Original Article

Molecular Detection of Tn7-Like Transposons and *IntI2* Integron Integrase in Multidrug-Resistant *Escherichia coli* and *Klebsiella* spp.

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Abstract

Background: Transposable elements are mobile DNA sequences that can move and change their location within the genome. **Objectives:** The aim of the study was to investigate class II integron and Tn7-like transposons and their relationship to multidrug resistance in commensal and pathogenic *Klebsiella* and *Escherichia coli* isolates. **Materials and Methods:** The disk-diffusion method was used to check the antibiotic susceptibility of 26 *Klebsiella* isolates and 76 *E. coli* isolates that had been isolated and preidentified using the VITEK 2 System from healthy stool, diarrhea, and urine in (urinary tract infection) specimens. The presence of the *intI2* and Tn7-like transposase genes was then examined using specific primer pairs by polymerase chain reaction. **Results:** A total of 76% of the total isolates harbored Tn7, of which 14% of these isolates appeared to harbor *intI2* in the Tn7 in three phylotypes. The other 62% of isolates had five patterns of Tn7. At the same time, the rest of the isolates (24%) were negative for the Tn7 and *intI2* genes. Overall, the *E. coli* and *Klebsiella* isolates contain different Tn7-like transposons and are associated with pathogenic bacteria ($P < 0.05$) though some normal flora harbor the transposons. The presence of *intI2* and Tn7-like transposons is associated with multidrug resistance in the studied species; *intI2* in Tn7-like transposon ($P < 0.029$) and Tn7-like transposition genes only ($P < 0.001$). A significant association ($P < 0.05$) was detected between *intI2* in the Tn7-like transposons and resistance to amoxicillin-clavulanate, piperacillin, cefotaxime, gentamicin, azithromycin, and nitrofurantoin. **Conclusions:** There is a correlation between multidrug resistance and the presence of Tn7-like transposition and *intI2* genes.

Keywords: Class 2 integron integrase, *Escherichia coli*, *Klebsiella*, multidrug resistance, Tn7-like transposon

Introduction

Escherichia coli is a member of the Enterobacteriaceae family of facultative anaerobic, nonspore-forming, Gram-negative bacteria. It is a component of the native gut flora of warm-blooded animals and people. Several dangerous *E. coli* can cause several illnesses, even though most are innocuous commensal organisms.[1] There are six pathogenic organisms, each with a unique set of virulence characteristics and mechanisms of pathogenicity. A typical serotype of *E. coli* that causes diarrheal illness and epidemics globally is O157:H7. It threatens public health and food safety worldwide, especially for vulnerable populations such as neonates, babies, and those with weakened immune systems.[2,3]

Klebsiella is a facultative anaerobic, nonspore-forming, nonmotile, straight rod with a diameter of 0.3–1.5 μm wide by 0.5–5.0 μm long. The rods can show up alone, in pairs, or brief chains. The cells have a covering. The ideal temperature for growth is 37°C. In addition to having a respiratory and a fermentative form of metabolism, *Klebsiella* spp. is chemoorganotrophic. Acid and gas are created due to the fermentation of glucose (more CO_2 is

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produced than H2). With less ethanol and less lactic, acetic, and formic acids produced compared with that in typical mixed acid bacterial fermentation, most strains primarily create 2,3-butanediol as a byproduct of the fermentation of glucose. There are no known needs specific to growth factors. Under anaerobic circumstances, some bacteria can repair molecule nitrogen.[4]

Transposable elements (TEs) are mobile DNA sequences that may move across a genome and alter its location. The most prevalent and prolific genes in nature, TEs, and the related transposase genes are crucial for biological diversity and adaptability.[5] It is recognized that many highly divergent bacteria that have adapted to different environments have Tn7 and related elements known as Tn7-like elements, which contain homologs of the Tn7 transposition proteins. Over the past 10 years, one important discovery has been 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 many different environments.[1,5] The size of Tn7 is often substantial, frequently 14 kb.[1,6] However, comparing some Tn7-like derivatives reveals that these elements have diverse genetic structures and accumulated several accessory resistance regions.[1,7] By transferring different resistance genes between bacteria using its transposase, the Tn7-like, a significant mobile genetic element of transposons, encourages the horizontal spread of bacterial antibiotic resistance. The two extremities of the Tn7 transposon, which are the class 2 integron system and the transposition module, have highly conserved sequences. The transposition module encodes five proteins: tnsA, tnsB, tnsC, tnsD, and in tnsABCDE, which is necessary for two transposition pathways. The unique bacterial transposon Tn7 encodes five transposition proteins: *Tns A, B, C, D,* and *E.* Surprisingly, Tn7 transposition is extremely specific, in contrast to other transposons, which inserts quite randomly into a wide variety of locations. Alternative target site-selectors TnsD and TnsE control Tn7 transposition into one of two distinct target DNAs. Therefore, a highly specific chromosomal or DNA binding site undergoes DNA replication.[7,8] The Tn7 transposition proteins, TnsA, which is required for the cleavage of the second strand; TnsB, the transposase; TnsC, an auxiliary protein for the transposase; TnsD, which is necessary for high-frequency transposition to the target location downstream of glmS; and TnsE, which is required for wider dispersion, are also required.[1,8] In most contexts, integrons constitute a complex mobilome. In addition, they can move across species during evolutionary time and have access to a large number of novel genes whose roles are yet unclear.[1,6,9] Therefore, this work aimed to identify the prevalence of Tn7-like transposons and their association with class 2 integron in commensal and pathogenic *E. coli* and *Klebsiella spp.* isolated from healthy individuals and patients with gastroenteritis and urinary tract infections in Hillah, Iraq.

Materials and Methods

The 26 *Klebsiella* isolates and 76 *E. coli* isolates that had previously been isolated from 200 samples of stools from newborns and children with gastroenteritis (aged 1–12 years) of both genders came to the hospitals in the city of Hillah and the Public Health Laboratory in Babylon, between November 2021 and January 2022. The control group includes 40 samples of healthy stools collected from infants and children. In addition, 90 urine samples from Urinary tract infection (UTI) patients (aged between 2 and 40 years) who visited the hospitals in Hillah City were of both genders. The isolates were identified using the VITEK 2 system. The isolates were examined for antibiotic susceptibility using the Kirby-Bauer disc diffusion method on Mueller Hinton agar. The antibiotic discs include amoxicillin-clavulanate (AMC) (30 µg), piperacillin (PRL) (100 µg), amikacin (30 µg), ceftazidime (30 µg), cefotaxime (CTX) (30 µg), impnine (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), nitrofurantoin (NIT) (100 µg), gentamicin (CN) (10 µg), azithromycin (AZM) (15 µg), tetracycline (10 µg), levofloxacin (5 µg), and chloramphenicol (30 µg).[10,11]

DNA extraction

FavorPrep Genomic DNA Mini Kit (for bacteria) was used to extract genomic DNA (1.8) according to the manufacturer's instructions (Favorgen, Taiwan, China).

Molecular detection of Tn7-like transposon

Tn7 transposition genes or related family members were detected using specific primer pairs by polymerase chain reaction (PCR). The final volume of the PCR mixture was 25 µL consisting of 4 µL of template DNA (1.8), 4.5 µL of ddH_2O, 12.5 µL of Taq PCR Master Mix, and 2 µL of each primer. The amplified PCR products were separated by electrophoresis in 1.5% agarose gels at 100 V for 60 min staining method; precast RED Safe stain [Tables 1 and 2].

Ethical considerations

All subjects gave their consent and agreed to study the scientific and ethically sound Babylon's Public Health Laboratory and hospitals in Hillah City. According to the document with the number 8220904 and the date September 28, 2022, the study protocols, subject information, and agreement form were examined and approved by a local ethics committee of the Biology Department at the College of Science at the University of Babylon.

Statistical analysis

All data were analyzed by the SPSS (IBM Corporation, New York, New York) statistical package (version 2010). Fisher's exact test of independence was applied to find a statistical association. P value <0.05 was considered significant.

Table 1: Lists the primer pair sequences, amplicon sizes

Gene	5′–3′ sequence	5′–3′ sequence	References
tnsA	F. TGGCTAACAGTACAAGAAGT	713	[1,7]
	R. CGCAACTCCTCCATATTCA		
tnsB	F. GGCTGAGTTGTTGCTAATG	845	[1]
	R. CCACCACATAAGACGGATT		
tnsC	F. TCGCATAATGGTTCGCTAA	893	[1]
	R. CTTGTCATCGTTGGATTCTG		
IntI2	F. CACGGATATGCGACAAAAAGGT	789	[8]
	R. GTAGCAAACGAGTGACGAAATG		

Table 2: PCR—thermal cycling conditions

Gene	Initial denaturant	Denaturant	Anneal	Extension	Final extension	Cycles
	°C (min)	°C (s)	°C (s)	°C (s)	°C (min)	
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 (50	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

Results

Of the 100 isolates, 74 were *E. coli*, and 26 were *Klebsiella* spp. collected from apparently healthy individuals and patients with gastroenteritis and Urinary tract infections. All *E. coli* and *Klebsiella* isolates exhibited high antibiotic resistance, including AMC (70.58%), CTX (58.96%), and ceftazidime (57.81%). Although all isolates were susceptible to imipenem (100%), meropenem had the lowest prevalence of resistance (4.86%), as shown in Figure 1. Generally, *E. coli* isolates were higher antibiotic-resistant relative to *Klebsiella* isolates. Most bacterial isolates in the current study were also found to be multidrug resistance (MDR) (72%) for at least three classes, including 75.68% of MDR-phenotype *E. coli* and 61.53% of MDR-phenotype *Klebsiella*. The remaining isolates were non-MDR–phenotype, and two *E. coli* isolates were sensitive to all antibiotics under investigation. Amoxiclline-clavulanate (AMC, $P = 0.002$), ceftazidime (CAZ, $P = 0.014$), Piperacilline (PRL, $P = 0.028$), as well as additional antibiotic classes as macrolides (AZM, $P = 0.001$), quinolones/fluoroquinolones (ciprofloxacin and levofloxacin), tetracyclines, and chloramphenicol ($P = 0.05$), most of the examined *E. coli* and *Klebsiella* isolates showed an MDR phenotype, where the percentages of both types of bacteria in the urine sample were higher than the stool samples in patient and healthy individuals. That may be related to the type of isolation sources as indicated by the Chi-square test, $P < 0.005$.

The presence of Tn7 transposition genes and class 2 integron integrase (*intI2*) gene was molecularly detected as the appearance of a single band of each tested gene in agarose gel, as shown in Figure 2. These PCR products included the tnsA gene (713bp), tnsB gene (845bp), and tnsC gene (893bp). Figure 3 showed the amplified fragment of the *intI2* gene (789bp). About 76% of total *E. coli* and *Klebsiella* isolates harbored Tn7-like transposition genes, some containing the *intI2* gene [Table 3]. Three of them (14%) harbor *intI2* in Tn7-like transpose, including *tnsABC* and *intI2*; *tnsAB* and *intI2* and *tnsB* and *intI2* patterns, as shown in Table 3. Figure 4 appeared prevalence of Tn7 transposition genes and integron class 2 gene (*intI2*) among *E. coli* (A) *and Klebsiella* (B) isolated from different sources. Therefore, five patterns (62%) of Tn7-like transposon, including *tnsABC, tnsAB, tnsAC, tnsA only*, and *tnsB* only were shown between *E. coli* and *Klebsiella*. At the same time, 24% of the total isolates were negative, which were not detected the presence of the Tn7-like transposon or *intI2* gene. These patterns were 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) and were associated with the source of isolation ($P = 0.029$; Chi-square test), and they related with pathogenic bacteria despite some normal flora harboring the mobile genetic elements.

The presence of *intI2* and Tn7-like transposon correlated with antibiotic resistances of the studied species ($P < 0.05$). Figure 5 revealed most of MDR-*E. coli* and MDR-*Klebsiella* harbored different patterns of Tn7-like transposons and *intI2* gene. Furthermore, this study also detected a considerable correlation between *intI2* in Tn7-like transposon and resistance

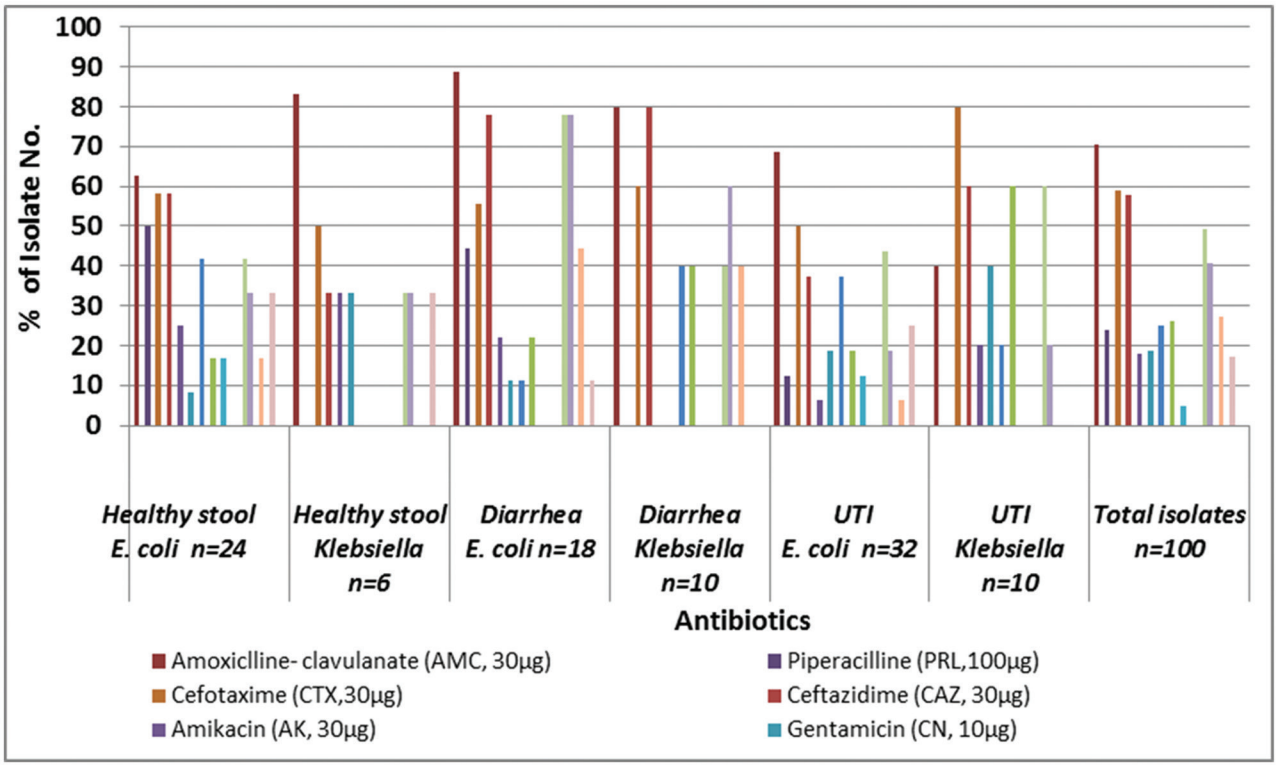

Figure 1: Antibiotic susceptibility prevalence in *Klebsiella* and *E. coli* isolated from different sources

to AMC, PRL, CTX, CN, AZM, and NIT ($P < 0.05$: Chi-square test).

Discussion

All *E. coli* and *Klebsiella* isolates showed a high rate of resistance to antibiotics such as AMC (70.58%), CTX (58.96%), ceftazidime (57.81%), and in general *E. coli* isolates were more resistant to antibiotics. Whereas all *E. coli* and *Klebsiella* isolates had the lowest frequency of meropenem resistance and imipenem sensitivity [Figure 2]. These findings are consistent with a prior study,[12] which discovered that *E. coli* was more resistant to antibiotics than *Klebsiella pneumonia*, with the lowest resistance to amoxicillin-clavulanic acid (4%) and CTX (33%), and the highest resistance to imipenem (2%). The varying rates of antibiotic intake in each nation account for the different resistance percentages across the globe.[13] Because *E. coli* can pick up antibiotic resistance traits from other bacteria in its environment, *E. coli* and *Klebsiella* were isolated from stool samples of apparently healthy people. Still, they showed high resistance to antibiotics, and one of them was resistant to eight antimicrobial classes. The human gut microbiota can contain over 1000 antibiotic-resistant genes, and transmitting these traits between intestinal commensals is an important process.[14] Accordingly, a study by Al-Zubaidi and Al-Salman[15] revealed that parents with higher education levels are better aware of UTI treatment and prevention than parents with lower levels of education.

The results of the current study discovered that *E. coli* resistance to ceftazidime and CTX varied depending on the locations, times, and sources of isolations, which did not match with the findings of this study. In 2015, Lee *et al*.,[16] in North Korea, discovered that *E coli* had a 6.8% and a 15.5% resistance to ceftazidime and CTX, respectively. While Kafilzadeh and Farsimadan[17] discovered that ceftazidime and cefotaxime resistance rates are 38.9% and 42.2%, respectively. Ceftazidime and CTX were reported to be resistant in Iran in percentages of 26.1% and 30%, respectively.[18] According to a study conducted in Egypt, 74.4% and 64.3% of *E. coli* bacteria were resistant to CTX and ceftazidime.[19] The two antibiotics' main cause of resistance may be because the bacteria possessed effective circulation pumps and efflux systems, which distribute antibiotics to bacteria outside their cells. In addition, most 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 the donor to the recipient cell.[20]

These outcomes include those by Abdu *et al*.[21] The formation of beta-lactamase enzymes, which break down the beta-lactam ring of antibiotics such as penicillin and cephalosporins, or change the target site of the antibiotic through mutation, reduce the permeability of the outer membrane of the bacterial cell, efflux pump systems, or RND family efflux systems (AcrAB-ToIC, MdfA, YhV).[22] In addition to the function of antibiotic use in selection for resistance, epidemiological studies

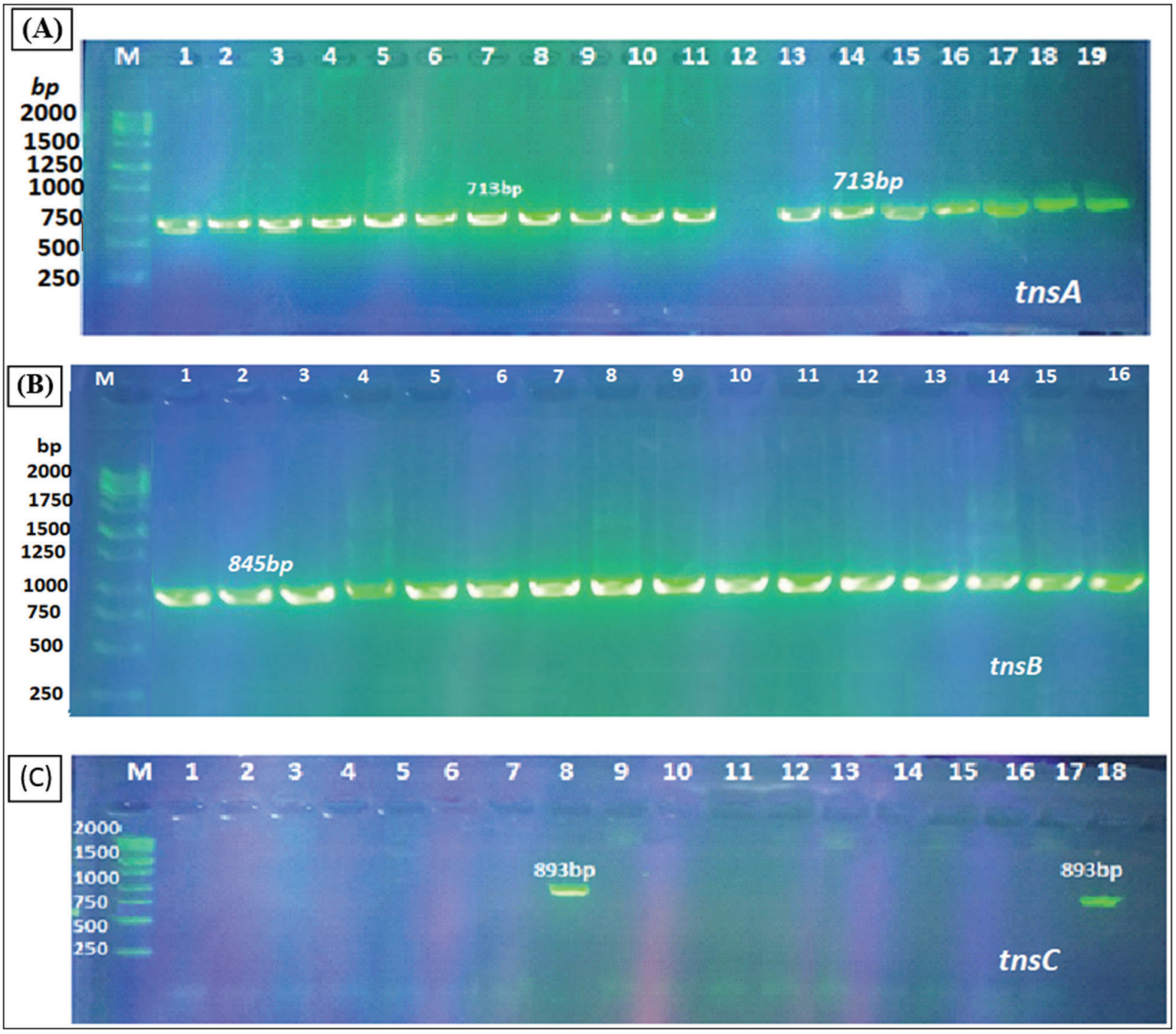

Figure 2: Agarose gel electrophoresis of *tnsA, tnsB*, *and tnsC* amplified product patterns of *E. coli* and *Klebsiella* isolates. M: refers to DNA size marker (250–20,000 bp); lanes 1–19 refer to PCR products of (A) PCR product of *tnsA* gene is 713 bp, (B) PCR product of *tnsB* gene is 845 bp, and (C) PCR product of *tnsC* gene is 893 bp 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

reveal a possible link between antibiotic resistance and the virulence phenotypes of diarrheagenic *E. coli*. They discovered that individuals with diarrhea used antibiotics more frequently before the onset of their symptoms. This link may indicate that drugs alter the gut flora, promoting the development of resistant infections.[23] Klein *et al*.[23] discovered that between 2000 and 2015, antibiotics consumption increased dramatically worldwide by roughly 39%. Additionally, they discovered that low- and middle-income groups in developing nations consumed the majority of antibiotics on average, influencing the prevalence of multidrug inflammatory strains and the greatest rates of antibiotic resistance. Making matters worse, the emergence of *E. coli* strains resistant to last-resort antibiotics such as carbapenems and colistin is linked to increased use of these drugs.[24] It was noted that carbapenem-resistant *E. coli* is rare, with prevalence varying depending on the research region of the world, but it does not exceed 3%. It was predicted that carbapenem resistance would emerge in the future. It can be seen in *E. coli* because the enzymes that break it down and subsequently render it inactive, the carbapenemases, are encoded primarily on paired plasmids and are highly transmittable, which is consistent with McEwen and Fedorka-Cray.[25] This is in line with the findings of the current investigation, which showed that meropenem resistance in *E. coli* isolated from Iraqi UTI patients with healthy stool had increased to more than 4.5%. Additionally, antibiotics are utilized in livestock to treat clinical illnesses, avoid and control common illnesses,

Figure 3: Agarose gel electrophoresis of *intI2* amplified product patterns of *E. coli* and *Klebsiella* isolates. M: refers to DNA size marker (250–20,000 bp); lanes 2 and 12 refer to PCR product of *intI2* gene (789 bp) 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

Table 3: Groups of Tn7 transposition gene patterns and class 2 integrase integron (*IntI*2) gene

Group No.	Tn7 gene patterns	*IntI*2	Total *E. coli*	Total *Klebsiella*	Total isolate
			n = 74	*n* = 26	*n* = 100
			No. (%)	No. (%)	No. (%)
1	*tnsABC*	+	8 (10.81)	2 (7.69)	10 (10)
2	*tnsAB*	+	2 (2.7)	0 (0)	2 (2)
3	*tns*B	+	2 (2.7)	0 (0)	2 (2)
4	*tnsABC*	−	20 (27.03)	2 (7.69)	22 (22)
5	*tnsAB*	−	4 (5.41)	10 (38.46)	14 (14)
6	*tnsAC*	−	6 (8.11)	0 (0)	6 (6)
7	*tnsA*	−	0 (0)	4 (15.38)	4 (4)
8	*tnsB*	−	12 (16.22)	4 (15.38)	16 (16)
Total positive isolates for Tn7 and *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 (72.97)	22 (84.61)	76 (76)
Total negative			20 (27.03)	4 (15.39)	24 (24)

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

The data were analyzed using the Chi-square test. Data with completely different letter labels showed a significant difference ($P = 0.001$; <0.05)

and foster animal growth.[26] A Human ingesting antibiotic through meat infected with antibiotics that enters the body and exerts selective pressure on the host's microbes is one of the unfortunate ways in which this usage of antibiotics has contributed to the proliferation and persistence of resistant bacteria in humans, while the second mechanism can tainted meat can spread resistant germs found in the intestines of food animals to humans. The overuse of antibiotics on food animals led to a high colonization rate of intestinal bacteria, particularly Enterobacteriaceae family members such as *E. coli* and *Klebsiella* spp., resistant to several antimicrobials. This is due to the fact that these substances apply the same selective pressure to microbes in livestock as they do when used by humans. Studies around the world have shown that ready-to-eat animal products contain *E. coli* strains resistant to several antibiotics, primarily Beta-lactam, through bacterial synthesis of Extended-spectrum beta-lactamases (ESBL).[27] According to earlier investigations, *E. coli* bacteria obtained from meat have also demonstrated resistance to last-resort antibiotics such as carbapenems[28] and colistin.[29] Humans would certainly consume this contaminated, undercooked meat, and it would populate the gut, creating a reservoir for future diseases resistant to antibiotics. Bourrel *et al.*[30] shown that participants with greater rates of intestine colonization of ESBL-producing *E. coli* strain present a higher risk of urinary tract infection with these strains. Antibiotic-resistant *E. coli* is commonly found in the intestines of humans and other animals. When these strains are eliminated through infected people, cattle, and pets' feces, they can enter humans through

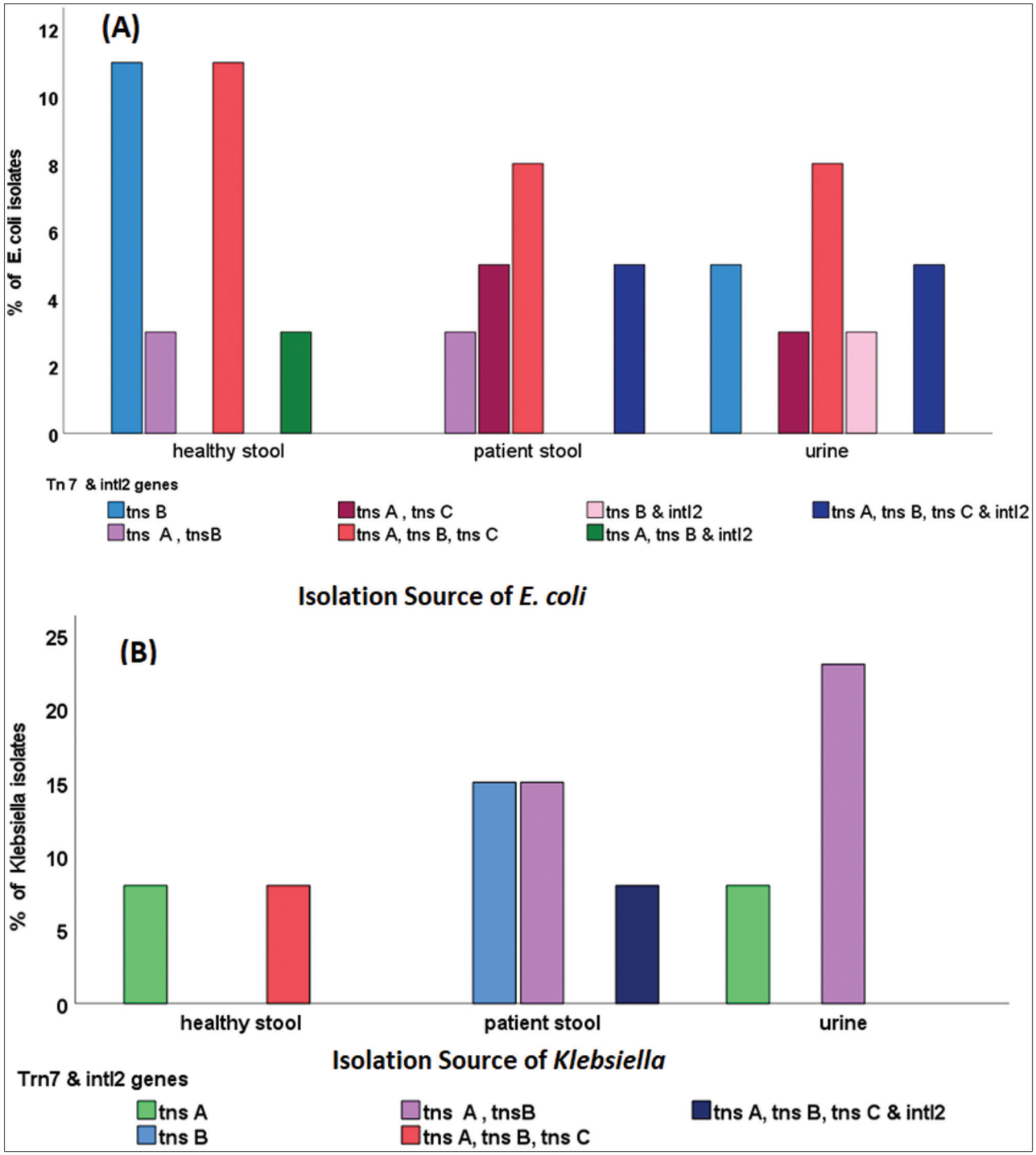

Figure 4: Prevalence of Tn7 transposition genes and integron class 2 gene (*intl2*) among *E. coli* (A) *and Klebsiella* (B) isolated from different sources

contaminated water and food. Due to the rise in the prevalence of fecal colonization by strains of *E. coli*, the number of human infections caused by them increases.[31] *E. coli* has an evolutionary advantage over other bacteria in its environment because of its capacity to colonize various environments, including the intestines of humans and other animals. This organism has also benefited from the ease with which these other bacteria can be transmitted via feces to humans. Over 1000 distinct antibiotic-resistant genes have been found in the human gut microbiome. It is a persistent phenomenon that certain characteristics are passed from intestinal commensals to others. The blaCTX-M gene, the most common ESBL gene in *E. coli* and *Klebsiella spp*, and OXA-48-type are an important example of resistance gene transfer between environmental bacteria, particularly gut symbionts and human diseases. There are more and more reports of carbapenem-hydrolyzing-lactamase

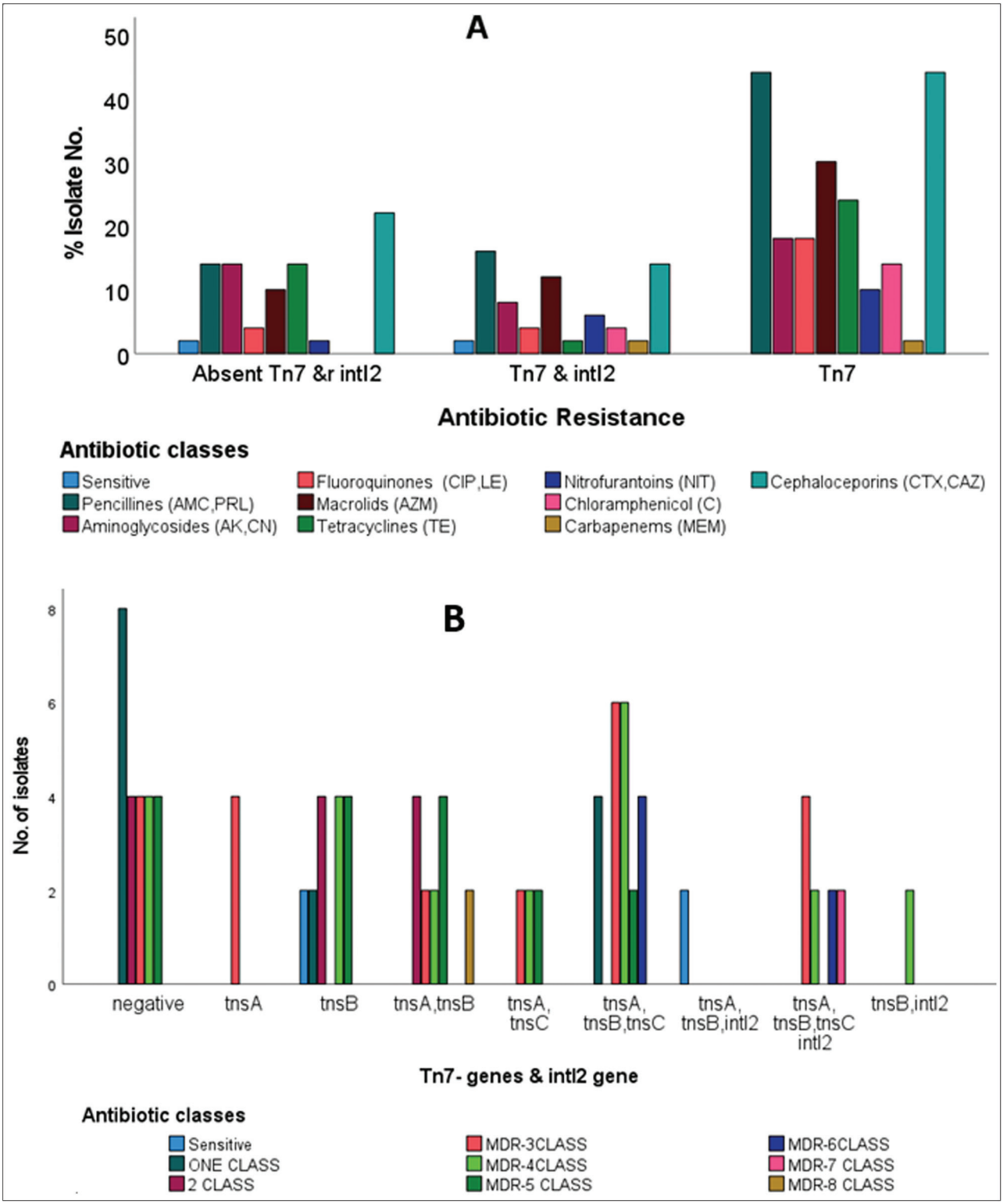

Figure 5: Prevalence of *intI2* gene and Tn7-like transposon among *E. coli* and *Klebsiella* isolates. (A) Percent of isolate numbers that associate antibiotic resistance with the presence of *intI2* gene and Tn7 transposition genes in bacterial isolates. (B) Isolate numbers that appeared the association of antibiotic resistance classes with the patterns of Tn7-like transposons

genes in Enterobacteriaceae in different parts of the world. The chromosomal DNA of various environmental Kluyvera species has been shown to include the potential source of the blaCTX-M β-lactamase genes.[32] While the Shewanella species identified in ambient water sources was the source of OXA-48.[33] Because many antibiotic-resistant genes are linked to elements such as plasmids or transposons, conjugation is frequently considered the

mechanism most likely responsible for the transmission of these traits, even though the transfer of these elements can also occur through transformation or transduction.[34] The previously mentioned ESBL and carbapenemases genes are excellent illustrations of resistance genes that have propagated across bacteria via plasmid conjugation and profoundly influence human health. A study from China[35] discovered that transmission of ESBL genes in *E. coli* occurs even in the food chain and accounts in part for the large fecal prevalence of ESBL-producing *E. coli* around the world. However, in light of the current level of globalization, resistant strains can easily move from one country to another. In a large cohort study of Dutch travelers visiting places with a high prevalence of ESBL-producing bacteria, 34.3% of those who were ESBL-negative before travel acquired these clones while abroad, with the majority of acquisitions taking place in the host country one of those who visited South Asia; after his return, the region remained colonized for a year.[36] The same study also revealed a 12% chance of transmission within families. Similar findings came from a Spanish investigation, where up to 66% of *E. coli* isolates from patients with ESBL-producing infections were identical to isolates from stool samples from their relatives. These findings suggest that acquiring *E. coli*-resistant strains while traveling is common and that transition within families can keep these strains around for a long time.

Antibacterial resistance in *E. coli* and other bacteria has been multifacetedly developed due to the therapeutic use of antibacterial medicines in human and animal medicine. The main consensus is that human activities and antibiotic chemotherapy are the only causes of antibiotic resistance. While not acquired through horizontal transmission, genomic investigations of environmental and human bacteria have revealed a number of resistance determinants or genes that extend back to the therapeutic usage of antibiotics in therapy. By blocking or eradicating rival bacteria for resources, intrinsic antibiotic resistance gives the strains that create a selective advantage. Exogenous antibiotic resistance, which was just found, differs from intrinsic prior until now; human behavior did not contribute to antibiotic resistance, primarily caused by antibiotics selection pressure.[36,37]

A core heteromeric transposase is composed of TnsB and TnsA, and a regulator protein called TnsC is related to Tn7-like transposons.[1,14] Most of the studied isolates from both species harbored only core transposition-encoding genes, and five variants (62%) appeared, including tnsABC, tnsAB, tnsAC, tnsA, and tnsB, as shown in Figure 4 and Table 3. These indicate about 22% of the isolates harboring genes encoding a complete Tn7-core transposase (tnsABC), and 20% of the isolates harboring truncated Tn7-core genes, whereas the haplotypes of the remainder (tnsA and tnsB) may be related to other TEs, for transposons or derivatives of Tn7-like transposons or truncated Tn7-core genes that have massive sequence variation that cannot be detected by the primer pairs used in this study. This conclusion is based on our observations of tnsE and tnsD gene detections because there is more than one sequence published in the NCBI database and most Tn7 transposases have been studied as protein sequences only using proteomic methods (https://www.ncbi.nlm.nih.gov/gene).

On the other hand, genes (tnsABCDE) are encoded by Tn7-like transposons, including an unusual heteromeric transposase. The two transposase subunits are TnsA and TnsB.[1,5] TnsA is related to endonucleases activity and has some homology with other transposon-endonucleases. At the same time, TnsB is a member of the vast family of bacterial transposase and is present other than Tn7-like transposons. A regulator protein called TnsC collaborates with several target site-selecting proteins to recognize various targets. While TnsE encourages horizontal transmission by concentrating on mobile plasmids, TnsD directs transposition into attTn7 (also detected in this study, data not shown). Tn7-like transposon's "core transposition mechanism" comprises the transposase TnsA and TnsB as well as the regulator protein TnsC.[1] One of the two target-selecting proteins, TnsD or TnsE, controls this fundamental machinery. By guiding transposition into the chromosome, the development of transposition with TnsABC + TnsD has increased the effectiveness of the element's vertical transmission. As a result of the TnsE protein's ability to detect features that were enriched during DNA replication on the lagging-strand template, transposition with TnsABC + TnsE happens preferentially into mobile plasmids.[1,5] Therefore, it is impossible to exclude the likelihood that these chromosomal Tn7-like transposons will try to transpose into mobile plasmids, making it simpler for Tn7-like transposons to spread among bacteria while the hosts are growing.

To transmit bacterial antibiotic resistance, Tn7-like transposons are crucial mobile elements. However, the information currently available on the thorough study of Tn7-like transposons in Enterobacterales isolates is still insufficient compared with studies about the transposition mechanism of Tn7-like transposons. Also, MDR intestinal bacteria such as *E. coli* are known to be associated with integrons.[1,6,12] In comparison with other investigations, the prevalence of integrons was comparatively greater; for instance, Ranjbaran *et al*.[38] reported 5.08% of class 2 integrons. A class 2 integron prevalence of 8% was observed in another study.[39] While Farshad *et al*.[40] reported that no integron class 3 was found, 47% of MDR isolates carried class 1 and 2 integrons. Research by Su *et al*.[41] found a frequency of 41.10% for class 2 integron.

As previously mentioned, there is a strong correlation between the presence of integrons and resistance to the

antibiotics quinolones, aminoglycosides, beta-lactam, trimethoprim, and chloramphenicol.[42] In this study, the class 2 integron integrase (*intI2*) was also found to be significantly correlated with resistance to the drugs AMC, PRL, CTX, CN, AZM, and NIT ($P = 0.05$: Chi-square test). This study is in line with earlier ones that, among 69 MDR-*E. coli* strains, found a significant correlation between 69 MDR-*E. coli* bacteria that had class 1 integrons and were resistant to amikacin, CN, chloramphenicol, ampicillin, tetracycline, nalidixic acid, and co-trimoxazole. Class 2 integrons were resistant to aminoglycosides, co-trimoxazole, cephalexin, ampicillin, and chloramphenicol.[41] Tn7-like transposons accumulate antibiotic resistance and most Tn7 transposons contain the *intI2* gene.

Comparing the genetic composition of various Tn7-like transposons allows us to surmise that the multidrug-resistant Tn7-like transposons share some evolutionary relationship, which has likely contributed to the critical function of Tn7-like transposons in the field of storing resistance genes. Despite the dissimilar assembly, each of these Tn7-like transposons, or portions of them, were identical to those discovered in other plasmids or chromosomes. This had to do with how mobile genetic elements could be used by transposons similar to Tn7 to gather genetic material and transmit it among strains.

Conclusion

This study is the first report to comprehensively analyze the incidence and antibiotic resistance characteristics of Tn7-like transposons in *E. coli* and *Klebsiella* isolates that were insulated from healthy subjects and patients with inflammation of the stomach and intestines and urinary tract infections in Hilla City, Iraq. Some *E. coli* and *Klebsiella* isolates were harboring integron genes in Tn7-like transposons. These isolates appeared eight patterns of transposon genes. Three of them are associated with the presence of the *intI2* gene; the other five patterns of Tn7 are similar to transposons.

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Conflicts of interest

There are no conflicts of interest.

References

1. He J, Li C, Cui P, Wang H. Detection of Tn7-like transposons and antibiotic resistance in Enterobacterales from animals used for food production with identification of three novel transposons Tn6813, Tn6814, and Tn6765. Front Microbiol 2020;11:2049.
2. Donnenberg M. *Escherichia coli*: Pathotypes and Principles of Pathogenesis. Academic Press; 2013. Doi: https://doi.org/10.1016/C2011-0-07692-5.
3. Mahmood IS, Al-Dohan JA, Al-Musafer MM. Effect of estradiol on the severity of lower urinary tract symptoms in men. Med J Babylon 2020;17:133-8.
4. Bennett JE, Dolin R, and Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. E-book. Elsevier Health Sciences; 2019. Doi: https://doi.org/10.1016/C2012-1-00075-6.
5. He J, Li C, Cui P, Wang H. Characterization of Tn7-like transposons and its related antibiotic resistance among Enterobacteriaceae from livestock and poultry in China. Research Square 2020;1:1-19.
6. Mazel D, Dychinco B, Webb VA, Davies J. Antibiotic resistance in the ECOR collection: Integrons and identification of a novel aad gene. Antimicrob Agents Chemother 2000;44:1568-74.
7. Pormohammad A, Pouriran R, Azimi H, Goudarzi M. Prevalence of integron classes in Gram-negative clinical isolated bacteria in Iran: A systematic review and meta-analysis. Iran J Basic Med Sci 2019;22:118-27.
8. Mitra R, McKenzie GJ, Yi L, Lee CA, Craig NL. Characterization of the TnsD-attTn7 complex that promotes site-specific insertion of Tn7. Mobile DNA 2010;1:1-14.
9. Hamidian M, Hall RM. Dissemination of novel Tn7 family transposons carrying genes for synthesis and uptake of fimsbactin siderophores among Acinetobacter baumannii isolates. Microb Genom 2021;7:mgen000548.
10. Tille MP. Bailey and Scott's Diagnostic Microbiology. 14th ed, St. Louis, Missouri: Elsevier; 2017.
11. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020
12. Mouanga-Ndzime Y, Onanga R, Kassa-Kassa RF, Bignoumba M, Mbehang Nguema PP, Gafou A, *et al*. Epidemiology of community origin Escherichia coli and Klebsiella pneumoniae uropathogenic strains resistant to antibiotics in Franceville, Gabon. Infect Drug Resist 2021;14:585-94.
13. World Health Organization. Global strategy for dengue prevention and control 2012-2020;2012.
14. Hu Y, Yang X, Qin J, Lu N, Cheng G, Wu N, *et al*. Met genome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. Nat Commun 2013;4:2151.
15. Al-Zubaidi OH, Al-Salman AR. Assessment of parents' awareness about urinary tract infections in children of Babylon Province. Med J Babylon 2022;19:50-7.
16. Lee JH, Subhadra B, Son Y-J, Kim DH, Park HS, Kim JM, *et al*. Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic Escherichia coli strains isolated from patients with urinary tract infections in South Korea. Lett Appl Microbiol 2015;62:84-90.
17. Kafilzadeh F, Farsimadan F. Investigating multidrug efflux pumps in relation to the antibiotic resistance pattern in Escherichia coli strains from patients in Iran. Biomed Res 2016;27:1130-5.
18. Maleki D, Jahromy SH, Karizi SZ, Eslami P. The prevalence of acrA and acrB genes among multiple-drug resistant uropathogenic Escherichia coli isolated from patients with UTI in Milad Hospital, Tehran. Avicenna J Clin Microbiol Infect 2016;4:39785-39785.
19. Hegazy EE, Alam El-Din RA, Amin AM, Mahgoub FM, El-Gamal SA. Microbiological profile of urinary tract infections with special reference to antibiotic susceptibility pattern of Escherichia coli isolates. Int J Curr Microbiol Appl Sci 2018;7:911-20.
20. Dobrindt U, Hentschel U, Kaper JB, Hacker J. Genome plasticity in pathogenic and nonpathogenic enterobacteria. Pathogenicity Islands and the Evolution of Pathogenic Microbes. Med Microbiol Immunol 2002;264:157-75.
21. Abdu A, Kachallah M, Yusuf-Bolus D. Antibiotic susceptibility patterns of uropathogenic Escherichia coli among patients with urinary tract infections in a tertiary care hospital in Maiduguri, North Eastern, Nigeria. J Biosci Biotechnol Discov 2018;3:14-24.

22. Paltansing S. Antimicrobial resistance in Enterobacteriaceae: Characterization and detection. Doctoral dissertation, Leiden University; 2015.
23. Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, *et al*. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci U S A 2018;115:E3463-70.
24. Nordmann P, Poirel L. Strategies for identification of carbapenemases-producing Enterobacteriaceae. J Antimicrob Chemother 2013;68:487-9.
25. McEwen SA, Fedorka-Cray PJ. Antimicrobial use and resistance in animals. Clin Infect Dis 2002;34:93-106.
26. Kaesbohrer A, Bakran-Lebl K, Irrgang A, Fischer J, Kämpf P, Schiffmann A, *et al*. Diversity in prevalence and characteristics of ESBL/pAmpC producing E. coli in food in Germany. Vet Microbiol 2019;233:52-60.
27. Köck R, Daniels-Haardt I, Becker K, Mellmann A, Friedrich AW, Mevius D, *et al*. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: A systematic review. Clin Microbiol Infect 2018;24:1241-50.
28. Joshi PR, Thummeepak R, Leungtongkam U, Pooarlai R, Paudel S, Acharya M, *et al*. The emergence of colistin-resistant Escherichia coli in chicken meats in Nepal. FEMS Microbiol Lett 2019;366:fnz237.
29. Ruppé E, Lixandru B, Cojocaru R, Büke C, Paramythiotou E, Angebault C, *et al*. Relative fecal abundance of extended-spectrum-β-lactamase-producing Escherichia coli strains and their occurrence in urinary tract infections in women. Antimicrob Agents Chemother 2013;57:4512-7.
30. Bourrel AS, Poirel L, Royer G, Darty M, Vuillemin X, Kieffer N, *et al*. Colistin resistance in Parisian inpatient faecal Escherichia coli as the result of two distinct evolutionary pathways. J Antimicrob Chemother 2019;74:1521-30.
31. Cantón R, Coque TM. The CTX-M β-lactamase pandemic. Curr Opin Microbiol 2006;9:466-75.
32. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: The phantom menace. J Antimicrob Chemother 2012;67:1597-606.
33. Von Wintersdorff CJ, Penders J, Van Niekerk JM, Mills ND, Majumder S, Van Alphen LB, *et al*. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol 2016;7:173.
34. Ye Q, Wu Q, Zhang S, Zhang J, Yang G, Wang J, *et al*. Characterization of extended-spectrum β-lactamase-producing Enterobacteriaceae from retail food in China. Front Microbiol 2018;9:1709.
35. Arcilla MS, van Hattem JM, Haverkate MR, Bootsma MC, van Genderen PJ, Goorhuis A, *et al*. Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): A prospective, multicentre cohort study. Lancet Infect Dis 2017;17:78-85.
36. Cox G, Wright GD. Intrinsic antibiotic resistance: Mechanisms, origins, challenges and solutions. Int J Med Microbiol 2013;303:287-92.
37. Al-Shibly IK, Alhamdany MH, Al-Kaif RAI, Al-Kaif LA. Immunological base behind the increased susceptibility of diabetic patients for infections. Indian J Public Health Res Dev 2019;10:3047-3051.
38. Ranjbaran M, Zolfaghari M, Japoni-Nejad A, Amouzandeh-Nobaveh A, Abtahi H, Nejad M, *et al*. Molecular investigation of integrons in Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infections. J Maz Univ Med Sci 2013;23:20-7.
39. Jones LA, McIver CJ, Rawlinson WD, White PA. Polymerase chain reaction screening for integrons can be used to complement resistance surveillance programs. Commun Dis Intell Q Rep 2003;27 Suppl:S103-10.
40. Farshad S, Japoni A, Hosseini M. Low distribution of integrons among multidrug resistant *E. coli* strains isolated from children with community-acquired urinary tract infections in Shiraz, Iran. Pol J Microbial 2008;57:193-98.
41. Su J, Shi L, Yang L, Xiao Z, Li X, Yamasaki S. Analysis of integrons in clinical isolates of *Escherichia coli* in China during the last six years. FEMS Microbiol Lett 2006;254:75-80.
42. Li Z, Craig NL, Peters JE. Transposon Tn7. In: Roberts A, Mullany P, editors. Bacterial Integrative Mobile Genetic Elements. Austin, TX: Landes Bioscience; 2012. p. 1-32.