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INTEGRONS DETECTION IN MULTI - DRUG RESISTANCE *ESCHERICHIA COLI* ISOLATED FROM CLINICAL AND ENVIRONMENT SOURCES IN BASRAH, IRAQ

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ABSTRACT

Aims: The study aimed to evaluate the type and frequency of integrons in multi-drug-resistant *Escherichia coli* isolated from clinical and environmental samples in Basrah, Iraq. **Methodology and results:** The MDR *E. coli* strains were identified using both conventional and molecular techniques. Antibiotic susceptibility was tested using the Kirby-Bauer method. The results revealed that out of 62 samples studied, 58 (93.54%) of the local *E. coli* isolates were resistant to at least three different antibiotics. These MDR *E. coli* strains showed high resistance to penicillin, erythromycin, and tetracycline, with rates of 62 (100%), 57 (91.93%), and 50 (80.64%), respectively. Additionally, we investigated the presence of integrons in MDR *Escherichia coli* using specific primers through the PCR technique. The results showed that 32 (51.61%) of the total isolates contained *IntI1* (491 bp in size), while 5 (8.06%) harbored *IntI2* and 6 (9.67%) harbored *IntI3* (788 bp and 600 bp, respectively). A significant association ($P \leq 0.05$) was also observed between the presence of *IntI1* and resistance to several antibiotics in MDR *E. coli*. **Conclusion, significance and impact of the study:** We concluded that integrons, particularly *IntI1*, are major genetic determinants in MDR *E. coli* strains. These elements play a crucial role in the horizontal transfer of drug resistance genes in clinical isolates of *Escherichia coli*.

***Key words*:** Antibiotic resistance, Integrons, MDR *Escherichia coli*

INTRODUCTION

Multidrug-resistant (MDR) *Escherichia coli* is a significant global health concern, increasingly found in both humans and animals. *E. coli* has a strong capacity to acquire resistance genes, primarily through horizontal gene transfer, despite being inherently sensitive to all clinically Important antimicrobial drugs (Poirel *et al*., 2018). Globally, and particularly in developing countries, pathogenic *E. coli* agents of gastrointestinal illnesses are significant contributors to occasional epidemics of diarrhea in humans and animals (Othman, 2018; Abd Al Wahid and Abd Al-Abbas, 2019; Hussein *et al*., 2023; Farhan and Al-iedani, 2021; Kareem and Khudaier, 2016). Due to the recent use of high dosages of antibiotics—which are necessary in the current food animal production system for disease prevention, management, and growth promotion—the frequency of extended-spectrum beta-lactamases (ESBLs) has been growing (Ojer-

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Usoz *et al*., 2017). Antimicrobial resistance has been found to arise and spread through a number of genetic mechanisms. According to Medini *et al*. (2005) and Tettelin *et al*. (2008), many of these mobile genetic elements (MGEs) form what is known as the mobilome, including integrons (intI), transposons (Tn), insertion sequences (IS), and introns. MGEs are transferred horizontally primarily through conjugation, transformation, and transduction (Woodford *et al*., 2011). Integrons are ancient structures that contribute to bacterial evolution by acquiring, storing, discarding, and utilizing gene cassettes, which are mobile elements (Osagie *et al*., 2019). Typically, each integron has three essential components: first, the integrase gene (*intI*); second, the promoter (*Pant*), which drives the expression of any gene correctly integrated; and finally, the attachment site (*attI*). The integrase is an enzyme that catalyzes the integration and excision of DNA units and belongs to the site-specific (tyrosine recombinase) family (Carattoli, 2001; Severino and Magalhaes, 2004). There are generally four types of integrons, each with a unique integrase gene. Almost all integrons from classes 1, 2, and 3 contain gene cassettes known to encode resistance to antibiotics and disinfectants (Diekema *et al*., 2004). Class 1 integrons are the most prevalent and well-characterized. Additionally, the *Vibrio cholerae* genome contains a unique class of integrons, class 4, which is not known to be associated with antibiotic resistance (Deng, 2015).

Numerous regional studies have investigated the distribution of integrons in isolates of multidrug-resistant (MDR) *Escherichia coli*. These studies found a significant correlation between antibiotic resistance and the presence of integrons (Khadhim and Kazaal, 2018; Abbas, 2015; Mohemmad *et al*., 2023). Moreover, a recent study by Hamza and Omran (2023) revealed a significant ($P < 0.05$) correlation between *IntI2* and resistance to several antibiotics, including cefotaxime, piperacillin, amoxicillin/clavulanate, gentamicin, and azithromycin. Despite these local studies, there is still insufficient knowledge about the spread of integron genes (*IntI1* , *IntI2*, and *IntI3*) and their correlation with multidrug-resistant *Escherichia coli* recovered from clinical and environmental samples in the regions studied. Consequently, the goal of the present investigation is to identify the types of integrons circulating in multi-drug-resistant *Escherichia coli* recovered from diarrheal patients, animals, and environmental sources in the Basrah region.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

Between September and December 2023, ninety samples were collected from various regions in Basrah province, southern Iraq. The samples were distributed as follows: stool specimens from diarrheal patients (30), fecal specimens from diarrheal cattle (30), and other samples (30) collected from local water supplies, desalination plants, animal enclosures, and drainage water. The samples were immediately transported to the Central Laboratory of Microbiology at the College of Veterinary Medicine, University of Basrah, in ice-cooled boxes for analysis and identification of *Escherichia coli* using standard procedures (Quinn, 2004).

The stool samples were first inoculated into Brain-Heart Infusion (BHI) broth and incubated for 24 hours at 37°C. A loopful of the broth culture was then streaked onto MacConkey agar and MacConkey sorbitol agar (MSA) and incubated at 37°C for 24 hours. The brilliant pink colonies (lactose fermenters) were subsequently subcultured on Eosin Methylene Blue (EMB) agar and incubated for 24 to 48 hours at 37°C. The Gram-negative bacteria exhibiting a metallic sheen were confirmed as *E. coli* using the VITEK 2 system (bioMérieux) to determine their antimicrobial resistance patterns.

DNA extraction and PCR analysis

For the detection of *E. coli* at the molecular level, a species-specific oligonucleotide primer was used (Table 1). A total reaction volume of 25 µl was prepared for the PCR reactions, which included 5 µl of PCR PreMix (Bioneer), 5 µl of extracted DNA, 1 µl of each primer, and 8 µl of nuclease-free water. The PCR conditions were as follows: initial denaturation for 4 minutes at 94°C, followed by 30 cycles of 45 seconds at 94°C, 1 minute at 57°C, and 1 minute at 72°C, with a final extension of 10 minutes at 72°C. The PCR amplicon size was checked under a UV transilluminator by loading 5 µl of PCR products into a 1.5% agarose gel stained with a safe dye. The size of the amplicons was then assessed by comparison with a 100 base pair DNA ladder (Promega).

Table 1: The oligonucleotides Primers used through the study.

Primer	Sequence (5'-3')	Size of amplicon (bp)	Ref.
Species specific	F: GCTTGACACTGAACATTGAG R:GCACTTATCTCTTCCGCATT	662	Riffon *et al*., 2001
IntI1	F:GGTCAAGGATCTGGATTT CG R:ACATGCGTGTAAATCATC GTC	491	Machado *et al* ., 2005
IntI2	F:CACGGATATGCGACAAAA AGG R:TGTAGCAAACGAGTGACGA AATG	788	
IntI3	F:AGTGGGTGGCGA ATG AGTG R:TGTTCTTGTATCGGC AGGTG	600	

PCR Detection of Integrons

All 62 positive DNA samples were used to identify the presence of integrons using PCR. The PCR master mix was prepared for the amplification reaction in a total volume of 25 µl, with reaction components and volumes similar to those used in the species-specific *E. coli* reaction. The PCR conditions began with an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of additional denaturation at 94°C for 30 seconds, an annealing step at 60°C for 30 seconds to detect *IntI1* , *IntI2*, and *IntI3*, and an elongation step at 72°C for 1 minute and 30 seconds. The reaction concluded with a final extension at 72°C for 10 minutes. A 1.5% agarose gel was used for electrophoresis to separate the PCR amplicons.

Antimicrobial susceptibility testing

The disc diffusion technique, as described by Kirby and Bauer (1966), was used to assess antibiotic susceptibility. The inoculum was prepared by suspending *E. coli* colonies, isolated from EMB agar plates after 18 to 24 hours of incubation, in saline solution. The suspension was then vortexed and adjusted to match the 0.5 McFarland turbidity standard. A sterile swab was dipped into the adjusted bacterial suspension and spread onto freshly prepared Mueller-Hinton Agar plates. Discs containing selected antibiotics were placed on the surface of the agar. The plates were then incubated for 20 hours at 37°C. After incubation, the minimum inhibitory concentration (MIC) was determined by measuring the diameter of the inhibition zones with a ruler. The results were categorized as susceptible or resistant based on the parameters approved by CLSI (2014).

Bio-statistical analysis

Version 21 of the SPSS program was used to perform the analyses. The chi-square test was employed to assess the correlation between the presence of integrons and antibiotic resistance, with a significance level set at $P \leq 0.05$.

RESULTS

Bacterial Isolation and Identification

The tagged bacteria were primarily identified by culturing on selective media. Suspected colonies of *Escherichia coli* appeared bright pink with a red halo on MacConkey agar and had a diameter of 2-3 mm with a dark black center and a distinctive metallic sheen under light on EMB agar (Figure 1). Furthermore, Gram staining was performed on all suspected colonies, revealing typical red short rod-shaped cells of *E. coli* (Figure 1-A).

Table 2 presents the percentage and distribution of positive samples ($P \leq 0.05$) based on culturing, VITEK-2 system, and PCR analysis. The results showed that 65 (72.22%) of the isolates examined through culturing were suspected to be *E. coli*. In contrast, the VITEK 2 system and PCR analysis confirmed that 62 (95.38%) and 62 (100%) of the isolates were *E. coli*, respectively. Additionally, clear bands of the expected size (662 bp) were observed on the agarose gel after electrophoresis, as shown in Figure 2.

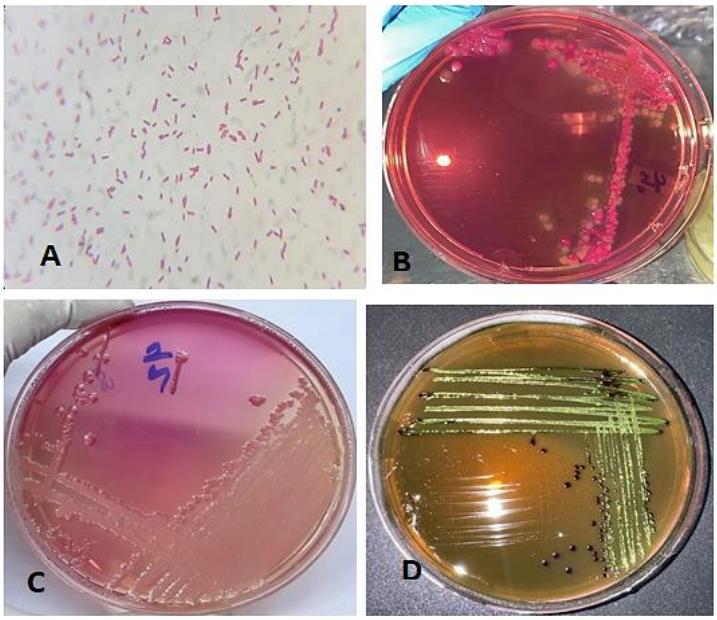

Figure 1: Primary Identification of *E. coli.* A: Gram stain *E. coli* under light microscope (1000X). B: Red-pink colonies of *E. coli* on MacConkey agar. C: *E. coli* colonies on MSA, presumed to be lactose fermenters, appearing as brilliant pink colonies with a crimson halo. D: *E. coli* colonies on EMB agar, displaying dark centers with a characteristic metallic greenish appearance.

Table 2: Isolation of *E. coli* from various sources using Culture media, VITEK 2 System and PCR

Source of samples	Examined Samples no= 90	Culturing no+ (%)	VITEK 2 no+ (%)	PCR no+ (%)
Fecal/ diarrheal cattle	30	29(96.66%)	28(96.55%)	28(100%)
Stool/ diarrheal patients	30	29(96.66%)	29(100%)	29(100%)
Other[1]	30	7(23.33%)	5(71.42%)	5(100%)
Total	90	65(72.22%)	62(95.38%)	62(100%)
P value[2]	0.00*	0.029*	0.03*	0.00

[1]Other samples were collected from local water supply, desalination plants, animal stripes and drainage water.[2] Indicate the presence of the significant at ($P \leq 0.05$)

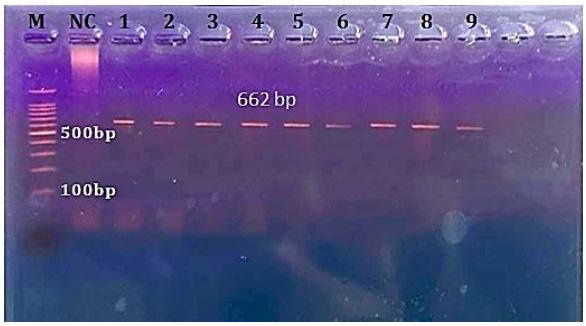

Figure 2: Conventional PCR amplification results of 23S rRNA gene (662 bp) for *E. coli* isolate. M: molecular marker (100bp), NC: negative control. Lanes 1-9: positive samples.

PCR Detection of Integrons

PCR was used to detect integrons (*IntI1*, *IntI2*, and *IntI3*) in local *E. coli* isolates. As shown in Table 3, there was a significant difference ($P \leq 0.05$) in the occurrence of *IntI1*, *IntI2*, and *IntI3* among different sample sources. The results revealed that 32 (51.61%) of the isolates contained *IntI1* (491 bp in size), while 5 (8.06%) harbored *IntI2* and 6 (9.67%) contained *IntI3* (788 bp and 600 bp, respectively) (Figures 3, 4, and 5).

Table 3: Percentage of Int1, Int 2 and Int3 in *E. coli* isolates by PCR.

Source of samples	Positive PCR	*Int1* no (%)	*Int2* %	*Int3* %
Fecal/ diarrheal cattle	28	12 (42.85%)	2 (7.14%)	2 (7.14%)
Stool/ diarrheal patients	29	18(62.06%)	2 (6.89%)	4 (13.79%)
Other*	5	2(40%)	1(20%)	0
Total	62	32(51.61%)	5(8.06%)	6(9.67%)
P value	0.00*	0.00*	0.00*	0.00*

[1]Other samples were collected from local water supply, desalination plants, animal stripes and drainage water.[2] Indicate the presence of the significant at P≤0.05)

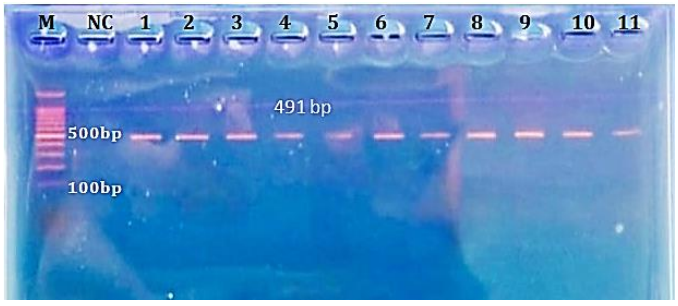

Figure 3: PCR amplification results of *Int1* gene (491 bp) detected in *E. coli* isolate. M: molecular marker (100bp), NC: negative control. Lanes 1-11: positive samples.

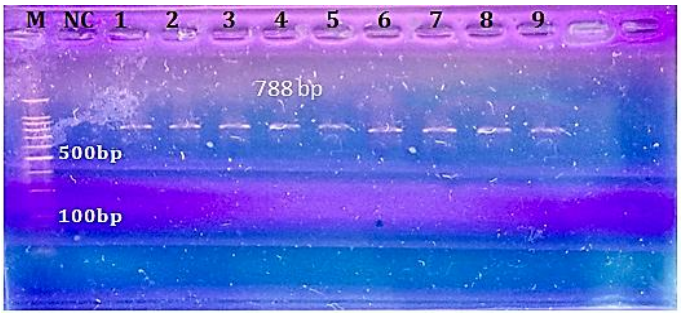

Figure 4: PCR amplification results of *Int2* gene (788 bp) detected in *E. coli* isolate. M: molecular marker (100bp), NC: negative control. Lanes 1-9: positive samples.

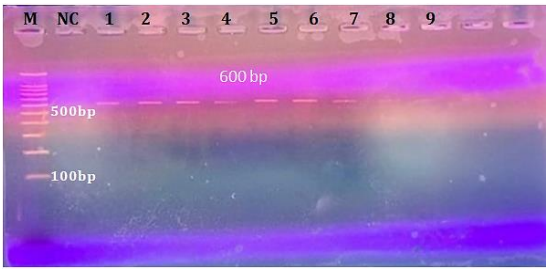

Figure 5: PCR amplification results of *Int3* gene (600 bp) detected in *E. coli* isolate. M: molecular marker (100bp), NC: negative control. Lanes 3-9: positive samples.

Antimicrobial susceptibility testing

Table 4 details the resistance frequency for each antibiotic used in the study. The table also reveals significant resistance ($P \leq 0.05$) to penicillin (62/100%), erythromycin (61/98.38%), and tetracycline (59/95.16%). The lowest levels of resistance were observed in cefotaxime (29/46.77%), chloramphenicol (29/46.77%), ciprofloxacin (23/37.09%), and cotrimoxazole (17/27.41%). Additionally, the antimicrobial susceptibility test showed that 58 out of the 62 studied *E. coli* isolates (93.54%) were resistant to three or more different antibiotics (Table 5, Figure 6). The MDR *E. coli* isolates were categorized into five phenotypes, as shown in Table 6.

Finally, Table 7 presents the types and numbers of integrons detected in MDR *E. coli*. The table shows that *IntI1* was present in all reported phenotypes [30/51.72%], with a significant association ($P \leq 0.05$) observed in phenotypes I [11/17.74%], II [6/9.67%], and IV [6/12.9%]. Additionally, *intI3* showed a significant association with phenotype I [4/8.06%], while *IntI2* was not significantly associated with any phenotypes recorded in this study.

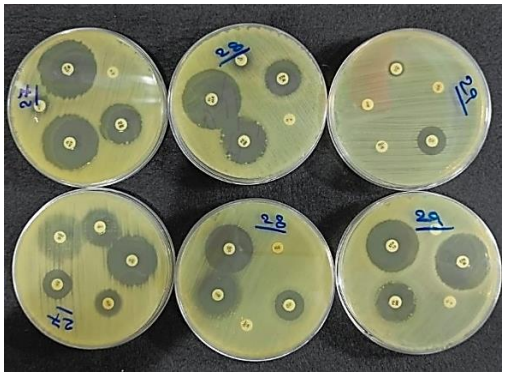

Figure 6: The antimicrobial susceptibility phenotypes of *E. coli* isolated from clinical and environment sources

Table 4: Numbers and percentage of *E. coli* resistant to different antibiotics used in the study

Antibiotics	Diarrheal cattle n=28 (%)	Diarrheal patients n=29(%)	Other* n=5(%)	Total n=62(%)
Penicillin (P)	28(100%)	29(100%)	5(100%)	62(100%)
Erythromycin(E)	28(100%)	29 (100%)	4(80%)	61(98.38%)
Tetracycline (TE)	26(92.85%)	29 (100%)	4(80%)	59(95.16%)
Gentamycin (GEN)	19 (67.85%)	25(86.20%)	2(40%)	46(74.19%)
Chloramphenicol (C)	10 (35.71%)	25(86.20%)	1(20%)	36(58.06%)
Cotrimoxazole (COT)	9(32.14%)	24(82.75%)	2(40%)	35(56.45%)
Ciprofloxacin (CIP)	11(39.28%)	22(75.86%)	1(20%)	34(54.83%)
Cefotaxime (CTX)	10 (35.71%)	19(67.85%)	1(20%)	30(48.38%)
Ertapenem (ETP)	1(3.57%)	20(68.96%)	1(20%)	22(35.48%)
Fosfomycin(FO)	2(7.14%)	18 (62.06%)	0(0%)	20(32.25%)
P value*	0.00*	0.00*	0.00*	0.00*

***Indicate the presence of the significant at (P≤0.05)**

Table 5: Phenotypes resistance patterns among the MDR E. *coli* isolated from diarrheal, local water supply, desalination plants, animal stripes and drainage water.

Antimicrobial resistance profiles	Number of strains showing profile
(I) ETP FO CTX E P CIP TE C COT GEN	20(34.48%)
(II) CTX E P CIP TE C COT GEN	11 (18.96%)
(III) E P TE C COT GEN	3(5.17%)
(IV) FO E P TE GEN	15(25.86%)
(IIV) E P TE GEN	9 (15.51%)
Total no. 62	58(93.54%)
P value	0.00*

*Indicate the presence of the significant at (P≤0.05) between the phenotype pattern of *E. coli* isolated from diarrheal, local water supply, desalination plants, animal stripes and drainage water

Table 6: Phenotypes numbers in MDR *E. coli* isolated from diarrheal, local water supply, desalination plants, animal stripes and drainage water.

Source of samples	No. Phenotype				
	I	II	III	IV	IIV
Fecal/ diarrheal cattle	1(5%)	6(40%)	9(81.81%)	5(55.55%)	3(100%)
Stool/ diarrheal patients	19(95%)	9(60%)	2(18.18%)	4(44.44)	0(0%)
Other*	0 (0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	20	15	11	9	3
P value	0.050*	0.00*	0.00*	0.00*	0.00*

Table 7: Type and number of integrons detection in MDR *E. coli* isolates

Phenotypes	Samples (No= 62) %	*IntI1* %	*IntI2* %	*IntI3* %
I	20(32.25%)	11 (17.74%)	2(3.22%)	4(8.06%)
(II)	11(17.74%)	6(9.67%)	1(1.61%)	1(1.61%)
(III)	3(4.83%)	2(3.22%)	0	0
(IV)	15(24.19%)	6(12.9%)	1(1.61%)	1(1.61%)
(IIV)	9(14.5%)	5(8.06%)	1(1.61%)	0
Total	58(93.54%)	30(51.72%)	5(8.62%)	6(10.34%)
P value	0.007 *	.015*	0.103**	.008*

*Indicate the presence of the significant at (P≤0.05) between the phenotypes of *E. coli* and *IntI1* and *IntI3*. **Indicate the insignificant at (P≥0.05) between the phenotypes of *E. coli* and *IntI2*.

DISCUSSION

Antimicrobial therapy is the primary treatment for bacterial infections. However, the rise of multidrug-resistant (MDR) strains has decreased the effectiveness of these medications. *E. coli*, a common clinical isolate, has increasingly exhibited new antibiotic resistances, making treatment more challenging (Chen *et al*., 2019). In Iraq, as in many other countries, the antibiotic misuse in hospitals and among farm animals is relatively common and widespread. Evidence suggests that resistance genes can spread between bacterial populations independent of antibiotic usage patterns. The development of MDR strains is currently believed to be largely attributed to integrons, which play a key role in the acquisition and dissemination of antimicrobial resistance genes in this bacterial species (Blancarte-Lagunas *et al*., 2020).

In the present study, we reported a high frequency of *IntI1* occurrence, with 32 (51.61%) of local *E. coli* isolates harboring this integron, particularly among those isolated from diarrheal patients (18/62.06%) and diarrheal animals (12/42.85%). This finding is consistent with Goldstein *et al*. (2001), who found that class *IntI1* was present in approximately 46% of Enterobacteriaceae isolates. Additionally, Dehkordi *et al*. (2020) reported that the frequency of class 1 integron in *E. coli* from domestic animals ranged from 23.8% to 52.4%. Conversely, our study did not detect *intI3* in *E. coli* isolates from local water supplies, desalination plants, animal waste, or drainage water, aligning with the findings of Laroche *et al*. (2009). The differences in these findings may be attributed to variations in the total number of *E. coli* recovered in the studies.

Table 4 outlines the resistance frequencies for each antibiotic used in the study. It reveals significant resistance ($P \leq 0.05$) to penicillin (62/100%), erythromycin (61/98.38%), and tetracycline (59/95.16%). This result is consistent with Abdul-Hussein et al. (2018), who reported high resistance to ampicillin (95.2%), and Ghaffoori and Suleiman (2022), who found significant resistance to ampicillin and tetracycline (95.2% and 85.7%, respectively). Khudaier *et al*. (2012) also reported high resistance to ampicillin and tetracycline (100% and 75%, respectively). However, our findings are inconsistent with those of Sakhi (2016), who reported lower resistance to erythromycin (12/14.2%).

On the other hand, the significant resistance observed in the current study to erythromycin, penicillin, and tetracycline may be attributed to irrational use of these antibiotics, the transmission of resistant isolates between individuals, and the consumption of food from antibiotic-treated animals. Additionally, the resistance rates for cefotaxime and chloramphenicol were 29 (46.77%), ciprofloxacin was 23 (37.09%), and cotrimoxazole was 17 (27.41%). These findings are higher than those reported by Rezaee et al. (2011), who found resistance rates of 5 (4.2%) for chloramphenicol, 15 (12.7%) for ciprofloxacin, and 26 (22%) for cotrimoxazole. The differences in resistance percentages could result from variations in antibiotic usage frequency across regions (WHO, 2012). The increase in antibiotic resistance in this community may be attributed to self-medication, noncompliance with prescriptions, and the sale of substandard drugs (Jafri *et al*., 2014; Endale *et al*., 2023).

The antimicrobial susceptibility test revealed that 58 out of 62 studied samples (93.54%) of local isolates were resistant to three or more different antibiotics, distributed across five phenotypes as shown in Table 6. Notably, phenotype I was prevalent, occurring in 19 (95%) of *E. coli* isolates from diarrheal patients. However, none of the isolates from diarrheal patients harbored phenotype IV. Furthermore, none of the phenotypes were observed in *E. coli* isolates from drainage water, animal waste, desalination plants, or local water supplies. This is likely due to the low number of *E. coli* recovered from these samples, reducing the likelihood of identifying specific resistance patterns. The high occurrence of multidrug-resistant *E. coli* may be associated with sample sources, particularly those linked to human disease and animals, suggesting that these sources might act as vectors for spreading antimicrobial-resistant bacteria into the environment (Olaru *et al*., 2023; Jalil *et al*., 2022).

Moreover, according to the results in Table 7, *IntI1* was detected in all reported phenotypes, with a frequency of 30 (51.72%). It was significantly associated ($P \leq 0.05$) with phenotypes I [11 (17.74%)], II [6 (9.67%)], and IV [6 (12.9%)]. Additionally, the *intI3* integron showed a significant association with phenotype I [4 (8.06%)], while *IntI2* did not show a significant association with the other phenotypes recorded in our study. Generally, the frequency of integrons detected in the

present study was relatively lower compared to other studies. For instance, Kargar *et al*. (2014) reported that class 1 and class 2 integrons were present in 78.26% and 76.81% of MDR isolates, respectively.

Moreover, according to Jones *et al*. (2003), classes 1 and 2 integrons were reported in 47% of MDR strains, while class 3 was not found in these strains. Rezaee *et al*. (2011) discovered 26.03% and 5.08% of class 1 and class 2 integrons, respectively, whereas Farshad *et al*. (2008) reported rates of 25.6% for class 1 integron and 41.10% for class 2 integron. The present study also revealed a clear correlation between class 1 integron and MDR E. coli isolated from the stool of diarrheal patients, aligning with the observation that class 1 is highly related to multiple resistances in Enterobacteriaceae in clinical settings (Martinez-Freijo *et al*., 1998). In contrast, our study reported *intI3* in E. coli isolates at a frequency of 6 (9.67%), which differs from findings in studies from Australia (White *et al*., 2001), Korea (Yu *et al*., 2003), France (Skurnik *et al*., 2005), Spain (Machado *et al*., 2005), China (Su *et al*., 2006), Iran (Rezaee *et al*., 2012; Farshad *et al*., 2008; Ranjbaran *et al*., 2013), Japan (Japoni *et al*., 2008), and Taiwan (Vinué *et al*., 2008). However, it aligns with the result of Kargar *et al*. (2014), who found class 3 integrons in 26.09% of MDR E. coli isolates.

Furthermore, based on Table 5 and Table 7, there is a significant correlation ($P \leq 0.05$) between *IntI1* and resistance to erythromycin, tetracycline, cefotaxime, and ciprofloxacin, and between *IntI3* and resistance to penicillin.

Several studies have investigated the occurrence of various integron classes and their association with drug resistance. Kargar *et al*. (2014) reported a substantial connection between class 1 integrons and resistance to ampicillin, gentamicin, ciprofloxacin, co-trimoxazole, and nalidixic acid. Other studies have also noted a correlation between resistance to quinolones, trimethoprim, chloramphenicol, and beta-lactams with the presence of specific integrons (Su *et al*., 2006; Chang *et al*., 2000; Singh *et al*., 2005). In contrast, our study found no connection between fosfomycin and *intI3*, cotrimoxazole with *IntI2* and *IntI3*, or chloramphenicol with *IntI3*. These discrepancies could be attributed to the limited occurrence and detection of *IntI2* and *IntI3* in our study.

Ploy *et al*. (2003) noted that class 3 integrons show limited presence in some bacteria, such as E. coli. However, a class 3 integron carrying *blaGES-1* has recently been associated with IncQ plasmids in some E. coli (Collis *et al*., 2002). Rowe-Magnus *et al*. (2001) reported that the incidence of class 3 integrons has varied, ranging from 0% to 10%, with sporadic reports of 7% in positive veterinary isolates.

CONCLUSION

We conclude that integrons, particularly *IntI1* , are major genetic determinants of multidrug-resistant (MDR) strains in *E. coli*. These components play a crucial role in the horizontal transfer of genes associated with antibiotic resistance, significantly impacting the prevalence of MDR *E. coli* isolated from clinical cases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

REFERENCES

Abbas, A.T. (2015). Detection of Integron CS Class 1 Type Metallo-β-Lactamase Gene in Clinical Isolate of Escherichia

Coli at Thi-Qar, Iraq. Thi-Qar Medical Journal (TQMJ):Vol.9 No.1: 49-55

Abd Al Wahid, Z. and Abd Al-Abbas, M.J. (2019): Detection of *E. coli* Strains Isolated from Water Sources and Diarrhea Cases by Random Amplified Polymorphic DNA in Basrah Governorate. J International Journal of Sciences, 8, 68-83. DOI: 10.18483/ijSci.1943

Abdul-hussein, Z.K.; Raheema, R.H. and Inssaf, A.I. (2018): Molecular Diagnosis of Diarrheagenic E. coli Infections among the Pediatric Patients in Wasit Province, Iraq, J. Pure Appl. Microbiol., 12(4):2229-2240. https://dx.doi.org/10.22 207/JPAM.1 2.4.62.

*Blancarte-Lagunas, M.I.; Castro-Escarpulli, G. Navarro-Ocaña ,A.; Ibáñez-Cervantes, G.; Marquez-Valdelamar, L.M.; Hernández-Carrillo, J.M.; Salazar-Salinas, J.; Mendoza-Vásquez, O.F.; Damazo-Hernández, G.; Sosa-Hernández O.; León-García, G.; Cureño-Díaz, M.A. and Bello-López, J.M. (2020):*Commensal and virulent Escherichia coli strains of vaginal origin are reservoirs of resistance cassettes in class 1 integrons. Journal of infection in developing countries, 14(1), 48–58. https://doi.org/10.3855/jidc.11940

Carattoli, A. (2001): Importance of integrons in the diffusion of resistance. Veterinary research, 32(3-4), 243–259. https://doi.org/10.1051/vetres:2001122

Chang, C.Y.; Chang, L.L.; Chang, Y.H.; Lee, T.M. and Chang, S.F. (2000): Characterisation of drug resistance gene cassettes associated with class 1 integrons in clinical isolates of *Escherichia coli* from Taiwan, ROC. Journal of medical microbiology, 49(12), 1097–1102. https://doi.org/10.1099/0022-1317-49-12-1097

Chen, M.; Wu, Y.; Yu, S.; Liu, S.; Wang, Y.; Huang, D.; Xu, X. and Lin, F (2019): Drug Resistance and Integron Genes in *Escherichia coli* Isolated from Urinary Tract Infection. Journal of nanoscience and nanotechnology, 19(9), 5989–5993. https://doi.org/10.1166/jnn.2019.16501

CLSI: Clinical and Laboratory Standards Institute (2014): Performance Standards for Antimicrobial Susceptibility Testing; twenty-first informational supplement. M100- S24, 34:1. Clinical and Laboratory Standards Institute, Wayne, PA.

Collis, C.M.; Kim, M.J.; Partridge, S.R. Stokes, H.W. and Hall, R.M. (2002). Characterization of the class 3 integron and the site-specific recombination system it determines. J Bacteriol. 84: 3017–3126. doi:10.1128/JB.184.11. 3017-3026.2002.

De Farhan, Z.A. and Al-iedani, A.A. (2021): The phylogenetic groupings of Escherichia coli isolated from human and farm animal feces in Basrah district, Iraq. Nat. Volatiles and Essent. Oils, 2021; 8(5): 8982-8990

Dehkordi, M.K.; Halaji, M. and Nouri, S. (2020): Prevalence of class 1 integron in *Escherichia coli* isolated from animal sources in Iran: a systematic review and meta-analysis. *Tropical medicine and health, 48,* 16. https://doi.org/10.1186/s41182-020-00202-1

Deng, Y.; Bao, X.; Ji, L.; Chen, L.; Liu, J.; Miao, J.; Chen, D.; Bian, H.; Li, Y. and Yu, G. (2015): Resistance integrons: class 1, 2 and 3 integrons. Annals of clinical microbiology and antimicrobials, 14, 45. https://doi.org/10.1186/s12941-015-0100-6

Diekema, D.J.; BootsMiller, B.J.; Vaughn, T.E.; Woolson, R.F.; Yankey, J.W.; Ernst, E.J.; Flach, S.D.; Ward, M.M.; Franciscus, C.L.; Pfaller, M.A. and Doebbeling, B.N. (2004): Antimicrobial resistance trends and outbreak frequency in United States hospitals. Clinical infectious diseases :

an official publication of the Infectious Diseases Society of America, 38(1), 78–85. https://doi.org/10.1086/380457

Endale, H.; Mathewos, M. and Abdeta, D. (2023): Potential Causes of Spread of Antimicrobial Resistance and Preventive Measures in One Health Perspective-A Review. Infection and drug resistance, 16, 7515–7545. https://doi.org/10.2147/IDR.S428837

Farshad, S.; Japoni, A. and Hosseini, M. (2008): Low distribution of integrons among multidrug resistant E. coli strains isolated from children with community-acquired urinary tract infections in Shiraz, Iran. Polish journal of microbiology, 57(3), 193–198.

Ghaffoori, HAH. and Suleiman, A.A.J. (2022). Isolation, Identification and antibiotic resistance profile distribution of clinical E. coli in Iraqi patients. Eurasian Medical Research Periodical, 8, 116–121.

Goldstein, C.; Lee, M. D.; Sanchez, S.; Hudson, C.; Phillips, B.; Register, B.; Grady, M;;Liebert, C.; Summers, A.O.; White, D.G. and Maurer, J.J. (2001). Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrobial agents and chemotherapy, 45(3), 723–726. https://doi.org/10.1128/AAC.45.3.723-726.2001

Hamza, O.A. and Omran, R. (2023): Molecular detection of Tn7-like transposons and *IntI2* integron integrase in multidrug-resistant *Escherichia coli* and *Klebsiella* spp. Medical Journal of Babylon 20(4): p 817-827,.DOI: 10.4103/MJBL.MJBL_353_22

Hussein, Z.M. and Naser, D.L.A. (2023): Phenotypic and Molecular Detection of *Escherichia Coli* in Patients with Diabetic Foot Infections in Basrah, Iraq. Central Asian Journal of Medical and Natural Science, 4(3), 216-228. https://doi.org/10.17605/cajmns.v4i3.1515

Jafri, SA.; Qasim, M.; Masoud, M.S.; Rahman, M.U.; Izhar, M. and Kazmi, S. (2014): Antibiotic resistance of *E. coli* isolates from urine samples of Urinary Tract Infection (UTI) patients in Pakistan. Bioinformation, 10(7), 419–422. https://doi.org/10.6026/97320630010419

Jalil, A.; Gul, S.; Bhatti, M.F.; Siddiqui, M.F. and Adnan, F. (2022): High Occurrence of Multidrug-Resistant *Escherichia coli* Strains in Bovine Fecal Samples from Healthy Cows Serves as Rich Reservoir for AMR Transmission. Antibiotics (Basel, Switzerland), 12(1), 37. https://doi.org/10.3390/antibiotics12010037

Japoni, A., Gudarzi, M., Farshad, S., Basiri, E., Ziyaeyan, M., Alborzi, A., & Rafaatpour, N. (2008). Assay for integrons and pattern of antibiotic resistance in clinical Escherichia coli strains by PCR-RFLP in Southern Iran. Japanese journal of infectious diseases, 61(1), 85-88.

Jones, L.A.; McIver, C.J. Rawlinson, W.D. and White, P.A. (2003): Polymerase chain reaction screening for integrons can be used to complement resistance surveillance programs. Communicable diseases intelligence quarterly report, 27 Suppl, S103–S110.

Kareem, R.A. and Khudaier, B.Y. (2016). Molecular Detection of tem Gene in Escherichia coli O157:H7 Isolated from Children and Buffaloes in Basrah Province. Basrah Journal of Veterinary Research, 15(3). doi.10.23975/BJVETR.2016.172806

Kargar, M.; Mohammadalipour, Z.; Doosti, A.; Lorzadeh, S. and Japoni-Nejad, A. (2014): High Prevalence of Class 1 to 3 Integrons Among Multidrug-Resistant Diarrheagenic Escherichia coli in Southwest of Iran. Osong public health and research

perspectives, 5(4), 193–198. https://doi.org/10.101 6/j.phrp.2014.06.003

Khadhim, M.M. and Kazaal, M.A. (2018): Association Between Antibiotic Resistance and Integron Class 2 Among Commensal *Escherichia coli* Genotypic Groups. Vol. 14 No. 25. : https://doi.org/10.28922/qmj.2018.14. 25.14-26

Khudaier, B.Y.; Abbas, B.A. and Khleel, K.A. (2012): Prevalence and antimicrobial susceptibility of *Escherichia coli O157:H7* isolated form human and animals sources in Basrah province. Bas.J.Vet.Res.Vol. 11, No.2, 2012

Kirby, W.M. and Bauer, A.W. (1966): Antibiotic Susceptibility Testing by a Standardized Single Disc Method .The American. J. Clin. Path., 45: 493-496

Laroche, E.; Pawlak, B.; Berthe, T.; Skurnik, D. and Petit, F. (2009): Occurrence of antibiotic resistance and class 1, 2 and 3 integrons in *Escherichia coli* isolated from a densely populated estuary (Seine, France). FEMS microbiology ecology, 68(1), 118–130. https://doi.org/10.1111/j.1574-6941.2009.00655.x

Machado, E.; Cantón, R.; Baquero, F.; Galán, J.C.; Rollán, A.; Peixe, L. and Coque, T.M. (2005): Integron content of extended- spectrum -beta-lactamase -producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain. Antimicrobial agents and chemotherapy, 49(5), 1823–1829. https://doi.org/10.1128/AAC.49.5.1823-1829.2005

Martinez-Freijo, P.; Fluit, A.C. Schmitz, F.J.; Grek, V.S.; Verhoef, J. and Jones, M.E. (1998): Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. The Journal of antimicrobial chemotherapy, 42(6), 689–696. https://doi.org/10.1093/jac/42.6.689

Medini, D.; Donati, C.; Tettelin, H.; Masignani, V. and Rappuoli, R. (2005): The microbial pan-genome. Current opinion in genetics and development, 15(6), 589–594. https://doi.org/10.1016/j.gde.2005.09.006

Mohemmad, H.J.; Alkhafaji, Y.A. and Alkafaji, H.R. (2023). Assessment of Beta-lactamases and Integrons Genes among Bacteria Isolated From Bladder Cancer Patients with Urinary Tract Infections. Journal of Population Therapeutics and Clinical Pharmacology, 30(8), 9–23. https://doi.org/10.47750/jptcp.2023.30.08.002

Ojer-Usoz, E.; González, D. and Vitas, A.I. (2017): Clonal Diversity of ESBL-Producing Escherichia coli Isolated from Environmental, Human and Food Samples. International journal of environmental research and public health, 14(7), 676. https://doi.org/10.3390/ijerph14070676

Olaru, I.D.; Walther, B. and Schaumburg, F. (2023): Zoonotic sources and the spread of antimicrobial resistance from the perspective of low and middle-income countries. Infect Dis Poverty 12, 59. https://doi.org10.1186/s40249-023-01113-z

Osagie, A.E. and Olalekan, S.H. (2019): Multiple Drug resistance: a fast growing threat. Bio Med. 21: 2574 - 1241. DOI: 10.26717/BJSTR.2019. 21.003572

Othman, R. (2018): Molecular Detection of the Virulence Genes in Escherichia coli Isolated from Healthy and Diarrheic Calves in Southern Iraq. Annual Research & Review in Biology, 26(6), 1-10. https://doi.org/10.9734/ARRB/2018/40765

Ploy, M.C.; Chainier, D.; Tran, Thi, NH.; Poilane, I.; Cruaud, P.; Denis, F.; Collignon, A. and Lambert, T. (2003): Integron-associated antibiotic resistance in *Salmonella enterica* serovar typhi from Asia. Antimicrob Agents Chemother. ;

47: 1427–1429. doi: 10.1128/AAC. 47.4.1427-1429.2003.

Poirel, L.; Madec, J.Y.; Lupo, A.; Schink, A.K. Kieffer, N.; Nordmann, P. and Schwarz, S. (2018). Antimicrobial resistance in *Escherichia coli*. Microbiology spectrum, 6(4), 10.1128/microbiolspec.ARBA-0026-2017. https://doi.org/10.1128/microbiolspec.ARBA-0026-2017

Quinn, P.J.; Cater, M.E.; Narkey, B.K. and Carter, G.R. (2004): "Enterobacteriaceae". In: clinic. Vet. Microb. 2nd ed.:210-236.

Ranjbaran, M., Zolfaghari, M., Japoni-Nejad, A., Amouzandeh-Nobaveh, A., Abtahi, H., Nejad, M., & Ghaznavi Rad, E. (2013). Molecular investigation of integrons in Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infections. Journal of Mazandaran University of Medical Sciences, 23(105), 20-27.

Rezaee, M.A.; Sheikhalizadeh, V. and Hasani, A. (2011). Detection of integrons among multi-drug resistant (MDR) Escherichia coli strains isolated from clinical specimens in northern west of Iran. Brazilian journal of microbiology: [publication of the Brazilian Society for Microbiology], 42(4), 1308–1313. https://doi.org/10.1590/S1517-83822011000400010

Rezaee, M.A.; Sheikhalizadeh, V. and Hasani, A. (2012): First report of class 1 and class 2 integrons in multidrug-resistant (MDR) Escherichia coli strains isolated from clinical specimens in northern west of Iran. Jpn J Infect Dis 2012; 65(3): 256e9.

Riffon, R.; Sayasith, K.; Khalil, H.; Dubreuil, P.; Drolet, M. and Lagacé, J. (2001): Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. Journal of clinical microbiology, 39(7), 2584–2589. https://doi.org/10.1128/JCM.39.7.2584-2589.2001

Rowe-Magnus, D.A. Guerout, A.M.; Ploncard, P.; Dychinco, B.; Davies, J. and Mazel, D. (2001): The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. Proc Natl Acad Sci USA. 98: 652–657. doi: 10.1073/pnas.98.2.652.

Sakhi, R.J. (2016): Isolation of *Escherichia coli* from Diarrhea and Pathogensity and Susceptibility Pattern for Antibiotic Test their. Science and Research (IJASR). Vol. 6, Issue 2, Apr, 29-34

Severino, P. and Magalhães, V.D. (2004): Integrons as tools for epidemiological studies. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 10(2), 156–162. https://doi.org/10.1111/j.1469-0691.2004.00769.x

*Singh, R.; Schroeder, C.M.; Meng, J.; White, D.G.; McDermott, P.F.; Wagner, D.D.; Yang, H.; Simjee, S.; Debroy, C.; Walker, R.D. and Zhao, S. (2005):*Identification of antimicrobial resistance and class 1 integrons in Shiga toxin-producing Escherichia coli recovered from humans and food animals. *The Journal of antimicrobial chemotherapy*, *56*(1), 216–219. https://doi.org/10.1093/jac/dki161

Skurnik, D.; Le Menac'h, A.; Zurakowski, D.; Mazel, D.; Courvalin, P.; Denamur, E.; Andremont, A. and Ruimy, R. (2005): Integron-associated antibiotic resistance and phylogenetic grouping of *Escherichia coli* isolates from healthy subjects free of recent antibiotic exposure. Antimicrobial agents and chemotherapy, 49(7), 3062–3065. https://doi.org/10.1128/AAC.49.7.3062-3065.2005

Su J.; Shi, L.; Yang, L.; Xiao, Z.; Li, X. and Yamasaki, S. (2006): Analysis of integrons in clinical isolates of *Escherichia coli* in China during the last six years. FEMS microbiology

letters, 254(1), 75–80. https://doi.org/10.1111 /j.1574-6968.2005.00025.x

Tettelin, H.; Riley, D.; Cattuto, C. and Medini, D. (2008): Comparative genomics: the bacterial pan-genome. Current opinion in microbiology, 11(5), 472–477. https://doi.org/10.1016/j.mib.2008.09.006

Vinué, L.; Sáenz, Y.; Somalo, S.; Escudero, E.; Moreno, M.A.; Ruiz-Larrea, F. and Torres, C. (2008): Prevalence and diversity of integrons and associated resistance genes in faecal Escherichia coli isolates of healthy humans in Spain. The Journal of antimicrobial chemotherapy, 62(5), 934–937. https://doi.org/10.1093/jac/dkn331

White, P.; McIver, C. and Rawlinson, W. (2001): Integrons and gene cassettes in the enterobacteriaceae. Antimicrob Agents Chemother 2001 Sep; 45(9):2658e61.

WHO (2012): The evolving threat of antimicrobial resistance: options for action. Geneva: World Health Organization. Available from:http://whqlibdoc.who.int/ publications/2012/9789241503181_eng. pdf [accessed].

Woodford, N.; Turton, J.F. and Livermore, D.M. (2011): Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS microbiology reviews, 35(5), 736–755. https://doi.org/10.1111/j.1574-6976.2011.00268.x

Yu, H.S.; Lee, J.C. Kang, H.; Ro, D. W.; Chung, J.Y.; Jeong, Y.S. Tae, S.H.; Choi, C.H.; Lee, E.Y.; Seol, S.Y.; Lee, Y.C. and Cho, D.T.(2003): Changes in gene cassettes of class 1 integrons among Escherichia coli isolates from urine specimens collected in Korea during the last two decades. Journal of clinical microbiology, 41(12), 5429–5433. https://doi.org/10.1128/JCM.41.12.5429-5433.2003

الكشف عن المُدمجات في الإشريكية القولونية المقاومة للأدوية المتعددة والمعزولة من المصادر السريرية والبيئية في البصرة / العراق

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هدفت الدراسة الى تقييم نوع ونسبة تواجد المُدمجات في الإشريكية القولونية المقاومة للعديد من المضادات والمعزولة من العينات السريرية والبيئية في محافظة البصرة. تم في البدأ الكشف عن بكتريا الإشريكية القولونية (المقاومة للعديد من المضادات) باستخدام كل من تقنيات التشخيص التقليدية والجزيئية. بالإضافة إلى ذلك، تم اجراء اختبار الحساسية للمضادات الحيوية من خلال استخدام تقنية كيربي -باور. وبينت النتائج أنه من مجموع ٦٢ عزلة تم تحليلها، كانت ٥٨ (٩٣,٥٤٪) من العزلات المحلية للإشريكية القولونية مقاومة لثلاثة مضادات حيوية مختلفة على الأقل. وقد أظهرت الإشريكية القولونية مقاومة عالية تجاه البنسلين، الإريثروميسين والتتراسيكلين ٦٢ (١٠٠٪)، ٥٧ (٩١,٩٣٪) و ٥٠ (٨٠,٦٤٪) على التوالي. علاوة على ذلك ، تم التحري عن وجود المدمجات في جراثيم الإشريكية القولونية (المتعددة المقاومة للمضادات) باستخدام بادئات محددة عن طريق تفاعل البوليميراز المتسلسل، وأظهرت النتائج أن ٣٢ (٥١,٦١٪) من العزلات الكلية التي تحتوي على المدمج ١ وبحجم ٤٩١ زوج قاعدة و ٥ (٨,٠٦٪) تحتوي على المدمج ٢ وبحجم ٧٨٨ زوج قاعدة و٦ (٩,٦٧٪) على المدمج ٣ وبحجم ٦٠٠ زوج قاعدة. كما اوضحت النتائج ارتباط كبير($P\leq0.05$) بين وجود المدمج ١ و المقاومة للعديد من المضادات في سلالات الإشريكية القولونية. وعليه نستنتج بان المدمجات وخاصة المدمج ١ تعتبر محددات وراثية رئيسية في سلالات الإشريكية القولونية المقاومة للعديد من المضادات الحيوية. وتلعب هذه المكونات دورا مهما بشكل واضح في النقل الأفقي للجينات المرتبطة بمقاومة المضادات الموجودة في جراثيم الإشريكية القولونية المعزولة سريريا.