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RESEARCH ARTICLE OPEN ACCESS

Using of Integrons as Biomarker to Assess Dissemination and Diversity of Antimicrobial Resistance Genes in Farm Animal Manure

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Abstract

Antimicrobial agents are widely used for treatment of animal and human diseases. Heavy use of antimicrobial agents permits bacteria to develop resistance to these agents specifically when a dose of antibiotic is insufficient or course of treatment is incomplete. Antimicrobial resistance genes (ARGs) are usually associated with mobile genetic elements (MGEs) including Integron therefore; these genes can transmit among bacteria via horizontal transmission. The current study was conducted to assess the possible role of manure in dissemination of antimicrobial resistance. The presence, quantitate, and diversity of resistance genes associated with Integron class 1 have been assessed using conventional and quantitative polymerase chain reaction (PCR) combined with sequencing of gene cassette within Integron and analysis of sequenced data by blast tool. Thirty-eight samples were found a positive for Integron and concentration of Integron in positive sample ranged from from 10^6-10^{10} copies/g of manure. High frequencies were detected to genes that encoded to sulphonamide and ammonium compound resistance. These genes were detected in 25% and 23% respectively of the total manure samples. In general, the detected genes in manure functionally belong to five protein families including Efflux pump, DNA repair, heavy metal resistance, membrane protein, and antibiotic resistance. Manure might act as a hotspot from which ARGs emerge and transfer to the environment and then to the animal and human environments.

Keywords: Antibiotic Rgene, Integrons, Manure and Mobile Genetic Elements

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INTRODUCTION

Resistance to antibiotics is a whole world thread to human and animal health. The global organization (OIE, FAO, and WHO) that deal with health and nutrition has reported that antibiotic resistance is one of three important risks that threaten human life.[1] By 2050, antibiotic resistant infections could cause 10 million deaths a year with accumulative cost reach to 10 trillion dollars.[2] Enteric Gram-negative bacteria are considered a major reservoir for antibiotic resistant genes. Transposons, plasmids, and Integrons are mobile genetic elements that mostly carry antibiotic resistance genes (ARGs) and are responsible for transferring these genes between bacterial species via a horizontal transmission (HT).[3-5]

Modification of cell components, an efflux pump, and enzymatic modification or degradation of antibiotic is such mechanisms that bacteria use to resist the harmful effect of antimicrobial substances. Some of these mechanisms specifically enzymatic ones rely on ARGs that are usually associated with mobile genetic elements; therefore, these genes are considered emerging contaminants and have a critical role in dissemination of antibiotic resistance.[5-11]

Using of manure as an organic fertilizer is involved in spreading of resistance genes to the human and animal environment.[12,13]

Based on WHO reports and several researches, further studies are needed to estimate the percentage of ARGs in the environment.[14,15] Therefore, information about the discharge pattern and diversity of ARGs in animal farms is needed.[16,17]

The current study critically examines the role of manure in spreading of ARGs to the animal and human environment.

MATERIALS AND METHODS

Sample collection

Four animal farms located within the AL-Qadisiyah governorate were selected and 30 manure samples were collected from each site in December 2021. Samples were collected from the deep layer of manure using sterile glass bottles.

Purification of DNA from manure

Soil DNA extraction kit from Biomedical Ana ™ (South Korea) was used to purification of Metagnomic-DNA from manure. Purification of DNAs was performed according to the manufacturer's instructions.

Primer, PCR and qPCR

Primers were designed to amplified class 1 Integron and 16s RNA (Table). Primers were synthesized by Integrated DNA Technologies (IDT). The presence of Integron and 16s rRNA genes in

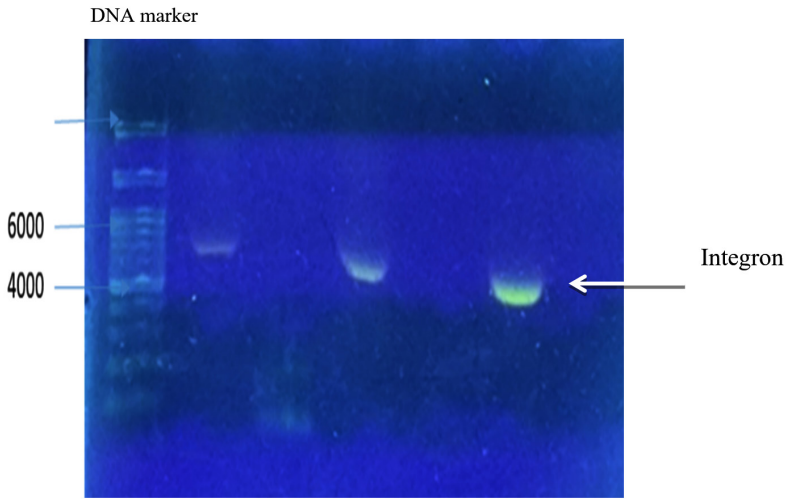

Figure 1. PCR Gel electrophoresis image (agarose 1%).Example of Positive result of integron 1 from DNA animal manure. line 1 represent DNA marker (1 KB) (NEB BioLabs®)

the tested sample were tested using Conventional PCR (Figure 1). The quantity of Integrons and reference gene in the manure were estimated using qPCR (Figure 2).

Genetic sequencing of Integron 1 gene cassette

PCR products that were positive to the presence of resistance genes within a gene cassette were further analyzed by sequencing. Sequencing of Gene cassette within Integrons performed by Macrogen (Seoul, South Korea). An Integron database (http://integrall.bio.ua.pt/?search#) and Blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) is an online tool used to analyse the sequence and diversity of resistance gene within cassette.

Data analysis

The statistic program, prism 5 was used to analyse data. Differences between groups were statistically performed using one-way ANOVA, when p value was less than 0.5 the difference was considered significant. The number of intregron1 integrase and 16S rRNA copies was calculated to estimate the amount of Integron to the total number of bacteria.

Ethical approval

The primary studies under which the samples were collected received ethical clearance from the Medical Ethics Committee of Al-Qadisiyah University (approval number 262/2022).

RESULTS

Integron identification

Pair of primers from the conservative area of interon1, Int1-F (GCCTTGATGTTACCCGAGAG) and Int1-R (GATCGGTCGAATGCGTGT) was used to detect the presence of the target gene in farm animal manure. As shown in Figure 1, 70-80% of farm animal manure was positive for integron1. Integrons were found in 100 out of 120 (83%) of total samples. Integron 1 was found in 43 of 50 (86%) sheep manure samples, 38 of 45 (84%) of the Cow manure samples, and 19 of 25 of the Goat samples (76%) (Figure 3).

The abundance of integrons1 in the farm animal manure

Quantitate of Integron 1 and 16S rRNA in manure was estimated using qPCR. As the result

Table. Oligonucleotides used in PCR, RT-PCR and sequencing of integrons 1

	Primer name	Target gene	Sequence	Ref.
1.	Int1 F	Integron integrase class 1	GCCTTGATGTTACCCGAGAG	
2.	Int1 R		GATCGGTCGAATGCGTGT	(25)
3.	GC F	Gene Cassette Integron 1	GGCATCCAAGCAGCAAG	
4.	GC R		AAGCAGACTTGACCTGA	

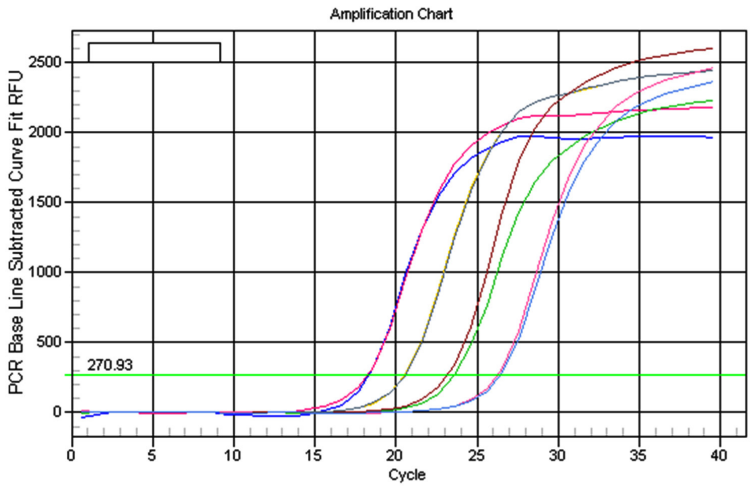

Figure 2. Examples of qPCR amplification plot of Positive result of integrons 1 from DNA animal manure

show, the concentrate of Integron - ranged from 10^6 - 10^{10} copies per gram of manure (Figure 4). The highest (but not significant difference $p<0.05$) of Integron was recorded in sheep manure as compared with other tested species manure, followed by cow manure, then goat. While the concentration of 16S rRNA in manure samples ranged from 10^8 – 10^{12} copies per gram of sample (Figure 4). The current study suggests that manure might act as a hotspot from which ARGs emerge and transfer to the natural environment.

High-throughput analysis of class 1 Integron GCs

The positive sample for Integron and their GCs were further analysed using High-throughput sequencing. 50% of Integron have no resistance gene within gene cassette. Also, 50% gene sequence within cassettes shows no hint in the NCBI gene bank.

Sequencing and blast analysis show that sulphonamide resistance gene (Sul1) and & quaternary ammonium compound were detected in 25% and 23% respectively of the tested sample

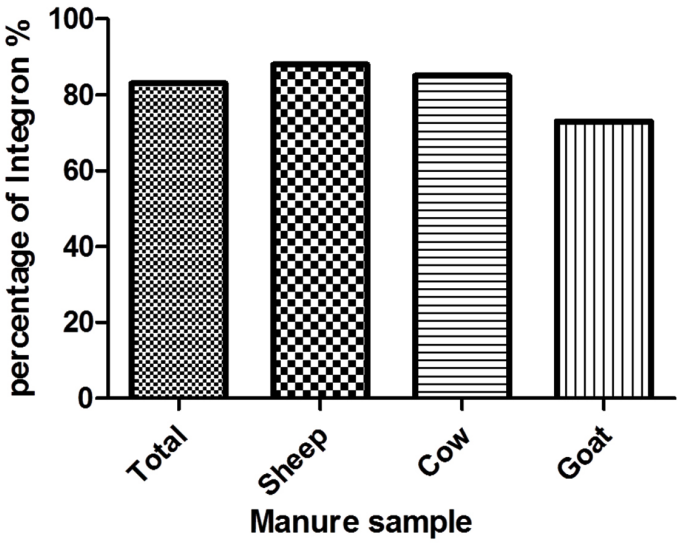

Figure 3. Class 1 Integron presence (%) in manure of some farm animal

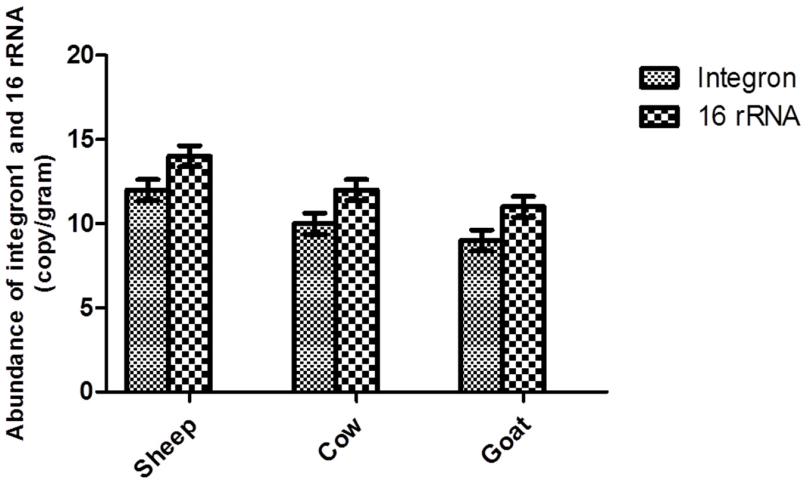

Figure 4. Amount of class one Intgron1 and 16s rRNA in some farm animal manure. The value represents log 10 of genes copy number

(Figure 5). Gene encoding resistant protein to Arsenic was recorded in 15% of the tested sample. As shown in Figure 5 the detected genes in manure at a percentage less than 5% included tetracycline genes (tetA and tetC), beta-lactamase (blaOXA-2), aminoglycoside (AadA), fluoroquinolone (aac) and Macrolide (Mph). Also, we found resist genes to heavy metals which include copper (pcoD), Cobalt-zinc-cadmium (silA), and Mercury (merC).

According to their function the detected genes belong to five protein families including Efflux pump, DNA repair, heavy metal resistance, membrane protein, and antibiotic resistance (Figure 6).

Our study suggests that manure represents a reservoir for ARGs and MGEs and one of the important source for emerge and spread of the genes to the animal and human environment.

DISCUSSION

A strong relationship between using manure as soil fertilizer and spreading of antibiotic

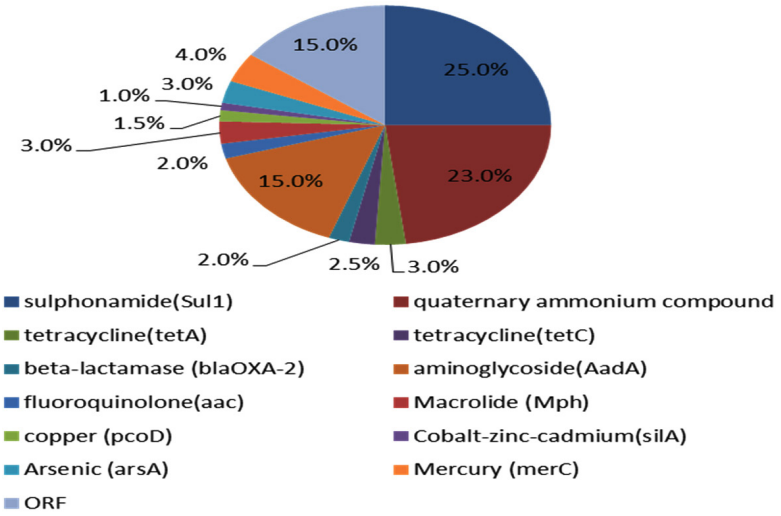

Figure 5. Diversity of integrons associated genes cassette in the tested animal manure samples

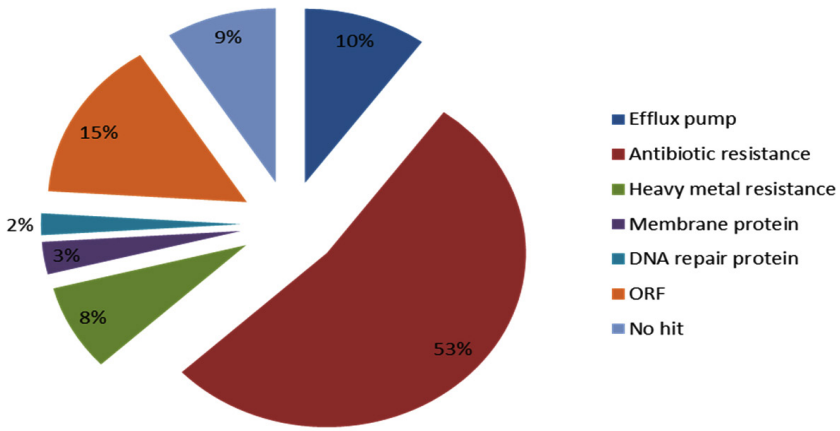

Figure 6. The relative abundance of protein family. Five protein family including heavy metal resistance, efflux pump, antibiotic resistance, DNA repair protein and membrane protein were show in functionally analysis of Integron gene cassette

resistance gene and bacteria have been reported in many studies.[16,17] Manure may represent a hotspot for dissemination of antibiotic resistance and as a big storage for ARGs and antibiotic resistant bacteria (ARB). Therefore manure has a critical role in role in emerging and disseminating ARGs & ARB in the natural environments.[18,19]

Integron is one of the mobile genetic elements that can capture, integrate, and express resistance and virulence genes. It is associated with plasmid and transposon and plays a critical role in the transfer of antibiotic resistance genes from resistance to sensitive bacteria. Furthermore, Integron represents a biological indicator for the assessment of ARGs and ARB in the environment than in animal and human.[15]

In the current study, PCR, qPCR, DNA sequencing, and the blast tool were used to assess the presence and abundance of Integron associated resistance genes in farm animal manure.

Results of the current study suggest that class one Integron is a dominant mobile genetic element in farm animal manure and gene cassette within Integrons carrying a wide range of antibiotic and metal resistance genes.

According to some studies, a strong relationship between the types of ARGs found in manure of certain farms and antimicrobial agents used in the same farm.[20-22]

Horizontal gene transfer (HGT) is the main mechanism in bacteria that facilitate transfer of ARGs from manure to soil microbes which can infect human or animal directly or by transfer to human or animal pathogen.[15,23] The evidence from this study indicates that manure is one of the important sources of Integrons and resistance genes.

Recently, ARGs classified as micro-contaminants that might hurt animal and human health.[24,25] Further researches are urgently needed to prevent the dissemination of ARGs to the environment. Find efficient methods for inactivation of ARGs in animal wastes, manure, and hospital wastes to reduce of emergence and dissemination of ARGS in the natural environments.[23,25]

CONCLUSION

Antibiotic resistance genes which are associated with mobile genetic elements including Integrons represent a potent risk factor for animal and human health. Manure has a high density of Integron and ARGs within the gene cassette of the Integron. Action is needed to prevent the spreading of these genes to various environments.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Medical Ethics Committee of Al-Qadisiyah University, Iraq, with reference number 262/2022.

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