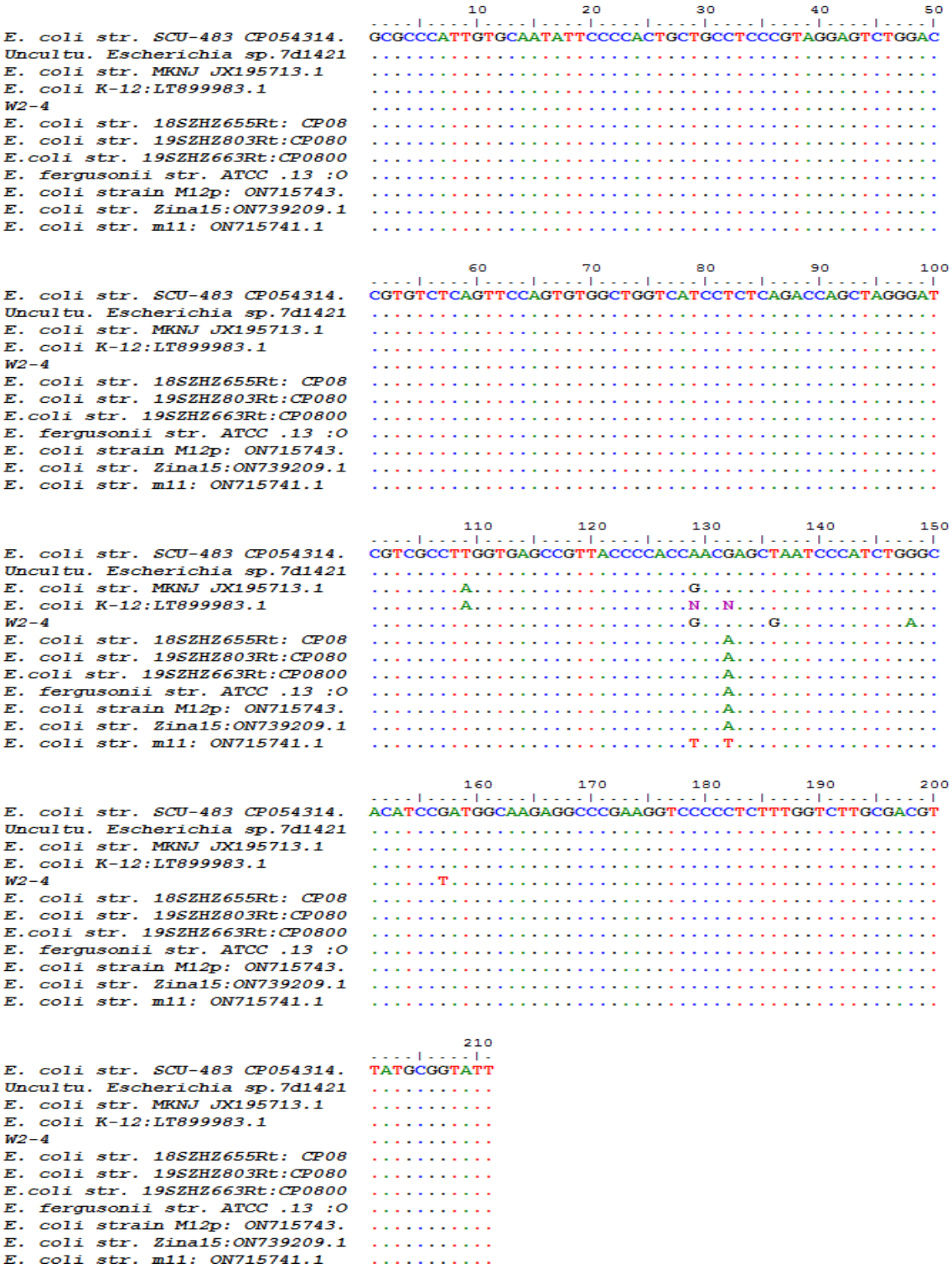


Figure (4-30): Nucleotides sequence of the 16S rRNA gene of the isolate *Escherichia coli* strain W2-4 among nucleotides sequence of those available in databanks. Data indicated that the position of nucleotides is different among isolates and identical data for all isolates are not shown.

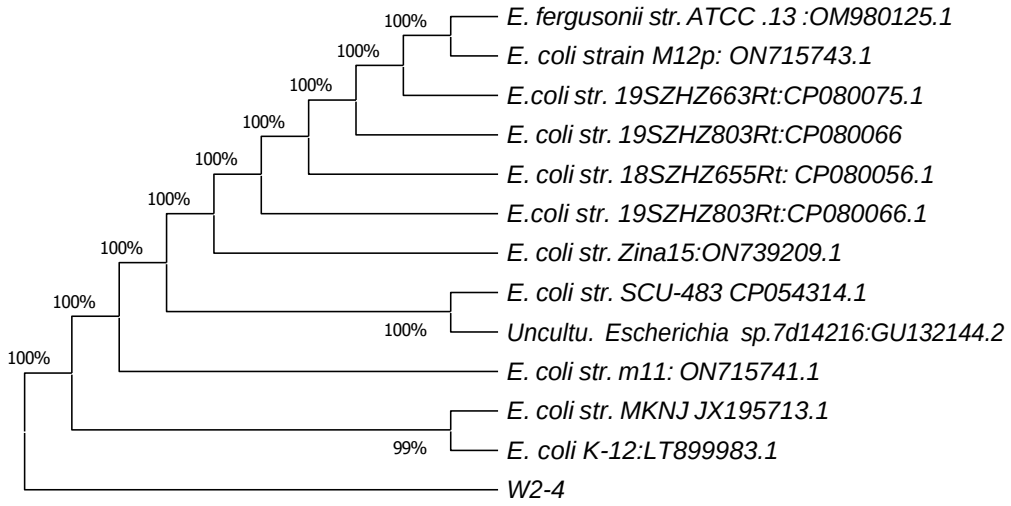
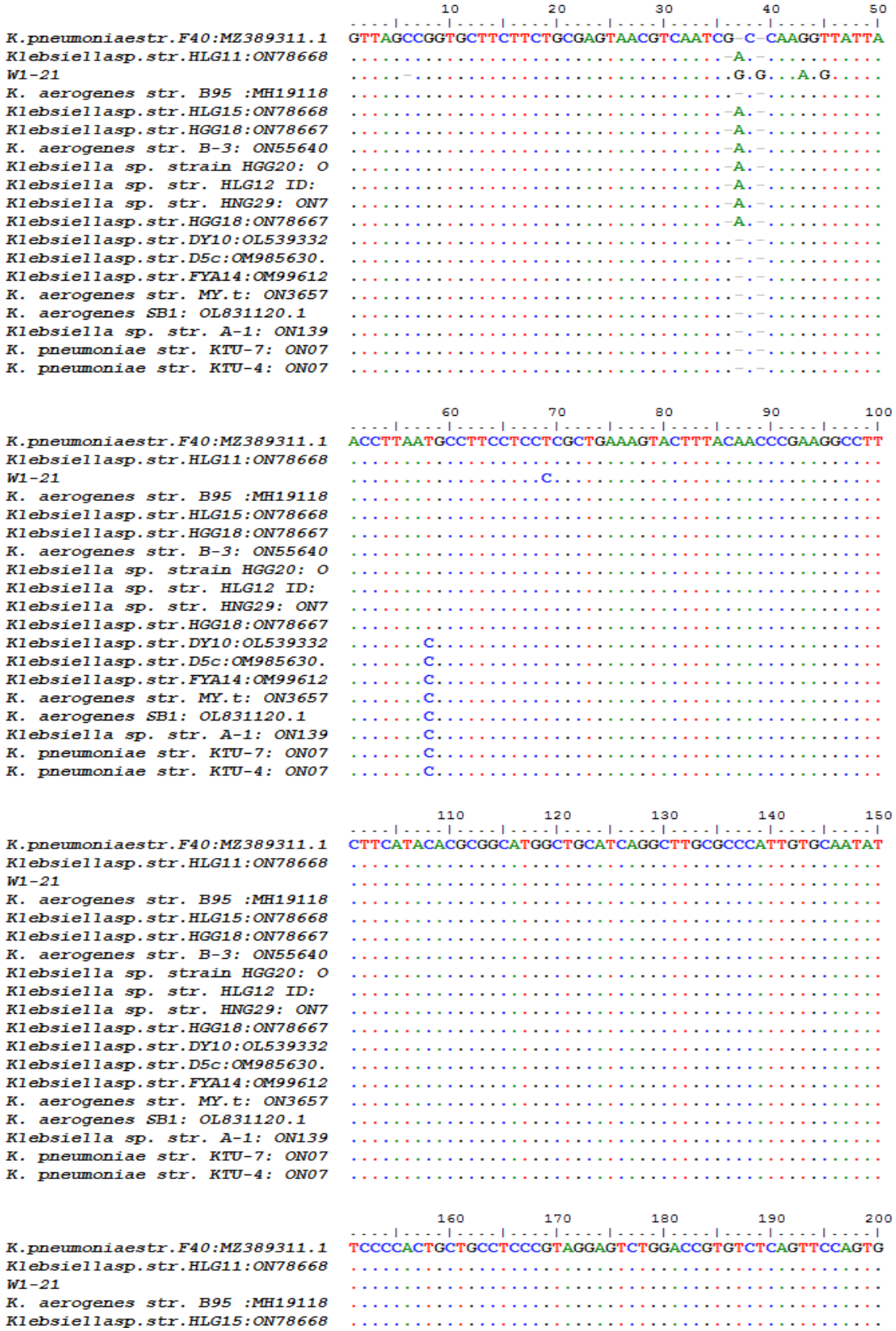


Figure (4-31): Phylogenetic tree of *E. coli* strain W2-4 with other NCBI databank strains

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

The local isolate named *Escherichia coli* strain W2-4 which was isolated from stool sample of a healthy person. It was identified according to microbial, cultural and biochemical characteristics (VITIC 99% probability). In addition to the results of molecular diagnosis and the Phylogenetic tree of 16SrDNA gene (Fig.4-31) revealed that its related to human-associated commensal *E. coli*. Which the local strain close clade with some strains of non-pathogenic *E. coli* and had 95.57% resembling to *Escherichia coli* K-12 strain JE2571; *Escherichia coli* strain SCU-483 of human-associated commensal *E. coli*, and uncultured *Escherichia sp.* clone 7d14216 which detected by PCR-DNA sequencing technique using 16SrDNA gene in Brazilian newborns to establishment of the bacterial fecal community during the first month of life. Also resemble to *Escherichia coli* strain MKNJ (isolated from aquatic Environments). However it was resemble to strains that isolated from enetic environment of healthy human including *Escherichia coli strain* 19SZHZ663Rt, *E. coli* strain 18SZHZ655Rt and *E. coli* strain 19SZHZ803Rt at 95.07% probability.


10 20 30 40 50
K.pneumoniaestr.F40:MZ389311.1 GTTAGCCGGTGCTTCTTCTGCGAGTAACGTCAATCG-C-CAAGGTTATTA
Klebsiellasp.str.HLG11:ON78668 ..........A.-..........
W1-21 .....-.....G.G...A.G.....
K. aerogenes str. B95 :MH19118 ..........-.-..........
Klebsiellasp.str.HLG15:ON78668 ..........A.-..........
Klebsiellasp.str.HGG18:ON78667 ..........A.-..........
K. aerogenes str. B-3: ON55640 ..........A.-..........
Klebsiella sp. strain HGG20: O ..........A.-..........
Klebsiella sp. str. HLG12 ID: ..........A.-..........
Klebsiella sp. str. HNG29: ON7 ..........A.-..........
Klebsiellasp.str.HGG18:ON78667 ..........A.-..........
Klebsiellasp.str.DY10:OL539332 ..........-.-..........
Klebsiellasp.str.D5c:OM985630. ..........-.-..........
Klebsiellasp.str.FYA14:OM99612 ..........-.-..........
K. aerogenes str. MY.t: ON3657 ..........-.-..........
K. aerogenes SB1: OL831120.1 ..........-.-..........
Klebsiella sp. str. A-1: ON139 ..........-.-..........
K. pneumoniae str. KTU-7: ON07 ..........-.-..........
K. pneumoniae str. KTU-4: ON07 ..........-.-..........
60 70 80 90 100
K.pneumoniaestr.F40:MZ389311.1 ACCTTAATGCCTTCCTCCTCGCTGAAAGTACTTTACAACCCGAAGGCCTT
Klebsiellasp.str.HLG11:ON78668 ....
W1-21 ....C.....
K. aerogenes str. B95 :MH19118 ....
Klebsiellasp.str.HLG15:ON78668 ....
Klebsiellasp.str.HGG18:ON78667 ....
K. aerogenes str. B-3: ON55640 ....
Klebsiella sp. strain HGG20: O ....
Klebsiella sp. str. HLG12 ID: ....
Klebsiella sp. str. HNG29: ON7 ....
Klebsiellasp.str.HGG18:ON78667 ....
Klebsiellasp.str.DY10:OL539332 .....C.....
Klebsiellasp.str.D5c:OM985630. .....C.....
Klebsiellasp.str.FYA14:OM99612 .....C.....
K. aerogenes str. MY.t: ON3657 .....C.....
K. aerogenes SB1: OL831120.1 .....C.....
Klebsiella sp. str. A-1: ON139 .....C.....
K. pneumoniae str. KTU-7: ON07 .....C.....
K. pneumoniae str. KTU-4: ON07 .....C.....
110 120 130 140 150
K.pneumoniaestr.F40:MZ389311.1 CTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATAT
Klebsiellasp.str.HLG11:ON78668 ....
W1-21 ....
K. aerogenes str. B95 :MH19118 ....
Klebsiellasp.str.HLG15:ON78668 ....
Klebsiellasp.str.HGG18:ON78667 ....
K. aerogenes str. B-3: ON55640 ....
Klebsiella sp. strain HGG20: O ....
Klebsiella sp. str. HLG12 ID: ....
Klebsiella sp. str. HNG29: ON7 ....
Klebsiellasp.str.HGG18:ON78667 ....
Klebsiellasp.str.DY10:OL539332 ....
Klebsiellasp.str.D5c:OM985630. ....
Klebsiellasp.str.FYA14:OM99612 ....
K. aerogenes str. MY.t: ON3657 ....
K. aerogenes SB1: OL831120.1 ....
Klebsiella sp. str. A-1: ON139 ....
K. pneumoniae str. KTU-7: ON07 ....
K. pneumoniae str. KTU-4: ON07 ....
160 170 180 190 200
K.pneumoniaestr.F40:MZ389311.1 TCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTG
Klebsiellasp.str.HLG11:ON78668 ....
W1-21 ....
K. aerogenes str. B95 :MH19118 ....
Klebsiellasp.str.HLG15:ON78668 ....

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Klebsiellasp.str.HGG18:ON78667  ..........
K. aerogenes str. B-3: ON55640  ..........
Klebsiella sp. strain HGG20: O  ..........
Klebsiella sp. str. HLG12 ID:   ..........
Klebsiella sp. str. HNG29: ON7  ..........
Klebsiellasp.str.HGG18:ON78667  ..........
Klebsiellasp.str.DY10:OL539332  ..........
Klebsiellasp.str.D5c:OM985630.  ..........
Klebsiellasp.str.FYA14:OM99612  ..........
K. aerogenes str. MY.t: ON3657  ..........
K. aerogenes SB1: OL831120.1    ..........
Klebsiella sp. str. A-1: ON139  ..........
K. pneumoniae str. KTU-7: ON07  ..........
K. pneumoniae str. KTU-4: ON07  ..........
```

Figure (4-32): Nucleotides sequence of the 16S rRNA gene of the isolate *Klebsiella sp.* W1-21 among nucleotides sequence of those available in databanks. Data indicated that the position of nucleotides is different among isolates and identical data for all isolates are not shown.

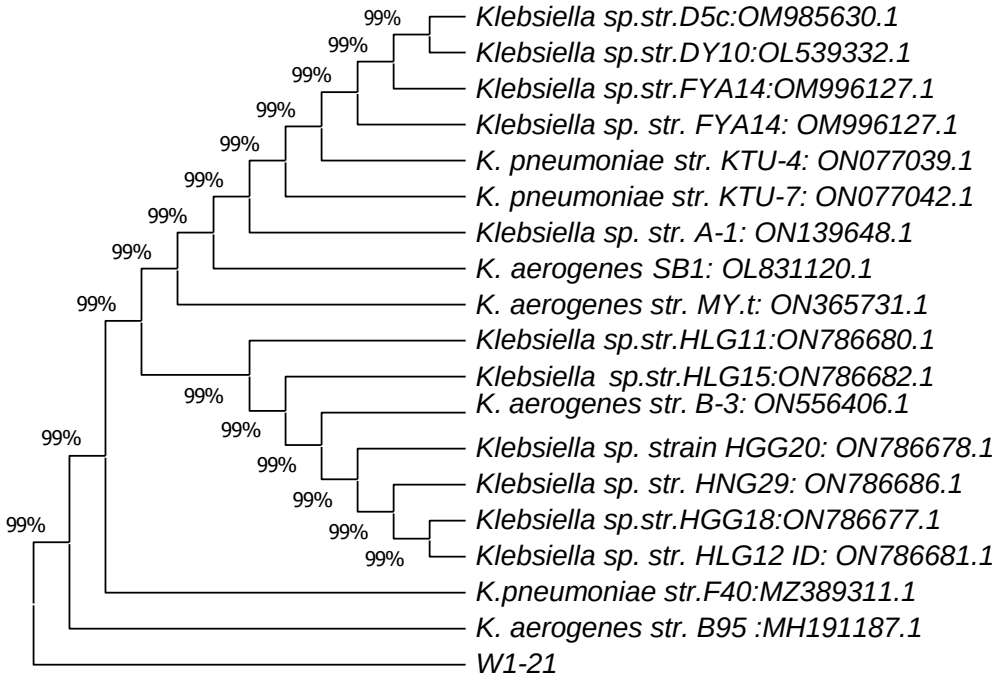

Figure (4-33): Phylogenetic tree of *Klebsiella sp.* W1-21 with other NCBI databank strains

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

Depending on the 16SrRNA Phylogeny tree (Fig.4-30), *Klebsiella sp.* W1-21which was isolated from a stool sample of a healthy person, it tendency to resemble *Klebsiella aerogenes* strain B95 ID: MH191187.1,and K. pneumoniae str. F40 ID: MZ389311.1 about 97.03 and with other bacterial strains mentioned in the phylogenetic tree about 96.53 that indicate may be of the same origin or linage. The local isolate W1-21 related to Klebsiella sp. depending on cultural, microbial, biochemical and molecular diagnosis, so was named Klebsiella sp. W1-21. Which it was different from other related bacterial strains such as Klebsiella aerogenes strains by indole, methyl red and Voges-Proskaure tests and the motility test, the related bacterial strains of Klebsiella aerogenes are motile whereas the local strain was non-motile as most Klebsiella spp.

Depending on the 16SrDNA molecular diagnosis (Fig 4-33) and phylogeny tree (Fig. 4-35), the isolate W4-19 which was isolated from patient stool sample, it tendency to resemble Klebsiella variicola strain TKV and Klebsiella variicola strain M34 about 90.18% and Uncultured Klebsiella sp. clone SF1-17 about 89.86% . Whereas, the Klebsiella pneumoniae strain BA1559 and other strains of Klebsiella pneumoniae that mentioned in the phylogenic tree be similar to W4-19 about 89.57. That indicted the local isolate W4-19 may be new strain of Klebsiella so it named Klebsiella sp. W4-19 and it bear a resemblance to Enterobacter cloacae complex in biochemical characteristics at 96% probability. But the local isolate was non-motile in compare with Enterobacter cloacae complex.

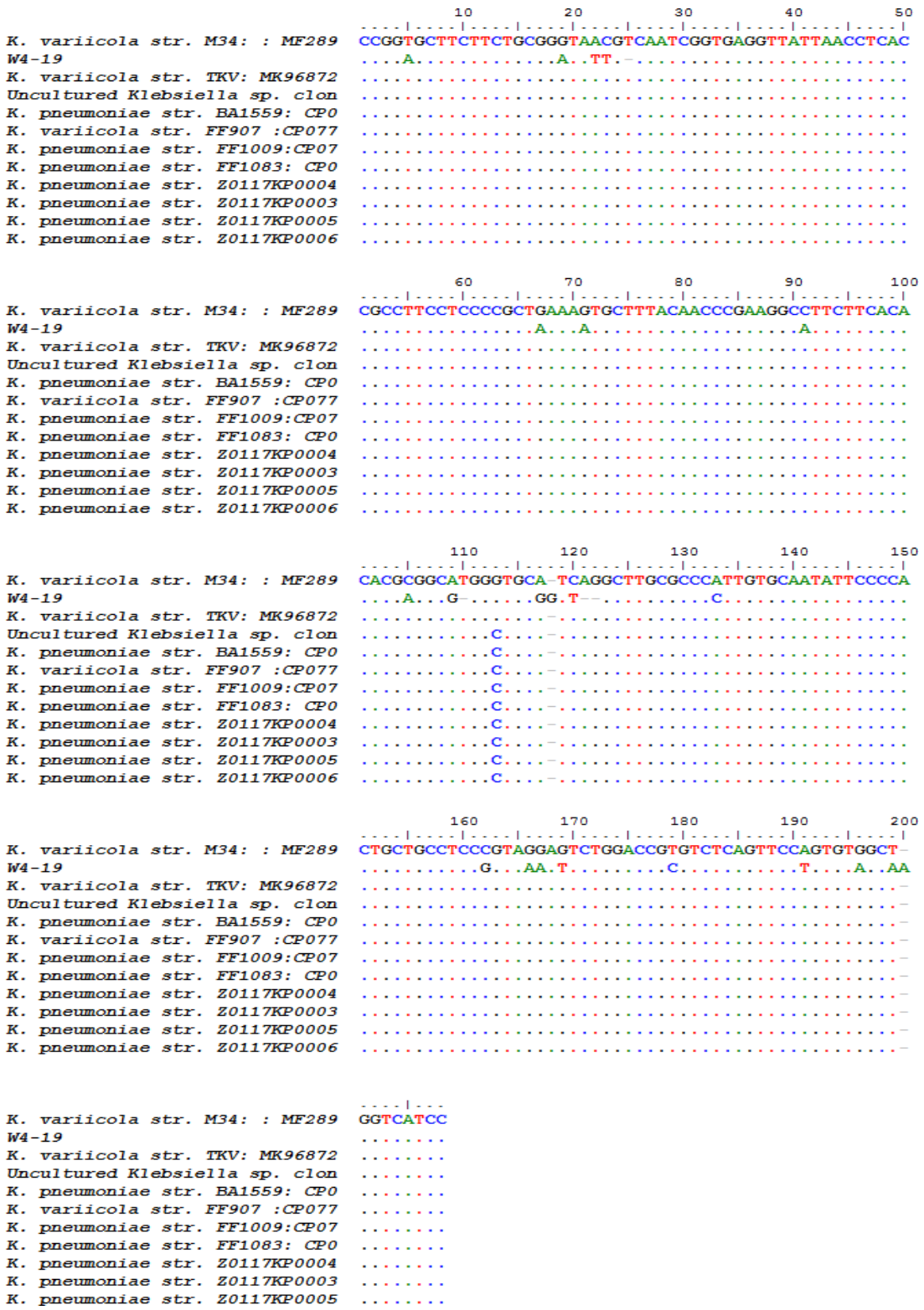

Figure (4-34): Nucleotides sequence of the 16S rRNA gene of the isolate *Klebsiella sp.* W4-19 among nucleotides sequence of those available in databanks. Data indicated that the position of nucleotides is different among isolates and identical data for all isolates are not shown.

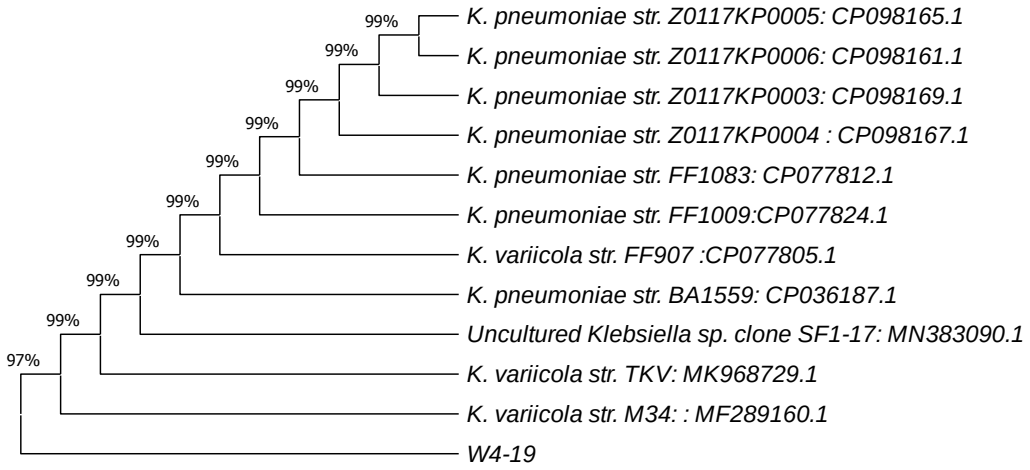

Figure (4-35): Phylogenetic tree of *Klebsiella sp.* W4-19 with other NCBI databank strains

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

Conclusion and Recommendation

Conclusions

Depending on the current study results, the works conclude the following

1. The highest rates of antibiotic resistance were observed in *E. coli* rather than *Klebsiella*. *E. coli* and *Klebsiella* were isolated from stool samples of healthy people, and they showed high resistance to antibiotics.

2. Both species had resistance to Amoxiclline-clavulanate ,Cefotaxime, and Ceftazidime. While the lowest frequency was meropenem ,and all strains were sensitive to imipenem.

3. *E. coli* and *Klebsiella* isolates were harboring integron gene in addition to Tn7-transposition genes. These isolates appeared to have variable patterns of transposon genes. There were eight patterns, three of them associated with the presence of intI2 gene. The other five patterns of Tn7 like transposon.

4. The present study revealed a correlation between the presence Tn7 like transposon and class 2 integron and the studied antibiotic resistances.

5. These results partly explain the high prevalence of antibiotic resistance observed in Iraq due to drug misuse. Most of the bacterial strains were multidrug-resistant, and they spread more in pathogenic strains than in commensal strains.

Recommendations

The results of this study encouraged the investigator to propose the following recommendations:

1. Comparing the genetic change in Tn7 like transposon with the severity of the disease with intestinal infection.
2. Study Tn7 like transposon in other types of bacteria and its relationship with disease.
3. Emphasis on the use of modern molecular techniques in diagnosing pathological isolates and distinguishing them from others.
4. Study other antibiotic resistance genes that are transmitted between intestinal commensal in healthy people.
5. The possibility of studying the effect of each variant on the function , structure and stability of the triple structure of the protein under study by using the great development in techniques in silico prediction tools and linking these result to the bacteria containing these proteins and the severity of the disease.

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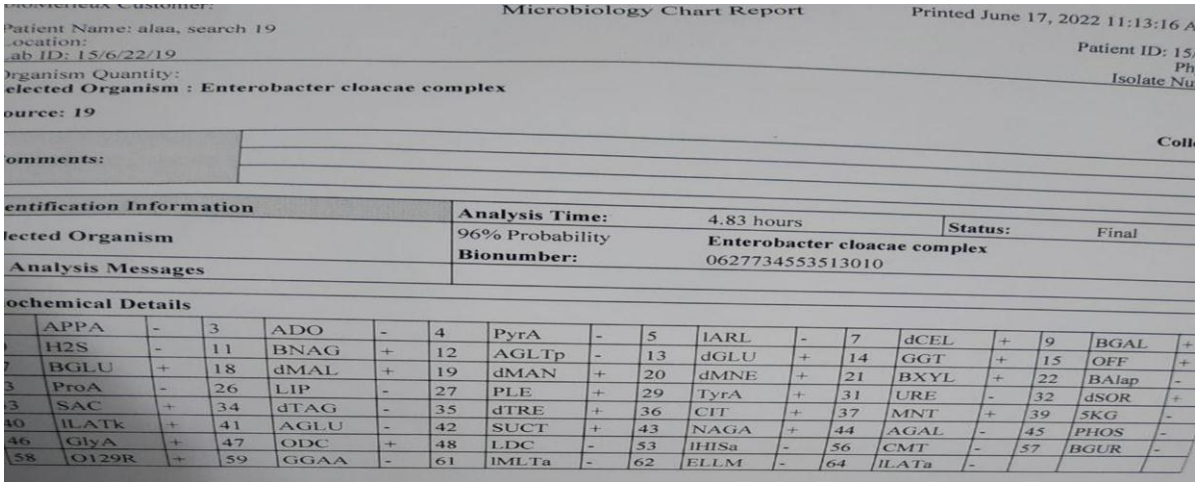
Appendix

Appendix(1) The result of VITEK 2 system for the diagnosis E.coli (98-99%probapility) and *Enterobacter cloacae* complex (88-96%) isolates



Microbiology Chart Report

Printed June 17, 2022 11:13:16 A

Patient Name: alaa, search 19
Location:
Lab ID: 15/6/22/19
Organism Quantity:
Selected Organism : Enterobacter cloacae complex
Source: 19

Patient ID: 15/
Ph
Isolate Nu

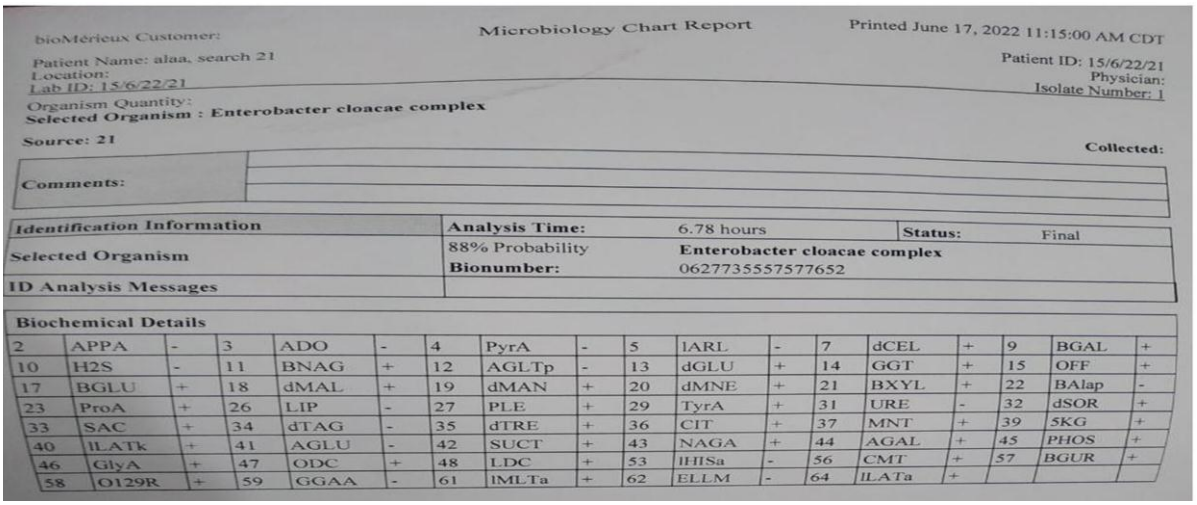
Comments:

Coll

Identification Information	Analysis Time:	4.83 hours	Status:	Final
Selected Organism	96% Probability	Enterobacter cloacae complex		
Analysis Messages	Bionumber:	0627734553513010		

Biochemical Details

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



bioMérieux Customer:

Microbiology Chart Report

Printed June 17, 2022 11:15:00 AM CDT

Patient Name: alaa, search 21
Location:
Lab ID: 15/6/22/21
Organism Quantity:
Selected Organism : Enterobacter cloacae complex
Source: 21

Patient ID: 15/6/22/21
Physician:
Isolate Number: 1

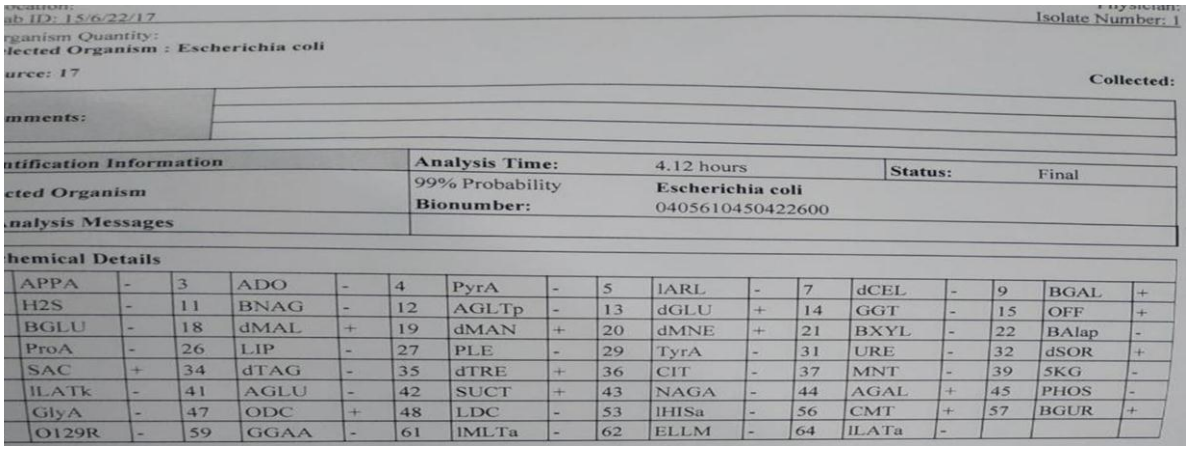
Collected:

Comments:

Identification Information	Analysis Time:	6.78 hours	Status:	Final
Selected Organism	88% Probability	Enterobacter cloacae complex		
ID Analysis Messages	Bionumber:	0627735557577652		

Biochemical Details

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



ab ID: 15/6/22/17
rganism Quantity:
lected Organism : Escherichia coli
urce: 17

Isolate Number: 1

Collected:

mments:

ntification Information	Analysis Time:	4.12 hours	Status:	Final
cted Organism	99% Probability	Escherichia coli		
nalysis Messages	Bionumber:	0405610450422600		

hemical Details

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

Appendix(2)): Summary of the 16S rRNA similarity analysis of NCBI databank strains homologous with *E. coli* strain R1-23, strain R2-5, strain R3-27 and strain W3-17

No.	NCBI Databanks Bacterial strain: GenBank Accession (Source of isolation)	*E. coli* isolates (Ident. %)			
		R1- 23	R2- 5	R3- 27	W3-17
1	*E. coli* strain C289: CP097426.1	99.49	100	98.63	98.66
2	*E. coli* strain C288 : CP097430.1	99.49	100	98.63	98.66
3	*Escherichia sp.* CA_136_AN_84: LC509433.2	100	99.49	99.30	99.32
4	*Escherichia sp.* CA_136_AN_58: LC509407.2	100	99.49	99.31	99.32
5	*Escherichia sp.* CA_136_AN_57: LC509406.2	100	99.49	99.31	99.32
6	*Escherichia marmotae* strain A51: ON688687.1	98.99	98.99	97.95	98.66
7	*E. coli* strain 19SZHZ663Rt: CP080075.1.	98.99	98.99	98.63	97.95
8	*E. coli* str. 19SZHZ869Rt: CP080085.1	98.99	98.99	97.99	97.95
9	*E. coli* O157:H7 str. SS17: CP008805.1	98.99	98.48	97.95	97.97
10	*E. coli* O157:H7 str. AmBaAl-9-IRAQ: ON680712.1	99.49	98.99	97.95	98.66
11	*E. coli* O157:H7 strain AmBaAl-7-IRAQ: ON680710.1	99.49	98.99	97.95	98.66
12	*E. coli* O157:H7 strain AmBaAl-5-IRAQ: ON680708.1	99.49	98.99	97.95	98.66
13	*E. coli* O157 str. AmBaAl-4-IRAQ: ON680707.1	99.49	98.99	97.95	98.66
14	*E. coli* O157:H7 strain AmBaAl-3-	99.49	98.99	97.95	98.66

	IRAQ: ON680706.1				
15	*E. coli* O145:H28 strain AmBaAl-2-IRAQ: ON680705.1	99.49	98.99	97.95	98.66
16	*E. coli* O104:H4 strain AmBaAl-1-IRAQ: ON680704.1	99.49	98.99	97.95	98.65
17	*E. coli* strain 02P2R3D1E6: ON054366	98.99	98.48	99.75	97.95
18	*E. coli* g8 : JQ661152	98.99	98.48	98.63	97.95
19	*E. marmotae* str. RHBSTW-00814:NZ_CP056164	98.99	98.48	97.95	97.95
20	*Escherichia sp*. str. PA18 :ON534344	98.99	98.48	97.95	97.95
21	*Escherichia sp*. str. PA8 :ON534341	98.99	98.48	97.95	97.95

E. coli R1-23 and *E. coli* sp. R2- 5 that originated from human feces with diarrhea; *E. coli* W3-17 and *E. coli* sp. R3- 27 that originated from human urine with UTI.

الخلاصة

أجريت الدراسة الحالية في مختبر التكنولوجيا الحيوية والهندسة الوراثية في جامعة بابل. اشتملت الدراسة على 240 عينة براز (200 مريض و 40 عينة تحكم) و 90 عينة بول. تم جمعها من براز الأشخاص (من 1 إلى 12 عامًا) والمسالك البولية (تتراوح أعمارهم بين 1 و 40 عامًا) من كلا الجنسين الذين حضروا إلى مستشفيات مدينة الحلة ومختبر الصحة العامة في بابل ، خلال فترة امتدت من نوفمبر 2021 إلى يناير 2022. تم عزل *E.coli* و *Klebsiella* من العينات وتم تشخيصهما وفقًا للخصائص المزرعة البكتيرية والكيميائية الحيوية. أيضًا ، تم تأكيدها بواسطة نظام VITEK® 2 وتسلسل16SrRNA. تم اختبار حساسية المضادات الحيوية بوساطة طريقة Kirby-Bauer. تم التعرف على الجينات القافزة الشبيهة بـ Tn7 والجينات *intI2* عن طريق تفاعل البلمرة المتسلسل (PCR) - التسلسل ، ثم بناء شجرة النشوء والتطور.

أظهرت النتائج أن الإشريكية القولونية من سلالات التنبيت الطبيعي تم عزلها من 60٪ من عينات البراز السليمة والعينات الباقية (15٪) تحتوي على بكتيريا الكلبسيلا. بينما كانت نسبة عزل بكتريا *E. coli* و *Klebsiella spp* من براز المريض المصاب بالتهاب المعدة والأمعاء 9٪ و 5٪ على التوالي والباقي (86٪) تحتوي على مسببات مرضية أخرى. بينما كانت نسبة عزل *E. coli* و *Klebsiella spp* , من بول المرضى المصابين بالتهاب المسالك البولية 35.56٪ و 11.11٪ على التوالي ، أما باقي العينات (53.33٪) فقد احتوت على مسببات اخرى التي تم استبعادها.

كانت عزلات *E. coli* و *Klebsiella spp* لديها قدرات متفاوتة لمقاومة مضادات الميكروبات المدروسة ، بما في ذلك 14 مضادًا حيويًا تنتمي إلى تسع فئات مختلفة لها أنماط أو آليات مختلفة في وقف نمو أو قتل الكائنات الحية الدقيقة. أظهرت جميع العزلات البكتيرية مقاومة عالية للمضادات الحيوية تقريبًا باستثناء الكاربابينيمات , فقد كان حوالي 72٪ من العزلات مقاومة للأدوية المتعددة (MDR) لأنها بدت مقاومة لثلاث فئات من المضادات الحيوية على الأقل واظهرت أربع عزلات فقط من الإشريكية القولونية حساسية لجميع المضادات الحيوية ، بما في ذلك عزلتان (8.3٪) تم استعادتها من عينات البراز السليمة والعزلتان الأخريان (6.25٪) من الادرار. على الرغم من عزل كلا النوعين من الأشخاص الأصحاء ، فقد أظهروا مقاومة عالية للمضادات الحيوية وواحدة منهم (*E. coli*) تقاوم ثمانية فئات من المضادات الحيوية .

كان لكلا النوعين البكتيريين (74 عزلة من *E. coli* و 26 عزلة من *Klebsiella spp*) معدلات مقاومة للأدوية عالية، لكن عزلات *E. coli* كانت أكثر مقاومة من *Klebsiella* مع Amoxiclline-clavulanate (70.58٪) ، Cefotaxime (58.96٪) و Ceftazidime (57.81٪). على الرغم من أن أقل تردد مقاومة كان meropenem (4.86٪) ، وكانت جميع العزلات حساسة imipenem (100٪).اظهرت حوالي 75.68٪ من عزلات الإشريكية القولونية مقاومة متعددة للمضادات (MDR) و 61.53٪ من *Klebsiella* والباقي كانت عزلات مقاومة لأقل من ثلاث فئات من المضادات (non –MDR) . ان معظم العزلات المعزولة من عينات الادرار كانت من النمط MDR بشكل عام ، كانت بكتيريا MDR أكثر مقاومة بيتا لاكتام. (P <0.002) Amoxiclline-clavulanate، (P <0.014)، Ceftazidime (P <0.028) Piperacilline) وفئات أخرى من المضادات الحيوية مثل quinolones / fluoroquinolones (ciprofloxacin and levofloxiclines)، (P <0.005 و{ قيمة P-value <0.05}) macrolids (azithromycin: P <0.001)،

يرتبط وجود Tn7 بالمقاومة المتعددة للأدوية للبكتيريا المدروسة (P <0.001 ؛ اختبار Fisher exact). التي كشفت عن أن الإشريكية القولونية ذات المقاومة المتعددة كانت تؤوي أنماطًا مختلفة من Tn 7 والجين *intI2*. توجد علاقة ارتباط بين وجود Tn7 والفئة 2 integronمع مقاومة المضادات الحيوية المدروسة (P <0.029). كشفت هذه الدراسة أيضًا عن وجود ارتباط كبير بين Integron class 2 ومقاومة (AMC) Amoxiclline-clavulanate و (PRL) Piperacilline و (CTX) Cefotaxime و (CN) Gentamicin و (AZM) Azithromycin و (P <0.05) (NIT) Nitrofurantoin).

أظهر التحليل الجزيئي أن هناك ثمانية أنماط من Tn7 (خمسة أنماط تشمل *tnsABC* و *tnsAB* و *tnsAC* و *tnsA* و *tnsB*) موزعة على 62٪ من مجموع العزلات بينما كانت 24٪ من العزلات الكلية سلبية ولم يتم الكشف عن وجود Tn7 أو intI2. تتوزع هذه الأنماط بين عزلات *E. coli* و *Klebsiella*. بشكل عام ، تحتوي عزلات *E. coli* و *Klebsiella* على جينات قافزة مختلفة مثل Tn7 (كاملة أو مقطوعة) والتي ارتبطت بمصدر العزل (P 0.029 ؛ اختبار Fisher exact) وكانت مرتبطة بالبكتيريا المسببة للأمراض على الرغم من بعض normal flora التي تأوي الجينات العناصر المتنقلة . بعض عزلات *E. coli* و *Klebsiella* كانت تؤوي جينات integron بالإضافة إلى جينات نقل Tn7. ظهرت هذه العزلات بأنماط متغيرة من جينات الينقولات ، ثلاثة منها مرتبطة بوجود جين *intI2* تشمل (*tnsABC & intI2*

و *tnsAB&intl2* و *tnsB&intl2*) . تم تقدير تردد integrone من الصنف 2 بنسبة 14٪ المرتبطة بـ Tn7 لإجمالي العزلات المدروسة وبنسبة 18.42٪ للعزلات الإيجابية التي تحمل جينات Tn7.

أخيرًا ، لوحظ أن هذه النتائج تفسر جزئيًا الانتشار العالي لمقاومة المضادات الحيوية التي لوحظت في العراق بسبب إساءة استخدام الأدوية. كانت معظم السلالات البكتيرية مقاومة للأدوية المتعددة ، وتنتشر في السلالات المسببة للأمراض أكثر من السلالات المتعايشة. في هذه الدراسة ظهر أن هناك علاقة ارتباط بين وجود Tn7 مثل الترانسبوزون و Integron الصنف 2 مع مقاومة الأدوية المتعددة للبكتيريا المدروسة.

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الدراسة الجزيئية لجينات العناصر القافزة بين العزلات البكتيرية المقاومة للمضادات الحيوية

رسالة

مقدمة الى مجلس كلية العلوم/جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في العلوم/علوم الحياة

من قبل

علا عدنان حمزة حسن
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بأشراف

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