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Identification and characterization of class 1 integron among multidrug-resistant and Environmental Escherichia coli in Diyala

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ABSTRACT

This study examines the incidence and characteristics of the Class 1 integrons in the Escherichia coli isolates coming from clinical and environmental sources. 15 isolates were tested; thirteen (86.6%) of the 15 isolates tested positive for class 1 integron, whereas none of the other isolates, irrespective of class, had any introns found. The incidence of *HS*549/*HS*550 genes associated with 3'CS Clinical Integron among local Escherichia coli. Fifteen *E. coli* samples were screened for 3'CS Clinical Integron, a clinical class 1 intgrons marker. Eleven of the fifteen samples (73.3%) were positive with variable ranges and frequencies of MRG284/MRG285 genes associated with the environmental cassette among local *E. coli*. Fifteen *E. coli* samples were screened for the environmental cassette, a marker for environmental class 1 integrin. Ten out of fifteen clinical samples (66.6%) were integrons. After screening six of the isolates, the gene cassettes for class 1 integrons were detected in the six isolates, which include those encoding resistance to trimethoprim *dfr*A5, aminoglycosides *aad*A1, sulfonamides (*sul*1), mercury reductase (*mer* operon), and those encoding a novel transporter and a short chain (dehydrogenase/reductase) in the selected isolates, which confer resistance to trimethoprim/sulfamethoxazole and aminoglycosides, respectively. Fifteen different patterns or arrangements of the gene cassettes were found. Our results indicate that class 1 integrons are widely distributed among MDR-UPEC strains in Diyala, which may directly or indirectly contribute to the selection of MDR strains. These findings are important for better understanding the factors and mechanisms that promote multidrug resistance among UPEC strains. Also, more research is needed to understand the transfer of integrons from clinical to environmental bacteria. This study offers significant insights into disseminating integron-mediated resistance, emphasizing the critical role of integrons as a focal point in public health initiatives aimed at combating antibiotic resistance.

Keywords; *Integron, PCR detection, Gene cassettes, Antibiotic resistance, Escherichia coli*

1. INTRODUCTION

Escherichia coli is a type of bacteria usually present in the lower intestine of warm-blooded species. It is gram-negative, meaning it does not retain a certain stain, and it is facultative anaerobic, which means it may survive with or without oxygen. E. coli is rod-shaped and belongs to the genus Escherichia.Uropathogenic Escherichia coli (UPEC) is a diverse group of strains that falls under the broader category of extra-intestinal pathogenic E. coli (Desvaux et al., 2020). UPEC is the most prevalent type. The uropathogen is accountable for urinary tract infections obtained inside the community (Abad et al., 2019). UPEC strains exhibit a wide range of genetic regulation and diversity. The distribution of virulence factors enables them to produce urinary tract infections (Dadi et al., 2020). Antibiotic resistance in bacterial pathogens is becoming a big problem for public health. This is mostly because bacteria can change their genes to deal with environmental stress. The integron system, especially Class 1 integrons, is a crucial mechanism facilitating adaptability and has been recognized as a major factor in spreading the resistance genes among bacterial populations (Fonseca & Vicente, 2022). Integrons, like mobile genetic elements, serve as platforms for acquiring and expressing the genes, mostly through gene cassettes, that encode antibiotic resistance, thus imparting multidrug resistance to the host bacteria (Cambray et al., 2010).Class 1 integrons are notably significant upon account of the widespread occurrence in the clinical and environmental isolates of Escherichia coli (E. coli) and other dangerous bacteria. These integrons have a specific integrase gene, intI1, which enables the incorporation of the gene cassettes into the integron framework, hence generating a varied spectrum of resistance profiles among various bacterial strains (Sunde & Norström, 2003). Cassette integration, governed through the integrase enzyme, facilitates the acquisition and sequential placement of the resistance genes, enhancing bacterial resistance (Gestal et al., 2011). Integrons can assimilate several resistance genes inside a single locus, rendering them effective vectors for multidrug resistance, especially in pathogenic bacteria such as E. coli. Integrons are mobile genetic elements commonly present in plasmids, chromosomes, and transposons, allowing them to transfer between bacteria. They have a vital function in the intricate genetic

processes that result in the development of different phenotypes and bacterial adaptation (Najafi et al., 2022). With their platforms, integrons are very good at recombination systems that can grab, store, get rid of, and rearrange mobile genetic fragments called cassettes. The dynamic genetic machinery of these organisms provides them with a significant capacity for adapting to their host. They have quickly become a major cause of antibiotic resistance, making it easier for different types of bacteria to quickly develop multi-resistant traits. Integrons are commonly found in bacteria, with approximately 15% of all sequenced bacterial genomes harbouring these elements (Chen et al., 2023). Integrons are known to be the principal source of transferable resistance genes and it is hypothesized that they may also function as repositories of antimicrobial resistance genes within microbial populations. The intI1 integrase enzyme makes it easier for the gene cassettes for the attI locus to be put in place. This plan makes it easier to add the resistance genes one at a time. The promoter Pc helps transcribe these genes together (Gestal et al., 2011). This arrangement gives the bacteria a strong way to adapt, which lets them quickly pick up new resistance traits by adding extra cassettes (Mazel, 2006).

Research indicates that Class 1 integrons are extensively prevalent in both clinical and environmental contexts, implying that resistance genes can transfer readily between human-associated bacterial populations and also environmental reservoirs (Halaji et al., 2020). This movement worries us because it means that antibiotic-contaminated soil and water could be hiding places for bacteria that are resistant to antibiotics. These bacteria could then get into clinical settings. Studies in diverse regions, such as Norway and also the Netherlands, have recorded the occurrence of the integrons in food and environmental samples, demonstrating the significant intersection between environmental and clinical bacterial populations (Sunde et al., 2015; van Essen-Zandbergen et al., 2007). The polymerase chain reaction (PCR) technique is the main molecular method used to find the Class 1 integrons in the Escherichia coli isolates. This technique emphasizes the amplification of the particular DNA sequences inside the intI1 gene, a recognized genetic marker for Class 1 integrons. The intI1 gene encodes an integrase enzyme crucial for the integration and rearrangement of the gene cassettes, frequently harbouring antibiotic resistance genes. By going after this gene, researchers can accurately find and measure how many Class 1 integrons are present in the bacterial samples. This study aimed to identify and characterize integrons among multidrug-resistant (MDR) and detect clinical environmental integrons. This study aimed to identify and characterize integrons among multidrug-resistant (MDR) uropathogenic Escherichia coli from outpatients in Baqubah City, Diyala. Polymerase chain reaction (PCR) tests were used to detect the presence of class 1 integrons, whose PCR products were sequenced to identify gene cassettes inserted within variable regions.

2. MATERIALS, AND METHODS

Activation of isolates

A cross-sectional study was conducted on 355 urine samples from patients attending Baqubah General Hospital, Al-Batoul Teaching Hospital, and the Public Health Laboratory between December 1, 2023, and November 1, 2024. by Vitek-2 system activated and identified 15 isolates selected from 105 *E. coli* isolates. Blood agar, MacConkey medium, and Neutrinet medium were used to reactivate the isolates. The isolates were selected based on their resistance and virulence factors (Prokop *et al*., 2017).

DNA Extraction and PCR Amplification

DNA extraction was conducted utilizing DNA and plasmid extraction kits (Promega, USA) in accordance with a pretreatment approach for Gram-negative bacteria and adhering to the manufacturer's instructions. The DNA's quantity and quality were assessed using a NanoDrop 1000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA).The PCR amplification procedure for the genetic level to detecting *E.coli* local isolates table (3-10) by follows step: Final volume for PCR mixture was 25 µl (12.5 of Master Mix 2x, 5 µl template DNA, 1 µl primers for each forward and reverse primer, finally, 5.5 µl nuclease free water) in uniplex PCR Eppendorf tubes but amount changed in multiplex PCR, mixed briefly via vortex then been placed in thermocycler polymerase chain reaction.

Molecular diagnosis of the integron genes of *E.coli* bacteria

Primers were picked to focus on certain parts of the Class 1 integron structure. The *int*I1 gene, which makes integrase and is necessary for gene cassette integration and antibiotic resistance, was given extra attention. The primer pairs in Table 1 came from research that was checked, which means they are always correct and reliable at finding Class 1 integrons. These primer sets were made to boost certain integron-related areas. (Stalder *et al*., 2014; Marquez *et al*., 2008; Klindworth *et al*., 2013). PCR findings were evaluated through examining band patterns that align alongside anticipated amplicon sizes. Figure 3 (Mono-plex PCR profiles) illustrates, that lanes exhibiting 311 bp bands corroborate the existence of the Class 1 integrons in the positive clinical isolates, consistent alongside previous research (Stalder *et al*., 2014). Variable-sized bands in the environmental samples exhibited various gene cassette arrays, indicating integron-mediated resistance gene diversity.

Table 1. Primers Utilized for Integron Detection

Primer	Sequence (5′→3′)	Target gene(s) or region	PCR product size (bp)	Annealing temp (°C)	References
intI1F165	CGAACGAGTGGCGGAGGGTG	Clinical class 1 integron	311 (clinical only)	55	Stalder *et al*,2014
intI1R476	TACCCGAGAGCTTGGCACCCA				
HS549	ACTAAGCTTGCCCCTTCCGC	3'CS Clinical integron	V	65	Marquez C. *et al*. 2008
HS550	CTAGGCATGATCTAACCCTCGG				
HS458	GCAAAAAGGCAGCAATTATGAGCC	Clinical cassette array	V	55	
HS459	GTTTGATGTTATGGAGCAGCAACG				
MRG284	GTTACGCCGTGGGTCGATG	Environmental cassette	V	55	Klindworth *et al*,2013
MRG285	CCAGAGCAGCCGTAGAGC				

Table (2): Amplification program of primers

Amplified gene	Initial denaturation	No. of cycle	Denaturation	Annealing	Elongation	Final extention
IntI	94°C/ 5min	35	94°C/ 1 min	55°C/45 sec	72°C/1 min	72°C/7min
HS5	94°C/ 5min	35	94°C/ 1 min	65°C/45 sec	72°C/1 min	72°C/7min
HS4	94°C/ 5min	35	94°C/ 1 min	55°C/45 sec	72°C/1 min	72°C/7min
MGR	94°C/ 5min	35	94°C/ 1 min	55°C/45 sec	72°C/1 min	72°C/7min

Analysis of the PCR Outcomes, and Significance to the Integron

PCR findings were evaluated through examining band patterns that align alongside anticipated amplicon sizes. Figure 3 (Mono-plex PCR profiles) illustrates, that lanes exhibiting 311 bp bands corroborate the existence of the Class 1 integrons in the positive clinical isolates, consistent alongside previous research (Stalder *et al*., 2014). Variable-sized bands in the environmental samples exhibited various gene cassette arrays, indicating integron-mediated resistance gene diversity.

Detection of integron Sequences

Gene sequencing refers to determining the order of nucleotides in a DNA molecule. Specifically, DNA sequencing of PCR amplicons involves determining the nucleotide sequence of the DNA fragments generated by the polymerase chain reaction (PCR) technique. The PCR amplicons were sequenced commercially from their ends. According to the sequencing corporation's instruction manuals, the machine can be operated in both forward and reverse directions. Macro gen Inc. is located in Geumchen, Seoul, South Korea. Exclusively transparent Analysed were chromatographs derived from ABI (Applied Bio systems) sequencing files. Moreover, it guarantees that the annotation and discrepancies were not attributable to PCR or Sequencing artifacts. The virtual locations and other relevant data have been retrieved. PCR fragments were determined by comparing the observed DNA sequences of local samples with the retrieved DNA sequences of the bacterial database. Bio Edit ver. 7.1 was used to compare DNA chromatograms with the deposited bacterial DNA sequences demonstrating the nucleic acid variations (DNASTAR, Madison). Snap Gene Viewerver.4.0.4 (https://www.snapg ene.com) annotated each detected variant inside the *E.coli*.

3. RESULTS

Detection of the Class 1 Integron in the Clinical, and Environmental Isolates

This work utilized PCR amplification to identify Class 1 integrons in the *E. coli* samples coming from clinical, and environmental sources. Figure (1) illustrates a distinct band for 311 bp in the lanes corresponding to the positive isolates, thereby affirming the presence of the *intI*1 gene, which encodes the integrase enzyme typical of the Class 1 integrons. The integrase gene serves like a vital indicator for identifying integrons, like it enables the insertion, and recombination of the gene cassettes into bacterial genomes (Fonseca & Vicente, 2022). The incidence of the Class 1 integrons used to the be significantly elevated among clinical isolates, alongside 86.6% testing positive, corroborating previous research upon the critical role of the clinical settings in the harboring, and disseminating antibiotic resistance genes via integron-mediated mechanisms (Al-Naimi, 2018; Bashir *et al*., 2015).

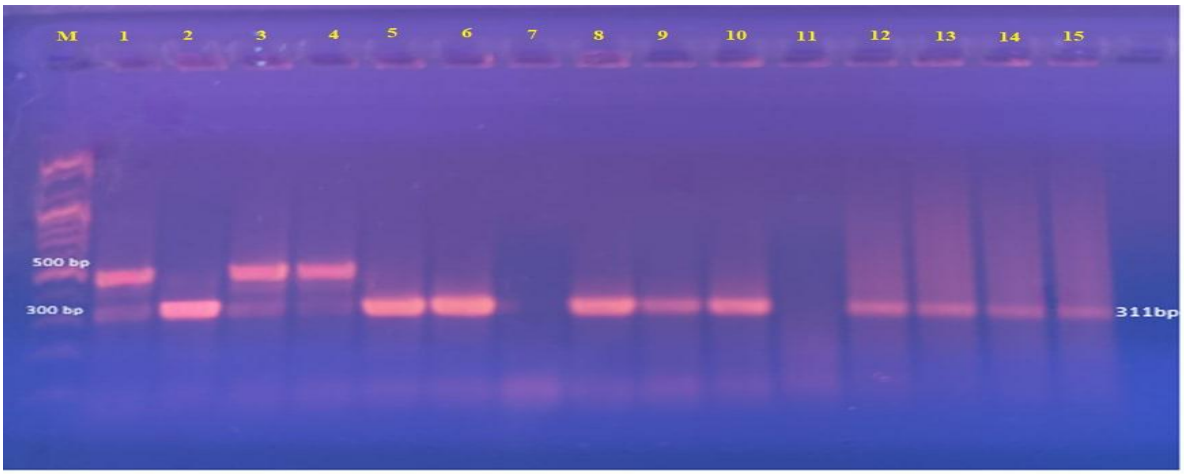


Figure (1): Gel electrophoresis of amplified *intI* gene of *Escherichia coli* isolates. Electrophoresis was done (1.5% agarose at 7 V for 1 h, Lane M: DNA ladder marker (1000); lane 2,5,6,8,9,10,12,13,14,15 (Positive for *intI gene*); lane 7 and11 (Negative for *intI gene*) ; lane 1,3,4 (different band pattern); lane16, N.C.(Negative control).

Characterization of the Gene Cassettes in the Class 1 Integrons

To clarify the variety of the resistance genes inside integrons, further PCR experiments were performed to the target the 3' conserved segment (3' CS), and variable sections in the Class 1 integrons utilizing primers HS458, and HS459. The PCR profiles depicted in the Figure (2) exhibit a variety of the band patterns among various isolates. Each pattern represents a distinct combination of the gene cassettes, underscoring the structural adaptability of the integrons in the integrating diverse resistance genes.

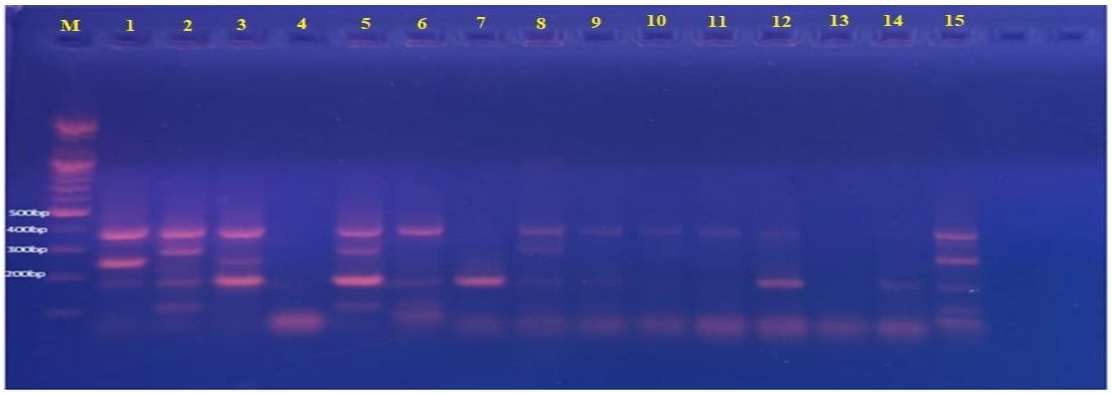


Figure (2): Gel electrophoresis of amplified *H4* gene of *Escherichia coli* isolates. Electrophoresis was done (1.5% agarose at 7 V for 1 h, Lane M: DNA ladder marker (1000); lane 1,2,3,5,6,7,8,9,10,11,12,14,15 (Positive for *H4 gene*); lane 4 and13 (Negative for *H4 gene*) ; lane16, N.C.(Negative control).

The incidence of *HS*549/*HS*550 genes associated with 3'CS Clinical Integron among local *E. coli*. Fifteen *E. coli* isolates were screened for the presence of 3'CS Clinical Integron, markers for clinical class 1 integrons. Eleven from fifteen (73.3%) clinical isolates were positive with variable bands. Figure (3).

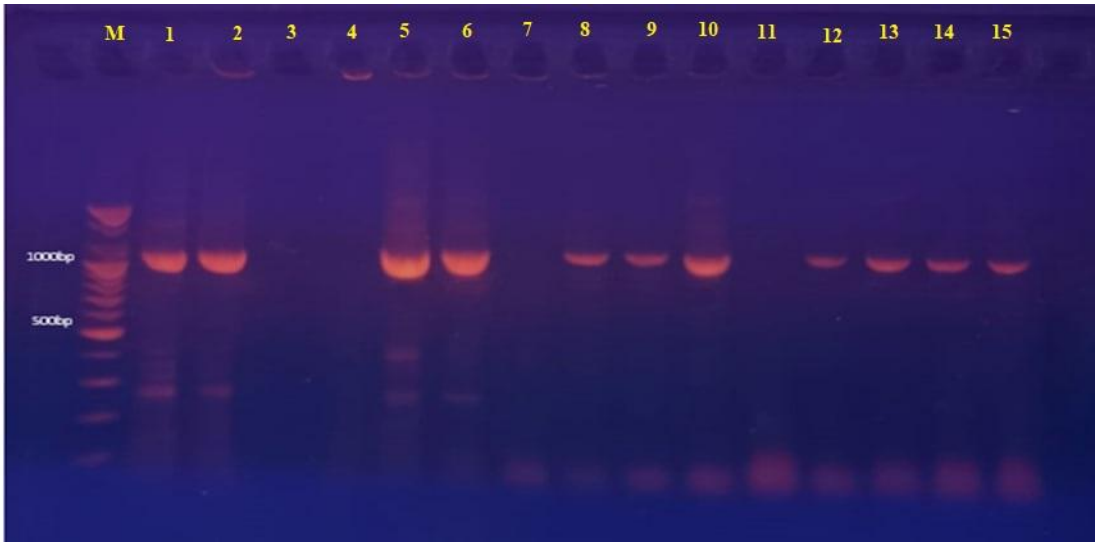


Figure (3): Gel electrophoresis of amplified *H5* gene of *Escherichia coli* isolates. Electrophoresis was done (1.5% agarose at 7 V for 1 h, Lane M: DNA ladder marker (1000); lane 1,2,5,6,8,9,10,12,13,14,15 (Positive for *H5 gene*); lane 3, 4, 7 and11 (Negative for *H5 gene*); lane16, N.C. (Negative control).

The heterogeneity in the gene cassettes highlights the adaptive characteristics of the integrons, particularly in the environmental isolates beneath various selective pressures. Certain isolates had bands linked to the *mer*A gene, which confers mercury tolerance, highlighting the significance of the integrons in the facilitating bacterial survival in the contaminated habitats. The structural variety enables bacteria to adapt, and endure both antibiotic, and environmental stresses, increasing the likelihood of the resistance gene transfer to the pathogenic strains (Sütterlin *et al*., 2020).

Variability of the Gene Cassettes, and Structural Analysis of the Integrons

The PCR amplification of the integron variable sections demonstrated significant variability in the cassette structure among the isolates. The standard architecture of the Class 1 integrons, comprising the 5', and 3' conserved segments (CS), used to be frequently disrupted through unique gene cassettes in the environmental isolates, resulting in configurations not commonly observed in the clinical samples. Figure (4) illustrates the gene cassette arrays in the specific environmental isolates, emphasizing their capacity to acquire numerous resistance determinants via recombination events. The incidence of *MRG*284/*MRG*285 genes associated with Environmental cassette among local *E. coli*. Fifteen *E. coli* isolates were screened for the presence of Environmental cassette, markers for Environmental class 1 integrons. Ten from fifteen (66.6%) clinical isolates were positive with variable bands. Figure (4).

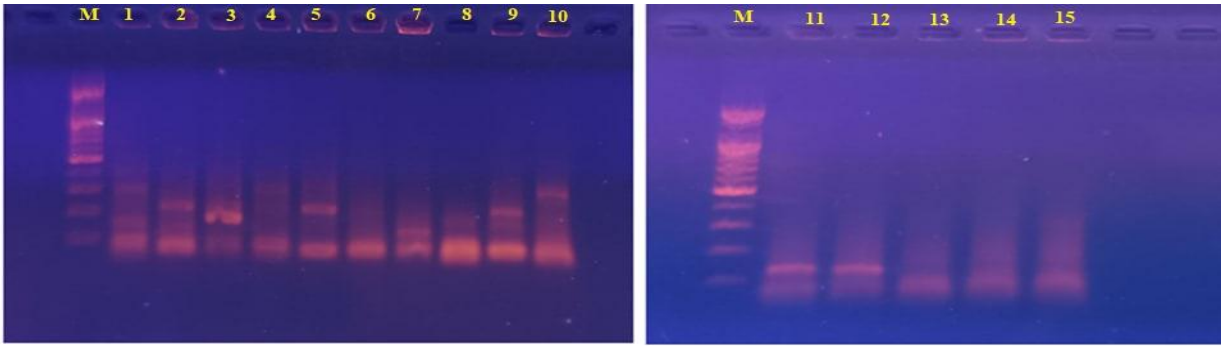


Figure (4): Gel electrophoresis of amplified *MGR* gene of *Escherichia coli* isolates. Electrophoresis was done (1.5% agarose at 7 V for 1 h, Lane M: DNA ladder marker (1000); lane 1,2,3,4,5,7,9,10 (Positive for *MGR gene*); lane 6 and 8 (Negative for *MGR gene*) lane11, N.C.(Negative control). B: lane 11 and 12 (Positive for *MGR gene*); lane 13, 14, 15 (Negative for *MGR gene*) lane 6, N.C. (Negative control).

Comparative Analysis of the Integron Variants in the Clinical, and Environmental isolates

the research additionally categorized integrons according to the content, and types of the gene cassettes found in each isolate. Table 1 presents a summary of the distribution of the integron types in the clinical, and environmental *E. coli* isolates. Each sample was used to be examined for the presence of the intI1 integrase gene, the quantity of the gene cassettes, and particular resistance genes contained inside each cassette. This table delineates the unique patterns of the resistance genes in the isolates

derived coming from clinical, and environmental sources. Clinical isolates often included numerous antibiotic resistance genes in the single integron, whereas environmental isolates exhibited a wider variety of the cassette types, including those conferring resistance to the heavy metals, and other environmental pollutants. The table depicts the frequency of the specific cassette types in the clinical samples, which include *dfr*A5, *sul*1, and *qac*EΔ1, which are typically linked to the resistance against trimethoprim, sulfonamides, and also the quaternary ammonium drugs, respectively. Conversely, the environmental samples displayed a greater diversity of the resistance elements, including the *mer*A gene, which facilitates survival in the mercury-contaminated environments. The existence of the gene cassettes in the environmental isolates indicates the significance of the integrons in the environmental adaptation, which is possibly facilitating the transmission of the resistance genes to the clinical contexts isolates (Carmona-Salido, López-Solís, López-Hontangas, & Amaro, 2024). (Barraud *et al*., 2013; Partridge *et al*., 2009).

Table 1 shows that the distribution of the Class 1 integrons among clinical *E. coli* isolates, works for emphasizing the diverse genotypic patterns seen throughout the isolates. Each pattern, indicated in the 'Genotype' column, comprises the unique gene combinations linked to antibiotic resistance, which include *dfr*A5, *aad*A1, and *qac*ΔE1. The table illustrates the quantity of the isolates, that tested positive for each genotypic pattern, offering insight into the incidence, and also diversity of the integrons in the clinical samples. This distribution demonstrates the variety of the resistance genes and also highlights the genetic intricacy that works to contribute to the multidrug resistance in the clinical *E. coli* isolates (Mohamed, & Rasheed Al-Taai, 2023).

Table (3): Distribution of class 1 integrons among clinical *E. coli* isolates.

Pattern No.	Genotype	No. of the positive integron
1	*rep intI1 DfrA5 qacΔE1 sul1 Tn6217*	8
2	*rep intI1 DfrA5 qacΔE1 sul1*	12
3	*intI1 DfrA5 qacΔE1 sul1*	15
4	*intI1 DfrA5 qacΔE1UshA*	10
5	*intI1 DfrA5 qacΔE1 ABC transporter*	9
6	*intI1 IS3 DfrA5 qacΔE1*	6
7	*intI1 aadA1 qacΔE1 sul1*	7
8	*intI1 TidD ABC transporter*	4
9	*intI1 DfrA5 qacΔE1*	11
10	*intI1 merA merD*	5
11	*intI1 qacΔE1 sul1*	13
12	*intI1 HP fsbD*	6
13	*hol UshA*	3
14	*intI1*	14
15	*merA*	8

Detection of the integrons in the environmental isolates exhibited significant variability, indicating varied cassette architectures that may represent responses to the varying ecological stressors. The heterogeneity in the environmental samples happens to the illustrated through bands of varying diameters, signifying varied integron cassette compositions among isolates. Environmental integrons seem to harbor gene cassettes, that impart resistance to both antibiotics, and several environmental stresses, including heavy metals, and toxins (Gillings, 2014).

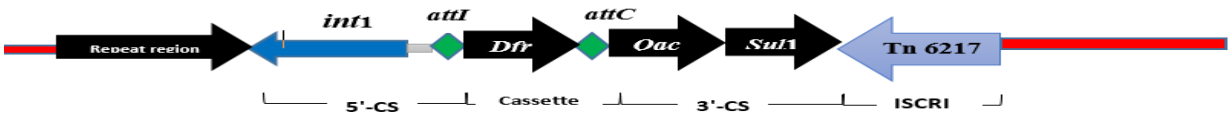
Table (4): Distribution of class 1 Integrons among environmental *E. coli* isolates

Genotype	No. of positive integron
intI1	6

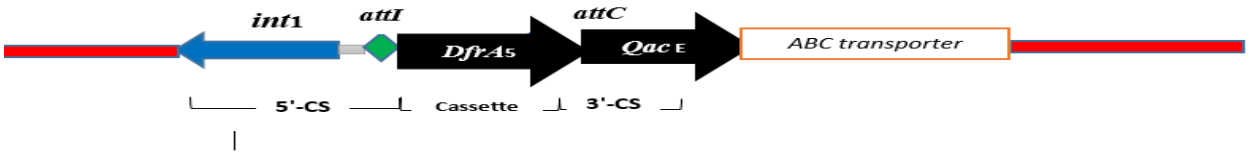
intI1 Ton	1
intI1dfrA aadA1	1

Table 5.Integrons, and the associated gene cassettes, identified in the E. coli isolates.

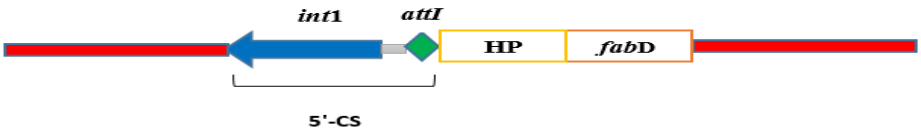
Sample	Integrase Gene		No. of the Gene Cassettes Present in the *intI1*	Name of the Gene Cassettes Present in the *IntI1*
	IntI1	Type *IntI1*		
E1	+		-	
E2	+	complet	+	*rep intI1 DfrA5 qacΔE1 sul1 Tn6217; intI1 DfrA5 qacΔE1 ABC transporter ;intI1 HP fsbD*
E3	+	complet	+	*intI1 aadA1 qacΔE1 sul1, intI1 aadA1 qacΔE1 sul1,intI1 merA merD, merA*
E4	+		_	
E5	+	complet	+	*intI1 IS3 DfrA5 qacΔE1 ,intI1 DfrA5 qacΔE1UshA*
E6	+		_	
E7	_		_	
E8	+		_	
E9	+	complet	+	*intI1 qacΔE1 sul1, intI1 DfrA5 qacΔE1 ABC transporter, intI1, intI1, intI1*
E10	+	Calin, complet	+	*intI1 aadA1 qacΔE1 sul1, hol UshA*
E11	_		_	
E12	+	complet	+	*intI1 qacΔE1 sul1, intI1 TidD ABC transporter*
E13	+	complet	_	
		complet		
E14	+		_	
E15	+	complet	_	



E1-A (Complete integron: Integron including an integrase and at least one *attC* site)



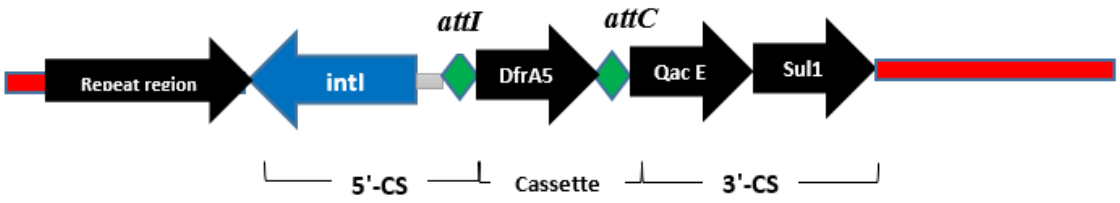
E1-B (Complete integron: Integron including an integrase and at least one *attC* site)



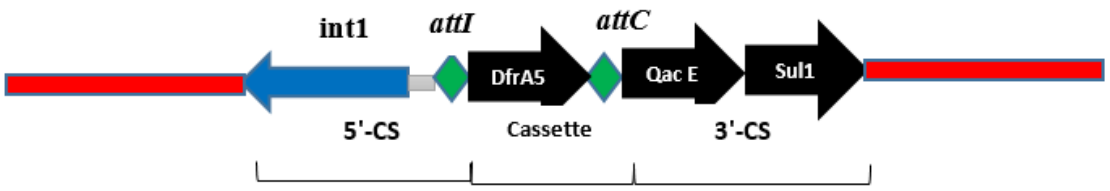
E1-C (Complete integron: Integron including an integrase and at least one *attC* site)

Figure (5): The complete structures of the integrons in the first isolate of *Escherichia coli*

E2-A (Complete integron: Integron including an integrase and at least one *attC* site)



E2-B (Complete integron: Integron including an integrase and at least one *attC* site)



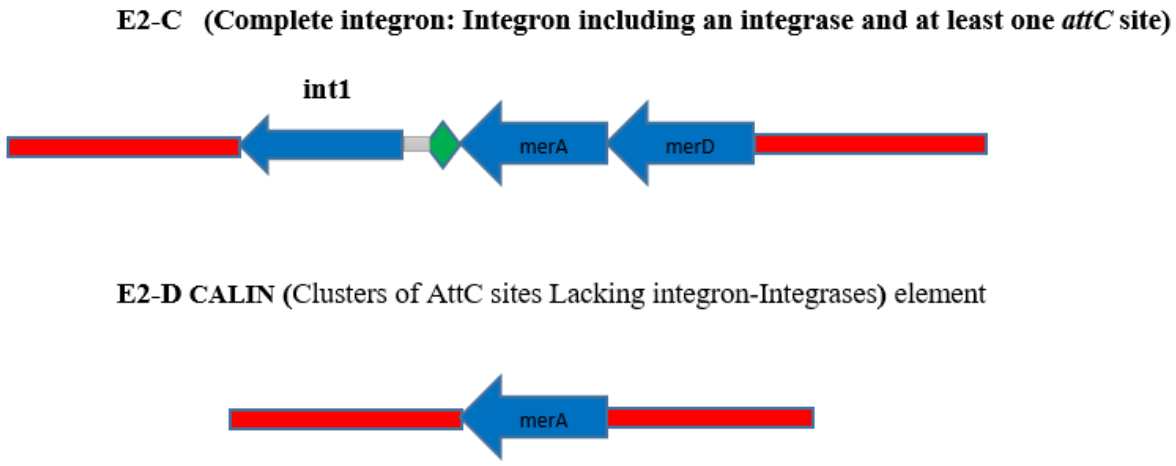


Figure (6): The complete structures of the Integrons in the second isolate of *Escherichia coli*

4. DISCUSSION

This work sought to discover and characterize Class 1 integrons in clinical and environmental *Escherichia coli* isolates, employing polymerase chain reaction (PCR), like the primary method for detecting integron presence, and related gene cassettes. PCR used to be selected for its sensitivity and specificity in amplifying relevant DNA sequences, especially for identifying resistance genes in the integrons, which turn out to be critical components in the spread of antibiotic resistance (Stalder *et al*., 2014; Holmes *et al*., 2016).PCR facilitated the targeted amplification of the intI1 gene, which encodes the integrase enzyme essential for gene cassette recombination in the Class 1 integrons. This enzyme enables the incorporation, and removal of the gene cassettes, frequently harboring antibiotic resistance determinants (González-Zorn & Escudero, 2012). Primers specific to the intI1 gene, and additional related resistance indicators were utilized. Utilizing PCR, we confirmed the existence of the Class 1 integrons in the various *E. coli* isolates, elucidating their abundance, and distribution in the clinical, and environmental samples (Gillings & Paulsen, 2014).

Analysis of the PCR Outcomes

The PCR amplification findings, illustrated in Figures 1 to 2, exhibited distinct band patterns that align alongside the anticipated amplicon sizes for the *int*1 gene, and other gene cassettes. The findings revealed, that 86.6% of the clinical isolates were positive for Class 1 integrons, in contrast to the reduced incidence in the environmental isolates. This mismatch indicates increased selective pressure in clinical situations, possibly resulting from the prevalent use of antibiotics, which promotes the formation, and retention of the integrons containing antibiotic resistance genes (Paterson, 2006; Holmes *et al*., 2016).

The mono-plex PCR profiles for multiple genes (e.g., *H4*, *intI*1, *MGR*) underscored the diversity in the presence of the resistance genes among isolates. Clinical isolates demonstrated a greater diversity of the resistance genes in their integrons, presumably upon account of the increased exposure to the various antibiotics in the healthcare environments. Environmental isolates, albeit positive for integrons, exhibited a reduced number of gene cassettes, suggesting potentially diminished selective pressure in the non-clinical settings (Gillings, 2014).

Distribution of the Gene Cassettes, and Variability of the Integrons

The gene cassettes linked to the Class 1 integrons in the isolates exhibited a range of resistance determinants, including trimethoprim (*dfr*A5), aminoglycosides (*aad*A1), and sulfonamides (*sul*1), among others. As indicated, a variety of resistance determinants, including trimethoprim (dfrA5), aminoglycosides (*aad*A1), and sulfonamides (*sul*1), were present in the gene cassettes connected to the Class 1 integrons in the isolates. As said, each sample had a different gene cassette arrangement, and clinical isolates had a wider variety of gene cassettes than environmental isolates. This distribution suggests that ambient bacteria may serve as reservoirs for integrons that could eventually be passed on to clinical pathogens through methods of horizontal gene transfer (Gillings & Paulsen, 2014; Barraud *et al*., 2013).

Consequences of the Integron-Mediated Resistance in the Bacterial Populations

Because these components facilitate the rapid spread of multidrug resistance among bacterial populations, the presence of Class 1 integrons—especially those carrying multiple resistance genes—poses a serious threat to public health. Through the acquisition and production of new resistance genes, integrons enable rapid bacterial adaptation to antibiotic stressors, making

infection management and treatment approaches more challenging (González-Zorn & Escudero, 2012; Al-Shabender&Al-Taai,2023). The results of this study demonstrate the need for strict monitoring of integron prevalence in clinical and environmental settings to anticipate and prevent the spread of antibiotic resistance genes.

Integron-mediated antibiotic resistance genes are prevalent in clinical Enterobacteriaceae linked to human diseases in this manner (Bhat *et al*. 2023). Lang (2015), says Tn21-like transposons, which harbor class 1 integrons, along with closely related In2 variants located in various independent sites (usually conjugative plasmids), contribute to the elevated prevalence of this element in commensal, environmental, and clinical bacterial isolates worldwide. PCR was employed to amplify and sequence the gene cassettes linked to the detected class 1 integron in *E. coli* for further characterization. Multiple *E. coli* isolates tested positive for *intI*1 and *qac*EΔ1. The DNA sequences of the PCR amplicons were almost identical to the nucleotide sequence of the *aad*A1 gene, which confers resistance to streptomycin and spectinomycin. The DNA sequence of the PCR amplicon exhibited 99% similarity with the *aad*A1 genes from *E. coli*. The studied class 1 integrons lacked the comprehensive resistance characteristic shown in the clinical *E. coli* isolates. This does not preclude the presence of other class 1 integrons in *E. coli* strains that we may have failed to identify. A significant proportion of *E. coli* isolates exhibiting resistance to streptomycin and sulfonamides were concurrently positive for the class 1 integrase gene *intI*1. All *E. coli* isolates that tested positive for *int*I1 and *qac*EΔ1 (n = 9) also possessed the *aad*A1 resistance gene in this manner (Sütterlin *et al*. 2020). These identical genetic markers were also apparent in the tested isolates. While evaluating the distribution of class 1 integron markers and *mer*A in avian *E. coli* isolates, we observed additional genotypes lacking the *int*I1, *qac*EΔ1, *aad*A1, or *mer*A genes. These atypical genotypes may signify recombinational occurrences within class 1 integrons and other genes (Adelowo *et al*. 2018). This research represents the inaugural documentation of the precise integron class I structure in pathogenic *E. coli* isolates. The spread of mercury resistance in *E. coli* seems to result from Tn21. Partridge *et al*. (2018) reported that Tn2 or a closely related variant containing antibiotic resistance genes integrated into the Tn21 mercury resistance module, resulting in the deletion of the adjacent sequence, while all four 38-bp inverted repeats associated with Tn21 family transposon termini were disrupted by an IS4321-like element. This element has also been documented in gram-negative clinical isolates linked to human sickness. Resistance to the heavy metal mercury is not uncommon among epidemiologically significant *E. coli* strains. Resistance to mercury in *E. coli* isolates, including pathogenic strains linked to sickness, has been documented; however, the genetic determinant responsible for this resistance remains unidentified. The selective benefit of mercury resistance for the organism remains ambiguous, as mercuric chemicals are not utilized in hatchery disinfectants. At now, the sole antibiotics authorized for use and demonstrated to be effective against infections are two fluoroquinolones: Ciprofloxacin and Levofloxacin. The efficacy of these medications may be transient, as *E. coli* is developing resistance to quinolones and fluoroquinolones. Alongside the ongoing development of novel antimicrobial drugs, a crucial aspect of addressing the issue of multidrug resistance may involve finding and leveraging situations that are detrimental to the survival and spread of integrons within bacterial cells (Baltazar *et al*. 2022). The method identifies the bulk of known attC sites and intI genes, remaining unaffected by genomic G+C content. The elevated sensitivity in identifying individual attC sites results in a minimal probability (0.02%) of overlooking all elements within a cluster of four attC sites. A genomic rearrangement that divides an integron will provide a CALIN and an integron, perhaps an In0 if the rearrangement occurs at the attI site. Identifies these two genetic elements, which are transcriptionally independent as PC cannot facilitate the expression of CALIN's cassettes. Conversely, these elements may retain functional connectivity as cassettes from the CALIN can be excised by the integron-integrase and then reintegrated at the integron's *att*I site. The status of the two parts as distinct entities or as a singular integron remains ambiguous. It is important to recognize that such instances may be challenging to differentiate from other evolutionary scenarios that include the loss of the integron integrase in one of several integrons within a genome. (Néron *et al*. 2022) delineate three categories of elements: Complete integron: An integron that encompasses an integrase and a minimum of one attC site, In0 element: An integron comprising just integrase and CALIN elements (Clusters of *Att*C sites devoid of integron-integrases): An integron that has a minimum of two attC sites (Néron *et al*., 2022).The search results for the integron elements are compiled to categorize the loci into three classifications (Figure E1: A, B, C). The components containing intI and a minimum of one *att*C site were designated as full integrons. The term complete denotes the existence of both elements; we cannot determine the functionality or expression of the integron. The In0 components possess intI but lack identifiable attC locations. We do not adhere completely to the original definition of In0, which encompasses the presence of an attI as noted by (Bissonnette 1992), as this sequence is unknown for the majority of integrons and so cannot be searched for. The cluster of *att*C sites devoid of integron-integrase (CALIN) contains a minimum of two *att*C sites and is absent of proximal *int*I. Specifically, if a locus encompasses integron-integrase and attC sites (full integron), the search is limited to the strand encoding attC sites between the terminus of the integron-integrase and 4 kb beyond its farthest attC. If other *att*C sites are identified beyond this one, the search will be extended by 4 kb in that direction until no further new sites are detected. If the element comprises solely *att*C sites (CALIN), the search is conducted on the same strand in both orientations. If the integron is In0, the search for attC sites is conducted on both strands inside the 4 kb region bordering the integron-integrase on each side. The sequences were effective for six *E. coli* isolates obtained from urinary tract infections out of a total of fifteen isolates. Six of the fifteen isolates exhibited positivity for the first class integrons (*int*I1), resulting in a 40% prevalence rate. The presence of bands with a 311 bp amplicon indicated a positive test result. Our analysis yielded various patterns of differing lengths. There were entire lengths of the intron and partial lengths referred to as calin. The whole intron was present in 19 of six isolates, and the CALIN was 33.3%.E1 comprises three

integrons: The first integron includes rep *int*I1, *DfrA*5, *qac*ΔE1, *sul*1, and Tn6217; the second integron contains *int*I1, *Dfr*A5, *qac*ΔE1, and an ABC transporter; the third integron consists of *int*I1 and HP *fsb*D. The presence of these genes within an integron would be atypical and may suggest a novel adaptation, wherein the integron plays a role in regulating non-antibiotic resistance features. This may influence how *E. coli* acclimatizes to various settings, especially if these genes provide a selection benefit in specific scenarios, such as biofilm development, persistence, or virulence. The isolate comprised three varieties of integron. Integrons comprise a collection of gene cassettes, including *intI*1, *Dfr*A5, *qac*ΔE1, and *sul*1. (Table 5). Integrons and related gene cassettes found in *E. coli* isolates.

5. CONCLUSION

The study discovered a significant presence of the int1 gene, essential for integron function, and resistance transfer, through PCR analysis. The prevalent presence of the gene in the clinical isolates suggests, that healthcare environments may act like reservoirs and possible enablers for the dissemination of antibiotic resistance. The integration of the next-generation sequencing alongside PCR can augment the comprehension of the genetic variety, and structure in the integrons, facilitating more accurate identification of the resistance profiles, and elucidation of the resistance mechanisms across bacterial populations.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The samples were obtained according to Scientific Research Ethics Committee approval in the College of Sciences, University of Diyala, and local Research Ethics Committee approval in the Iraqi Ministry of Health on December 20, 2023

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