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Prevalence and Characterization of Three Classes of Integrons as Antimicrobial Resistant Mobile Genetic Elements in Broiler-Chickens

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Abstract

Overuse of antibiotics in both agriculture/veterinary and medicine can caused the development of antimicrobial agent resistance and the dissemination of resistant microbes in the environment via the exchange of mobile genetic elements (MGEs) which carry resistant genes between numerous bacterial species. Chickens as well as animal farms release bacteria with antibiotic resistance genes (ARGs) into the environment. Our study has been focused on the spreading and presence of integrons in chicken fecal material and litters and to recognize the most common classes of integrons that can be related with the broilers wastes and intestinal contents. Results of study showed prevalence of integrons in collected samples especially class 1integron and followed by class 2 integron then class 3 consequently. Chickens fecal material and litters play essential role in the dissemination of antibacterial resistance throughout integrons. So, broiler feces and litter play a significant role in the development and spread of antibiotic resistance. Thus, it may be concluded that integrons are the common mediators of antimicrobial resistance among multidrug resistant microorganisms at important stages of poultry production.

Keywords: *Bacterial resistant, chickens, diagnosis, birds, molecular detection.*

Introduction

Food safety is one of the main problems of the poultry industry (Gushchin V.V., G.E. Rusanova, N.I. Riza-Zade). In the last years, it was observed increasing importance of poultry products in human nutrition, due to their high nutritional and dietary qualities, the speed of poultry reproduction and the relatively low cost of these products (Gushchin V.V., Rusanova G.E., Riza-Zade N .I., 2012).

In this respect, the consumption of poultry products is accompanied by a certain risk to human health. Hence, pathogenic microorganisms cause dangerous food poisoning (food poisoning), often fatal. So, ensuring the safety of poultry products is important tendency for the poultry industry (Gushchin V.V., Rusanova G.E., Riza-Zade N.I., 2012). Moreover, it is known that with an increase in the number of antibacterial drugs, the number of pathogens resistant to antibiotics also increases. Traditionally, the most acceptable method that increases the effectiveness of chemotherapy incase of bacterial infections and that increase the development of microorganisms resistance is the rational use of combinations of antimicrobial agents (Panfilova M.N., Zhukova N.N., Sazonov A.A., Kazakova E.S., Kasyan I.A., Portenko S.A., 2012).

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The antibiotic resistance is a main public health threat worldwide. (Yuce, 2001; Tenover, and Hughes 1996; Gold and Moellering, 1996). Moreover, the problem of the emergence of antibiotic-resistant bacteria in foods of animal origin has arisen and is becoming more acute, which is associated with the excessive use of antibiotics in animal industry. In this regard, in many countries antibiotics-growth stimulants are gradually excluded from the diets of animals and birds. In the EU, their use is already prohibited (Gushchin V.V., Rusanova G.E., Riza-Zade N.I., 2012).

Antibiotics resistance genes play major role in antibiotics resistance (Pruden et al., 2006). However, these antibiotics resistance genes carry on mobile genetic elements (transposons, integrons, plasmids, phages) that transferred among various bacteria by horizontal gene transfer (HGT). (Bahl et al., 2004; Goren et al., 2010).

Integrons are genetic platforms that allow bacteria to evolve quickly by acquiring, stockpiling, excision, and reordering open reading frames found in cassettes, which are mobile elements. The evolutionary potential of integrons is based on the wide range of functions encoded in the cassettes, as well as the intricate coupling of integron activity to bacterial stress. (Escudero. et al., 2015). Anyway, antibiotic resistance bacteria is available in animal wastes e.g. fecal material (Agga et al.,2019; Wang et al., 2018; Xiong et al., 2018)

In present study, molecular methods were used to identify the presence and spreading of integrons in poultry fecal material and litters and to recognize the most common classes of integrons can be related with the broilers wastes and intestinal contents. Furthermore, the present study can focused on the necessity of restrict use of antibiotics in poultry industry. As well as, the study referring to the importance of treating the poultry waste to minimize risk of development and prevalence of antibiotic resistance.

Materials and methods

The present experiment was carried out between September until November 2022. A total of 200 samples have been taken from the feces and litter of broiler chickens farms in Al-Diwaniyah Province.

Ethical Management of the Study

The trail has been approved and conducted at the College of Veterinary Medicine, University of Al-Qadisiyah, with approval number (P.G, No. 1890 in 2020), from September to November 2022, in accordance with international criteria for animal care and use.

Sample collection

A total of 200 fecal materials and litter samples were collected from broilers farms. Moreover, all samples were transported directly to the laboratory. Specimens were marked with field information and were cultured in nutrient broth. Furthermore, samples transferred to the laboratory for freezing for molecular analysis. All samples were put in a nutrient media swab and brought to laboratory for examine.

DNA Extraction (Salting Out Method)

The DNA extraction carried according to procedure Pospiech and Neumann (1995)

Wizard Minipreps DNA Kit (Promega). The DNA was also purified using the genomic DNA purification Kit supplemented by Integrated DNA Technologies (IDT) Company (Canada).

Measuring the concentration and purity of extracted DNA

The extracted DNA was tested using a Nanodrop spectrophotometer, which evaluated DNA content (ng/L) and DNA purity throughout the absorbance reading at (260/280 nm).

Primers Suspension Preparation

By dissolving the lyophilized product primers, the primers were resuspended., preparing the stocked primer by adding PCR water (free nuclease water) according to Instructions from the manufacturer. After that the working primer was prepared to equal to 10 pmol/µl by diluted of 10 µl from stock primer with 90 µl of PCR water, then mixed well with the vortex.

PCR was performed using specific primers that shown in the table (1) (Raviv et al., 2007).

Table (1): Primers Used for Detection of Virulence Genes and Integrons

Gene	Sequences (5 - 3-)		Product size (bp)	Reference
16S rRNA	F	AGAGTTTGATCCTGGCTCAG	1500	(Raviv et al., 2007)
	R	GGTTACCTTGTTACGACTT		

Polymerase Chain Reaction (PCR) assays.

The components of the Polymerase Chain Reaction (PCR) were used (Maxime PCR Abm Kit) and the process was carried out according to company instructions.

Agarose Gel Electrophoresis

The PCR products were examined using agarose gel electrophoresis according to the manufacturer's instructions (Plus science / UK). According to Sambrook and Rusell (2001).

Preparation of Agarose Gel

Based on the Brody and Kern approach, the Agarose gel was made. (Brody & Kern, 2004).

Statistical Analysis

All the information were analyzed using the statistical software for social science (SPSS) for Windows version 32 (Inc., Chicago, IL, USA). To evaluate the relationship between the variables being investigated, the Chi-square test was performed. Field (2005) defined statistical significance as a p-value of 0.05.

Results

Pathogens that are resistant to antibiotics have a considerable negative economic impact and have hampered the poultry industry expansion. Anyway, through molecular research, multiple strategies for microorganisms to develop antibiotic resistance have been identified.

Total positive and negative specimens and percentage of total integrons has been showed in table (2), where it was 117 (58.5%) positive while 83 (41.5) negative result.

Table (2) Show total positive and negative samples and percentage of total integrons

Total samples	*intI P+*		*intI N-*	
	No.	*%*	*No.*	*%*
200	117	58.5	83	41.5

In the table (3) appear positive samples of each integrons, where it was intI1 P+, intI2 P+ and intI3 P+ 47, 42 and 28 samples, moreover the percentage was 23.5% , 21% and 14% respectively. The results were significant at P<0.05.

Table (3) Show positive samples and percentage of each integrons

Total samples	*intI1 P+*		*intI2 P+*		*intI3 P+*	
	No.	*%*	*No.*	*%*	*No.*	*%*
200	47	23.5	42	21	28	14
Calculated X^2	6.17					
Calculated P value	0.046*					

* Significant difference at P<0.05

From results of table (4) it was demonstrated that the prevalence of various types of integrons in different regions of Al-Diwanyiah Province where the highest percentage of positive samples of intI P+ in the Alshafiya where it was 80%, Nufer it was 70% , Alhamza 66.66%, Alsanya, Alsader, Aldaghra and Almhanawiya it was 60%, Albder 53.33% while the lowest in Al-Dewaniya city was 51.11%. The statistical analysis referring that there was no significant (P>0.05) between the included regions.

Table (4) Show positive samples and percentage of total integrons in different regions in province

No.	The region	Total samples	*No. of intI P+*	*%*
1.	Alsanya	30	18	60
2.	Al-Dewaniya city	45	23	51.11
3.	Albder	30	16	53.33
4.	Alhamza	15	10	66.66
5.	Alsader	25	15	60
6.	Aldaghra	25	15	60
7.	Almhanawiya	15	9	60
8.	Nufer	10	7	70
9.	Alshafiya	5	4	80
10.	Total	200	117	58.5
11.	Calculated X^2	3.33		
12.	Calculated P value	0.911*		

* No significant difference at P<0.05

Based on the results of table (5), statistically it can demonstrate that in Alsania there was no significant in value of intI1 P+, intI2 P+, intI3 P+. Furthermore, results of Al-Diwanyiah city, Albder, Alhamza, Alsader, Aldaghra, Almhanawiya, Nufer and Alshafiya, there was no significant in these indicators.

As well as, in all regions of Province the differences between intI1 P+, intI2 P+, intI3 P+ were no significant (P>0.05) among these value.

Table (5) Show positive samples and percentage of each integron according to regions of province

The region	Total samples	*intI1 P+*		*intI2 P+*		*intI3 P+*		X^2	*P value*
		No.	*%*	*No.*	*%*	*No.*	*%*		
Alsanya	30	6	20	8	26.66	4	13.33	1.66	0.435
Al-Diwaniya city	45	9	20	10	22.22	4	8.88	3.24	0.197
Albder	30	8	26.66	7	23.33	1	3.33	6.53	0.038
Alhamza	15	4	26.66	3	20	3	20	0.257	0.879
Alsader	25	4	16	5	20	6	20	0.500	0.779
Aldaghra	25	6	24	5	20	4	16	0.500	0.779
Almhanawiya	15	5	33.33	1	6.66	3	20	3.33	0.189
Nufer	10	3	30	2	20	2	20	0.373	0.830
Alshafiya	5	2	40	1	20	1	20	0.682	0.711
Total	200	47	23.5	42	21	28	14	6.17	0.04
Calculated X^2		3.34		2.62		7.32			
Calculated P value		0.911		0.956		0.502			

Molecular trail in addition to integrons detection by PCR test:

PCR reaction has been used to detect integrons in broiler chickens in Al-Diwaniyah province. DNA has been extracted from specimens then electrically migration has been conducted to fix the integrons presentation in the specimens after that it was preserved in the necessary refrigeration to using it nextly.

The tests have been done throughout utilization of PCR to copy and amplifying a specific piece of DNA. The length of bundle was 1500 bp that it used in current study PCR on DNA taken from these specimens.

Sequencing test of 16S ribosomal RNA gene in broiler chickens: -

Sequencing test has been conducted by Mega 11.1 software and appeared the changes presentation in nucleotide sequence of 16S ribosomal RNA gene in comparison to many that registered worldwide. These changes are listed in table.

However, one of limitations of phenotypic method for bacterial identification is in ability to identify the bacterium on species level in some cases .in the present work, the identification key led to an assignment of the bacterial strain of quest on genus level only.

This in turn addressed the indispensable need to identify the bacterial strain of quest on aspecies level via amolecular approach via 16Rdna sequencing. Rsults revaled the successful amplification of the full lengh (1500bp) of 16SrDNA gene as show in figure (1).

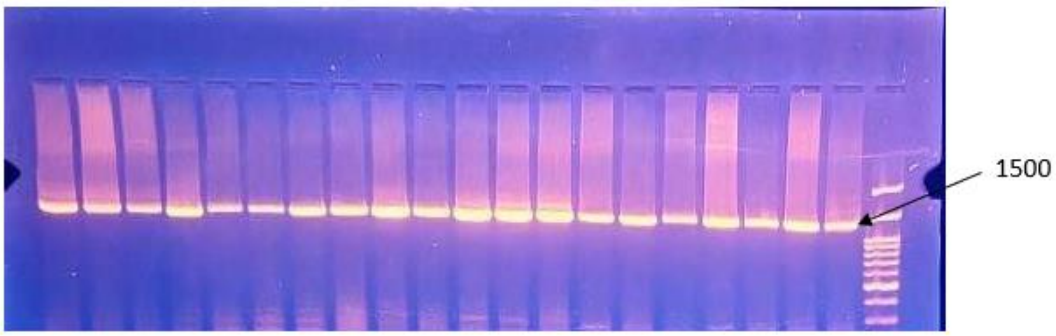
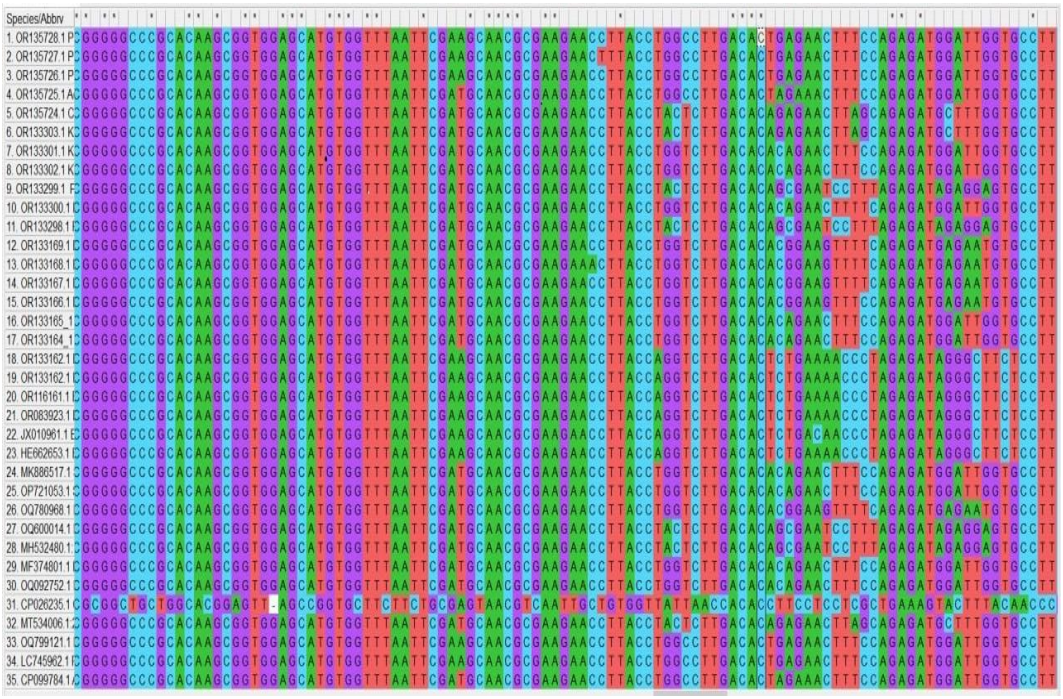


Figure (1) agaros gel electrophoresis for unknown bacteria 16rRNA gene with product size 1500bp lane (L):DNA ladder (100-1500),lanes(1-20): PCR product of feces and litter in 1.5% agarose gel and TBE (1X) at (70volt for 90 min).

After sequencing of 16SrDNA gene apartial fragment (20 nucleotides) were obtained (as show below). This nucleotide sequence was analyzed via BLASTN algorithm of NCBI. Results of BLASTN inferred that the query sequence is well matched with asset of 16SrDNA deposited in the international nucleotid databases with 97-100% identity 100% qures coverage and e-value of 0.00 , all belonging to 16S rDNA (table 6).Additionally , the query sequences of the test isolated matched at 97-100% identity 100% query coverage and e-value of 0.00 with one of the subject (hits) with the accession number (OR133162.1 , OR133162.1, OR135725.1 , OR135726.1 , OR135727.1 , OR135728.1 , OR133298.1 , OR133299.1 , OR133166.1 , OR133167.1 , OR133168.1 , OR133169.1 , OR133161.1 , OR133303.1, OR135724.1, OR133165 .1, OR133164.1 , OR133301.1, OR133302.1 , OR133300.1).

Table (6): Multiple sequence alignment test of the partial 16S ribosomal RNA gene according to ClustalW alignment examine by using (MEGA 11.1, multiple alignment analysis tool). The multiple alignment test symmetry (*) and variations in 16S ribosomal RNA gene, partial sequence nucleotide sequences.



Discussion

Poultry farming is particularly important in regions where alternative sources of protein may be limited or more expensive) Wu et al., 2022(. Because antibiotics are commonly used in poultry farming to promote growth and prevent disease, therefore, there are increasing concerns about the negative effects of antibiotic use on human and animal health. However, one of the most important of these negative effects is the increasing emergence of antibiotic resistance (Guetiya et al., 2016). Firstly, number of collected samples from various region of province depend on number of active chicken farms that distributed in various cites of province.

Results of prevalent of Integrons classes

Increasing the times and spectrum of antimicrobial-resistant diseases was a significant public health importance. (Mahmud et al., 2022)

Based on a recent data, antibiotic resistance genes can be transported among bacterium regardless of antibiotic consumption pattern. The acquisition of resistance genes through horizontal transport was a significant role in developing multidrug-resistant strains (MDR strains) (Kargar et al., 2014).

Anyway, present trail aimed to assess the role of class I, II, and III integrons in developing antibiotic resistance in some of intestinal bacterial isolates. The study results showed the prevalence of integrons I II and III frequency 47 (23.5%), 42 (21%) and 28 (14%), respectively. Moreover, the percentage of total integrons was 117 (58.5%) positive while 83 (41.5) negative result. Where it was found that a high percentage contains integrons responsible for rearranging and forming genes that carry antibiotic resistance, as the percentage of samples carrying integrons reached 58.5% according to Table No. 2, and there were three types of Integrons, which are intI, intII, and intIII respectively, of 23.5%, 21%, and 14%, according to Table No. 3. Hence, it becomes clear that the presence of these encronaments is due to the misuse and excessive use of antibiotics.

From Table No. 4, we find that the Al-Diwaniyah center has the lowest percentage of the presence of integron, because some of the fields from which samples were taken are the Zahoor Al-Watan company that uses correct scientific methods in feeding poultry and does not use antibiotics as growth stimulants.

It was noted from Table No. 4 that the city of Al-Shafia occupied the first place in the number of samples that carry Integron, as the percentage reached 80%, and this indicates the use of a poor diet and the wrong use of antibiotics. Based on many study, there is a comparatively high integron distribution specially class 1 among E. coli (Karimi et al., 2020). Researchers have showed that 23 strains (36.5 %) consist of at least one integron encoding gene, with Class I integron being the more prevalent type identified (Staji et al., 2018). Another study was noticed 26.5 % of the E. coli isolates possess class1 integrons, an important part. Class 2 and 3 integrons presentation was not detected (Huang et al., 2020). Class 1, as well as Class 2 integrons are founded in 78.26 % and 76.81 % of Multi drug resistance strains. Anyway, an integron of class 3 is presented in 26.09 % of Multi drug resistance strains (Kargar et al.,2014). Abbassi et al. (2021) found from classes 1 and 2, respectively, integrons in 57 and 2, and one isolate had both. Class 1 integrons had seven gene cassette arrays, while class 2 had one.

Conclusion

The present work demonstrates that Integron I, II and III were play important role in carrying the antimicrobial resistance gene in broiler-chickens. The current study shows links among the current identified local strains and some worldwide isolates of the bacterium.

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Conflict of interest

No conflict.

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