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Typing and Integron Distribution among Multi-Drug Resistance *Escherichia coli*

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ABSTRACT

The study aimed, through the use of taxonomic associations, to find the genetic relationship between strains of *Escherichia coli* bacteria in 152 samples of infection symptoms. The results of the susceptibility test for 42 isolates showed that 8 isolates were sensitive to most antibiotics, while 12 isolates had moderate resistance, ranging from 5-8 antagonists, while 22 isolates were resistant to 9-12 antibiotics. Through the use of phylo-group profiling system it was found that the vast majority of *Escherichia coli* isolates under study have high virulence factors and possess large genomes by belonging to group B2. The results of genotyping using the PCR-Integron system showed that the unty Pable isolates were only... and that the highest percentage was in the first type, which reached... which indicates that there is a relationship between resistance to multiple antibiotics and the presence of introns.

Keywords- taxonomic, *Escherichia coli*, PCR-Integron system.

I. INTRODUCTION

Escherichia coli bacteria are one of the most important pathogens that cause infections, and their importance has increased due to the serious infections they cause in hospitals (Bexiga *et al*, 2011). Because it possesses many virulence factors that qualify it to cause infection (Pena *et al*, 2014). Iraq is experiencing a significant annual increase in cases of urinary tract infections due to *E. coli* (Gagliotti *et al*, 2011). As an example of this, there were large infections in 2013, which amounted to about 61.3% (Abass *et al*, 2014) an increase of 27.5% over the previous year 2012, in all reports of urine samples from all reports of urine samples (Salman *et al*, 2013). Treating bacterial infections can be difficult, and the cause of recurrence is antibiotic-resistant bacteria (MDR), which has become a problem in treating diseases. Bacterial resistance to antibiotics is one of the challenges facing researchers (Moura *et al*, 2013). The increase in resistance to multiple antibiotics came through genetic and physiological changes that developed as a result of selection pressures and the misuse of these antibiotics in medical treatments, which led to the development of bacterial resistance to these antibiotics (Koo *et al*, 2011).

Multidrug resistance (MDR) is encoded by resistance genes located on integrons (Betteridge *et al*, 2011). To evaluate the genetic diversity of bacteria, several molecular techniques have been developed. Because the 16S rRNA gene sequence is the region least susceptible to mutations and the most conserved, the molecular identification of bacteria was carried out (Kaushlesh *et al*, 2012). PCR fingerprinting methods, such as integrons, were done using Integrons-PCR to study the genetic relationship, as they are genetic structures that have the ability to express genes and acquire the exchange of gene cassettes (**Xu** *et al*, 2011).

II. MATERIALS AND METHODS

2.1 E. coli sample collection and identification

42 *Escherichia coli* isolates were isolated from 150 different clinical sample sources in the Medical City and Al Kindi Hospitals (n = 42; 36 from urine samples, 3

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from sputum samples, and 3 from wound samples**).** The isolates were diagnosed using the API 20 E system in hospitals and were also confirmed in microbiology laboratories at Al-Mustansiriya University, Baghdad. To identify these isolates, several tests were performed by culturing them on MacConkey and EMB agar, using the Gram stain, and other biochemical tests such as IMViC tests (indole production, methyl red test, Voges-Proskauer test, and citrate use) and H_2S production (Chess, 2012).

2.2 Detection of antibacterial agents susceptibility

All isolates were tested for antibiotic susceptibility by the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2016). The antibiotic tablets used in this study are: Levofloxacin (5µg) ،Kanamycin (30µg), Cefepime (30µg), Cefoxitin (30µg), Tetracycline (30µg), Imipenem (10µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Nalidixic acid (30µg), Gentamicin (10µg), Cefotaxime (30µg), Augmenten (30µg). Each concentration of antibiotics was applied to the surface of Mueller-Hinton agar plates inoculated with *Escherichia coli* isolates and incubated at 37°C for 24 hours.

2.3 Bacterial DNA preperation for PCR

E. coli DNA was prepared for PCR according to the method described previously (Adwan *et al*, 2013). Briefly, cells were scraped off an overnight nutrient agar plate with a sterile loop, washed with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), then the pellet was re-suspended in 0.5ml of sterile distilled H2O, and boiled for 10-15min. The cells then were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11.500X g for 5min. DNA concentration was determined using a spectrophotometer at 260nm and 280nm and the samples were stored at -20ºC until use for further DNA analysis.

2.4 Rep-PCR profile and dendrogram construction

An individual bacterial colony grown on tryptic soy agar (TSA) (Becton, Dickinson, San Jose, CA) was inoculated into 5ml of tryptic soy broth (TSB) (Becton, Dickinson) and incubated at 37°C for 6hours with shaking. One ml aliquot of bacterial culture was subjected to genomic DNA (gDNA) extraction. Quantification and intactness of extracted gDNA were performed using 0.8% agarose gel-electrophoresis with standard DNA size markers (1Kb).

Rep-PCR was performed using The class 1 integron forward primer (5'-CAGTGGACATAAGCCTGTTC- 3') and reverse primer(5'-CCCGAGGCATAGACTGTA- 3') class 2 integron forward primer(5'-CACGGATATGCGACAAAAAGGT- 3') reverse primer(5'-GATGACAACGAGTGACGAAATG- 3') class 3 integron forward primer(5'-GCCTCCGGCAGCGACTTTCAG- 3') reverse primer(5'-ACGGATCTGCCAAACCTGACT- 3') (**Koeleman** *et al*, 2001).

were used to amplify repetitive equences present in the chromosomal DNA of *E. coli* isolates. Integrons-PCR was carried in 25ul, volume comprising of 100ng of *E. coli* DNA, 4.5µl (10 pmol) of each primer and 12.5ul Mastermixe. Filtered water was added to the mixture to make a final volume of 25ul. Reactions were carried out using a programmable thermocycler according to the following thermocycling conditions: 94°C for 5 minutes, 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minutes and final step of 72°C for 7 minutes. Amplicons (15ul) were analyzed PCR products were detected in 1% agarose gel for 1hr. at 50V, stained with ethidium bromide and visualized by transilluminator. Dendrograms were constructed using unweighted pair-group method of arithmetic average (UPGMA) (BioProfile software, Waltham, MA).

2.5 Phylogenetic analysis

The distribution of phylogenetic groups amongst *E. coli* isolates was determined as recently described by Clermont and colleagues (Clermont *et al*, 2013). Briefly, a single reaction mixture contained 2µl of 10x buffer (supplied with Taqpolymerase), 2µL of DNA (approximately 100ng), 20pmol of each appropriate primer (except for AceK.f (40 pmol), ArpA1.r (40pmol), trpBA.f (12pmol) and trpBA.r (12pmol)) (Shanghai Generay Biotech Co., Ltd.), 2mM of each dNTP and 2U of Taq DNA polymerase (Fermentas, Lithuania) in a total volume of 20µL. PCR amplifications were carried out on a thermal cycler Mastercycler gradient under the following conditions: initial denaturation at 94°C for 4 min and 30 cycles for each denaturation at 94°C for 5sec, annealing at 57°C for 20sec (group E) or 59°C for 20sec (quadruplex and group C), amplification at 72°C for 1min, and final extension at 72°C for 5 min (Clermont *et al*, 2013). PCR products were analyzed by electrophoresis with a 2% agarose gel and visualized using transilluminator.

III. RESULTS AND DISCUSSION

42 isolates of *E. coli* bacteria were obtained from 152 samples, representing 27.63% of the total samples collected from the Medical City Department (Baghdad Educational Department, Children's Department, Burns Department, Educational Laboratories) and Al-Kindi Hospital. *E. coli* identified known through the classical diagnostic and genetic diagnostic test.

Our current study showed that the *Escherichia coli* bacteria were very large in urine isolation rates, reaching 36 isolates, or 73%, from a total of 111 urine samples, while the total isolation rates from other sources amounted to 6 isolates, including 3 (16.4) sputum isolates from A total of 25 sputum samples, and wound swabs 3 (10.5) out of a total of 16 wound swabs samples, as shown in Table 1:

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Table 1: Number and percentage of total isolation of *E. coli* isolated from different clinical sources.

Insulation source	Number of isolates	Total number of samples	Insulation ratio
Urine	36	111	73
Sputum	3	25	16.4
Swab Wounds	3	16	10.5
Total number of isolates	42/ Rate 27.63		
Total number of samples	152		

A test was conducted for antibiotics, as there was a widespread difference for the 42 *E.coli* against 12 different antibiotics: Imipenem, Levofloxacin, Tetracycline, Gentamicin, Nalidixic acid, Ciprofloxacin, Cefepime, Cefotaxime, Cefoxitin, Ceftazidime, Kanamycin, Augmenten.

In general: It was divided into three levels: a lower division for the antibiotic Imipenem

While almost the same rate of resistance to antibiotics appeared Gentamicin, cefoxitin, kanamycin, Augmentin, While it was resistant to antibiotics Levofloxacin, tetracycline, nalidixic acid, ciprofloxacin, cefepime, cefotaxime.

The percentage of resistance to the antibiotics used as follows: Imipenem 04.76% , Levofloxacin 73.80% , Tetracycline 59.52%, Gentamicin 35.71% , Nalidixic acid 80.95%, Ciprofloxacin 73.80%, Cefepime 83.33%, Cefotaxime 88.09%, Cefoxitin 40.47%, Ceftazidime 85.71%, Kanamycin 57.14%, and Augmenten 52.38% as shown in chart 1.

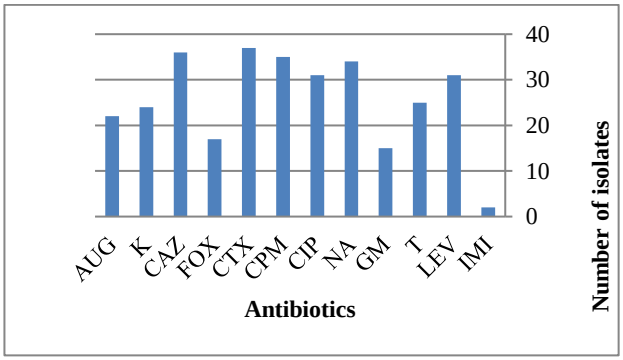

Figure 1: A diagram showing the resistance level of isolates for each antibody.

The results of the current study included our multiple local isolates. Where 36 isolates showed varying resistance, ranging from 3 to 11, against hot light, while 2 isolates were sensitive to all vital fires, 3 isolates were resistant to the antibiotics used, and only 1 isolate was resistant to 12 different antibiotics used as shown in Table 3: In the current study, we divided the isolates into three groups according to the number of their resistances, as shown in Table 2:

Table 2: The isolates under study are divided into three groups based on the number of antibodies they resist.

%	Antibiotics resistance isolates	Number of antibiotic resistance	Group
19	8	0-----4	**1**
28.6	12	5-----8	**2**
52.4	22	9 ----12	**3**

The results showed that the first group had 8 isolates that were resistant to 0-4 antibiotics, at a rate of 19%. The second group had 12 isolates that were resistant to 5-8 antibiotics, while the third group was the dominant group, which included 22 isolates that were resistant to 9-12 antibiotics, at a rate of 52.38%.

It is important to mention that isolates 1 and 6 were the most sensitive among all the isolates, as their resistance to the antibiotics used was 0, while isolate 9 was resistant to all 12 antibiotics used under study, as shown in Table 3:

Table 3: Multiple antibiotic resistance that showed local isolates of *E. coli*.

Isolates code	Isolates number	Number of antibiotic resistance
E1 , E6	2	0
E14 , E17, E40	3	2
E2	1	3
E28 , E36	2	4
E12 , E22 , E23 , E39	4	5
E5 , E8 , E30	3	6
E37	1	7
E16 , E20 , E26 , E32	4	8
E4 , E15 , E19 , E21 , E27, E29 , E31 , E33 , E38	9	9
E3 , E7 , E10 , E11 , E13 , E18 , E25 , E34 , E41 , E42	10	10
E24 , E35	2	11
E9	1	12

Our isolates under study were classified according to the Integron system into four groups, and the fifth group is the untyped isolates. The first class, Class1, came in first place, with the largest percentage of isolates, 31 (73.80%), followed by the untypable isolates, 7 (16.66%), and then the second class, Class2, and the first class is related to the second class.Class1+2 with the same number and percentage 2 (4.76%), then the third class Class3 with the last rank 0 (0.00%) as shown in Table 4:

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Table 4: Classification of *E.coli* isolates by the Integron system

Typing\	A Group	Group B	Group C	D Group	E Group
	Class1	Class2	Class3	Class1+2	Untypable
Isolates	E4,E5,E7,E8,E9,E10,E11,E12,E13,E14,E15,E16,E17,E18,E19,E20,E21,E23,E24,E25,E26,E28,E29,E30,E32,E33,E34,E35,E38,E40,E42	E27,E39	0	E22,E36	E1,E2,E3,E6,E31,E37,E41
No.	31(73.80%)	2(4.76%)	0(0.00%)	2(4.76%)	7(16.66%)
Total	42				

The study of Zheng *et al*. (2015) indicated that the largest percentage of isolates were from the first class, while the second class was found in a small number of isolates, while the third class was not found in any of the isolates. In the study of Shin *et al*. (2015), it was stated that the first type was the predominant percentage of isolates. The study by Bashir *et al*. (2015) also indicated that class 1 Integron was widespread in pathogenic *E.coli* bacteria isolated from clinical urine samples, as it was found in 17 (65%) isolates out of a total of 26, while class 2 Integron was found in one isolate and at a rate of only 4%. There were no isolates belonging to the third class Integron, while the first class Integron was associated with the second class, and the number of untypable isolates was 8(31%). The dominance of the first class of Integron in most local isolates is due to the multiple resistance characteristic of the resistant isolates. Isolates of this class are generally characterized by their high resistance to antibiotics, as they ranged from 13 isolates resistant to Cefoxitin to 28 isolates resistant to Ceftazidime, with the exception of Imipenem, which amounted to 2 (6.5%) isolates. El-Sokkary and Abdelmegeed (2015) explained that the widespread use of antibacterials clinically is linked to the increase in *E.coli* strains with multiple resistance, which are distinguished by their possession of genetic elements that have an important role in the development of resistance, and class 1 Integron is considered one of these elements. It indicated a direct correlation of the first class of Integron with resistance, as isolates of the Integron class amounted to 51.2% of the total of 84 clinical isolates of *E.coli* bacteria. Resistance to antibiotics was very high, ranging from 53% in Ceftazidim and 60% in Gentamicin, except for the antibiotic. Imipenem, as the resistance rate was 0%. Moghaddam *et al*. (2015) also explained that class 1 anticorrosion and ESBL in *E.coli* bacteria are very common. Many studies have indicated that the prevalence of class 1 Integron in *E. coli* bacteria in various studies ranges from (22-59%) and this large percentage may be due to differences in the spread of the gene in different geographical regions, the number of samples and the uncontrolled use of antibiotics (Sung and Oh, 2014). It is worth noting what was mentioned in the study of Ahangarkani *et al*. (2014) that 22% of *E.coli* out of a total of 100 isolates carried class 1 Integron , and 95% of them were resistant to well-known and widely used antibiotics, including Ciprofloxacin, which is high compared to isolates that do not contain this gene, and given the low The spread of Integrons in this and other studies and the rise in resistance may be likely because the resistance genes could be carried on other transposable elements such as transposons or prophages instead of Integrons , which were found, despite this, to be strongly linked to resistance to some antibiotics. Vitals such as Ciprofloxacin (Yang *et al*., 2004). The rapid emergence of antibiotic resistance among bacteria is mainly due to the spread of antibiotic resistance genes via horizontal gene transfer (Barlow *et al*., 2004). In intestinal bacteria, conjugative plasmids and transposons are the main factors for the spread and maintenance of multidrug resistance genes within and between species (Olsson-Liljequist *et al*., 1997).

There is an important phenomenon, which is the separation of resistance genes located on gene cassettes and their entry into other Integrons. This contributes to the creation and distribution of new resistance cassettes and the development of plasmids and transposons. The association between the presence of multidrug resistance and the presence of Integrons plays an important role in the development of that resistance (Rao *et al*., 2006). Many resistance genes encode for different mechanisms of drug resistance in bacterial genomes and in outer chromosome segments of DNA (White *et al*., 2001). Multiple drug resistance (MDR) is encoded by resistance genes located on Integrons ; Betteridge *et al*., 2011). Increasing the random use of antibiotics has led to an increase in multiple resistance in bacteria, and the policy must be changed by giving appropriate antibiotics that may prevent the transmission of resistance genes through Integrons (Farrell *et al*., 2003). Understanding the molecular mechanism of resistance genes may help to introduce strategies new antimicrobials and some preventive measures to prevent further spread of resistance determinants among pathogens (Shapiro, 1999). The original type of antechrons in *E.coli* bacteria refers to horizontal transfer in this type of bacteria. According to the Class 1 Integron classification, the second class Integron is usually associated with the Transposon Family (Tn7) and its derivatives such as Tn1825, Tn1826 and Tn4132 (Cambray *et al*., 2010).

In the current study, no isolation was found to be possessed because it was the third category, meaning the percentage was 0%. This is consistent with what Yu (2013) reported that class 3 appears to be much less common in the spread of antibiotic resistance. Ranjbar *et al*. (2011) also indicated that no class 3 Integron was detected in any of the bacterial species examined, including *E.coli*. While other studies showed the presence of small percentages of this species, ranging from 0 to 10%, as in the study of Rowe-Magnus *et al*. (2001) who indicated the presence of high resistance to ceftazidime

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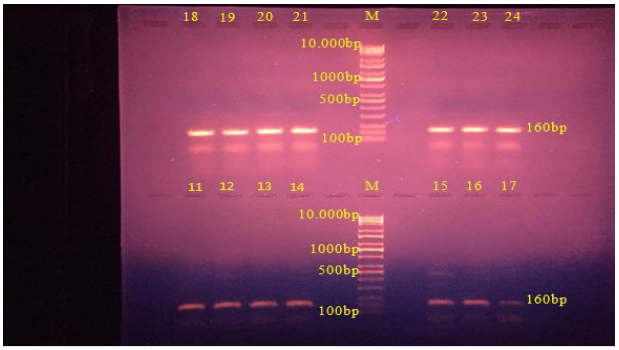
from 587 samples tested for Gram-negative bacteria. While the study by Mohammadalipour *et al*. (2017) stated that of a total of 164 isolates of *E.coli* bacteria, 24 of them carried class 3 Integron , and that its presence was significantly associated with resistance to the antibiotics Gentamicin, Kanamycin, and Tetracycline , The results of the same study indicated that Integrons are widespread in Diarrheagenic *E.coli* and their transmission contributed significantly to the emergence of resistance among Diarrheagenic *E.coli*.

The results of the current study showed that out of a total of 42 isolates, only two isolates belonged to the class 1 Integron associated with the second class, at a rate of 4.76%. This study was similar to the result of the study of Kheiri and Akhtari (2016), as they indicated that the connection between the first and second Integron classes was 14% of the total isolates. In the same context, Shams *et al*. (2015) indicated that the isolates' possession of the intI1 and intI2 genes qualifies them to belong to the common group, which is distinguished by its high resistance to antibiotics, as 13.2% of its total isolates were multiple-resistant. It was found in the results of the current study that 7 isolates were unable to find a genetic relationship with the rest of the isolates, because they did not possess any of the class 1, second and third Integron genes. This means that the infections caused by *E.coli* were not from genetically identical strains (That is, it was not clonal) as in the figure1:

The resulting packets of PCR- Integron reaction to some isolates of *E.coli* bacteria. Fig 2: (using electric relay technology for 1.5 hours and a voltage of 50 volts. The letter M denotes the volumetric guide 1kb. Numbers 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 represent isolates).



IV. CONCLUSION

The current study indicates that the resistance shown by bacteria has a close relationship with the presence of introns, especially class I introns, which appear through the distribution of isolates for each class.

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