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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 ≤ 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

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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-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**

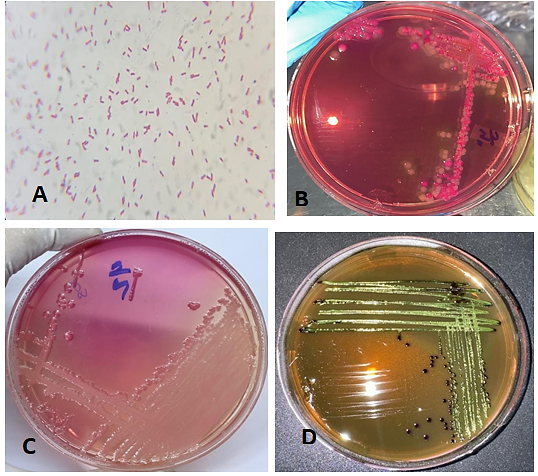
Version 21 of the SPSS program was used to perform the analyses. The chi-square test was employed to assess the correlation between the presence of integrons and antibiotic resistance, with a significance level set at P ≤ 0.05.

**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 ≤ 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.

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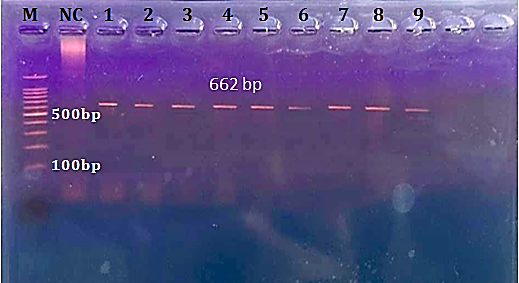
**Figure 1:** Primary Identification of *E. coli.* A: Gram stain *E. coli* under light microscope (1000X). B: Red-pink colonies of *E. coli* on MacConkey agar. C: *E. coli* colonies on MSA, presumed to be lactose fermenters, appearing as brilliant pink colonies with a crimson halo. D: *E. coli* colonies 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%) |
| Other1 | 30 | 7(23.33%) | 5(71.42%) | 5(100%) |
| Total | 90 | 65(72.22%) | 62(95.38%) | 62(100%) |
| P value2 | 0.00\* | 0.029\* | 0.03\* | 0.00 |

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

**PCR Detection of Integrons**

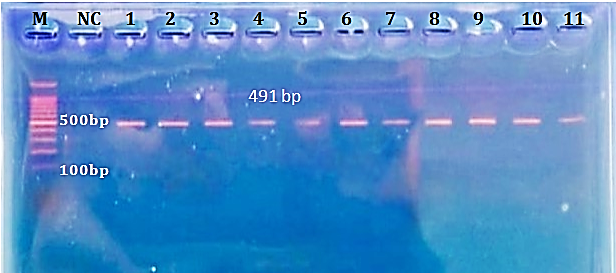
PCR was used to detect integrons (*IntI1*, *IntI2*, and *IntI3*) in local *E. coli* isolates. As shown in Table 3, there was a significant difference (P ≤ 0.05) in the occurrence of *IntI1*, *IntI2*, and *IntI3* among different sample sources. The results revealed that 32 (51.61%) of the isolates contained *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* isolatesby PCR.

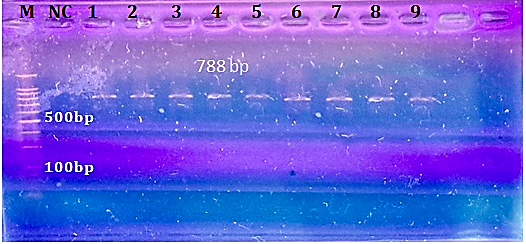
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Source of samples** | **Positive PCR** | ***Int1* no (%)** | ***Int2* %** | ***Int3* %** |
| Fecal/ diarrheal cattle | 28 | 12 (42.85%) | 2 (7.14%) | 2 (7.14%) |
| Stool/ diarrheal patients | 29 | 18(62.06%) | 2 (6.89%) | 4 (13.79%) |
| Other\* | 5 | 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\* |

1Other samples were collected from local water supply, desalination plants, animal

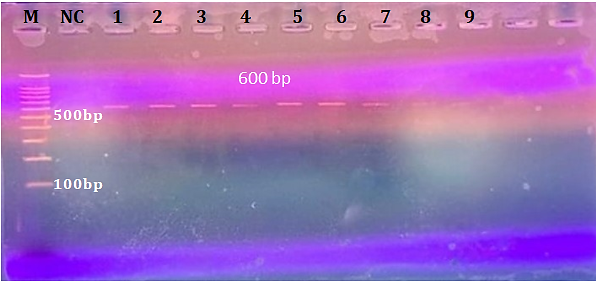
stripes and drainage water.2 Indicate the presence of the significant at P≤0.05)



**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.

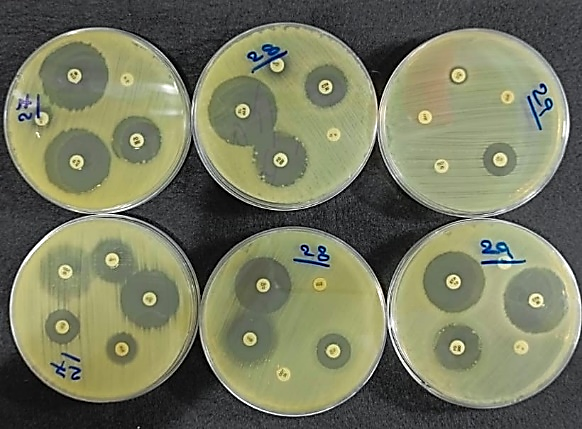


**Figure 5:** PCR amplification results of *Int3* gene (600 bp) detected in *E. coli* isolate. M: molecular marker (100bp), NC: negative control. Lanes 3-9: positive samples.

**Antimicrobial susceptibility testing**

Table 4 details the resistance frequency for each antibiotic used in the study. The table also reveals significant resistance (P ≤ 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 ≤ 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.

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**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.

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

**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 ≤ 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 ≤ 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 ≤ 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

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**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 62 عزلة تم تحليلها، كانت 58 (93.54٪) من العزلات المحلية للإشريكية القولونية مقاومة لثلاثة مضادات حيوية مختلفة على الأقل. وقد أظهرت الإشريكية القولونية مقاومة عالية تجاه البنسلين, الإريثروميسين والتتراسيكلين 62 (100٪), 57 (91.93٪) و 50 (80.64٪) 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 32 (51.61٪) من العزلات الكلية التي تحتوي على المدمج 1 وبحجم 491 زوج قاعدة و 5 (8.06٪) تحتوي على المدمج 2 وبحجم 788 زوج قاعدة و6 (9.67٪) على المدمج 3 وبحجم 600 زوج قاعدة. كما اوضحت النتائج ارتباط كبير (P≤0.05) بين وجود المدمج 1 و المقاومة للعديد من المضادات في سلالات الإشريكية القولونية. وعليه نستنتج بان المدمجات وخاصة المدمج 1 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.