



Role of integron in antibiotic resistance by microbial communities isolated from Iraqi soil

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ABSTRACT

The proliferation of antibiotic-resistant microorganisms is a significant public health concern. Recent decades have discovered numerous genetic pathways that contribute to the transmission of resistance genes among bacteria. One of these processes is the use of integrons, which are genetic components that can exchange and express genes from gene cassettes (GC). In particular, Gram-negative bacteria spread integrons widely and move them between bacterial populations using plasmids, transposons, and other mobile genetic parts. However, the unaltered collection of environmental DNA (eDNA) is necessary to precisely determine the soil's biodiversity. This study aims to isolate environmental DNA from soil in Iraq and detect their resistant genes for antibiotics.

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Introduction

Integrons are old structures that aid in a development of bacterium by collecting, keeping, getting rid of and using cassette-like mobile components as reading frames, they are found in around 17% of the chromosomes of bacteria (Osagie & Olalekan 2019). These formations may be found in a variety of habitats, including forests, soils, plant surfaces, biofilms, sediments, deep-sea sediments, hot springs, desert soils, and river sediments Today, the term "integron" is used to describe an extensive set of genetic components that can all trap gene cassettes (Sabbagh et al. 2021). Nevertheless, over a third integrons that lack gene cassettes have been discovered in the bacterial genome (empty integrons) according to Sütterlin et al. (2020). Despite the fact that first bacterium was

resistant to antibiotics was initially discovered in the middle of the 1950s, it remained unclear until the 1970s which resistance phenotypes were related to elements or plasmids that are transferable. Integrons were found in the late 1980s. Particularly in Gram-negative bacteria, integrons are crucial in the spread of antibiotic resistance. Because antibiotic resistance leads to significant morbidity and death rates as well as the spread of multidrug resistant bacteria, understanding antibiotic resistance and management measures is difficult. An alarming number of cases of bacteria expressing extended spectrum beta lactamases (ESBL) have been reported recently worldwide; the countries of the Middle East and Iraq are affected. Treatment delays are caused by bacteria that have multi-drug resistance and beta lactam resistance genes. Numerous investigations have been conducted to

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identify the reasons behind medication resistance and the effects of this issue in clinical settings (Rahim et al. 2020). An Action Plan is connected to genetically mobile components like plasmids or transposons in resistant integrons, enhancing transmission within and between species. Integrons significantly contribute to Multiple resistance in gram negatives bacteria more than gram positives bacteria, as is now well known (Partridge et al. 2009).

All integrons are made up of three main parts: a centrally changeable area and a conserved 5' and 3' segment. This is where integrons take in and express external genes that are part of the gene cassette (Osagie & Olalekan 2019). The integrase gene *intI* belongs to the tyrosine recombinase family and codes for a specific recombinant site (Hall 2012). An important part of the 5' and 3' zones in all integrons is where the *attI* receptor is located. This receptor is found by integration and is next to the *intI* gene, so *attI* is in front of *intI*. Another benefit of the protein *attI* is that it creates a recombinant between the inserted gene cassette and an integrase protein (Partridge et al. 2009). Either within or between *intI*, the *Pc* and *Pint* components of the promoter sequence, along with integrons, integrase, and *attI*, trigger the expression of the genes already present in the integrated gene cassette (Escudero et al. 2015).

The architecture of the 3' conserved segment varies among integron classes. In between the 3' and 5' zones are the gene cassettes, which allow integrons to acquire additional genes. Two key benefits of integron technology are that it incorporates the novel genetic composition into the bacterial genome at *attI*, allowing the integron promoters to generate the newly integrated genes without causing any abnormalities in the existing genes (Gillings 2014).

According to several studies, the reported cassette count varies from 0 to 100. The difference between *Vibrio cholerae* and certain other bacteria is evident in the number of cassette taps. Integron categories vary widely due to the acquisition of numerous gene cassettes (Xu et al. 2011). Numerous inquiries have been made, all in vain, about the origin of the gene cassettes. While the underlying mechanism for this ability remains unknown, the frequency and size of gene cassettes suggest that some species may possess them and have the ability to create gene cassettes from a single or pair of genes. These cassettes, which are movable components, contain many distinct genes that cause antibiotic resistance (Sabbagh et al. 2021).

Ke et al. (2011) reported a random assembly of the conserved portion of integrons in regions 3' and 5'. Between two recombination sites, the integrase gene integrates gene cassettes (*attI*, *attC*). The strands can be

united from integrons into free DNA, and this procedure is reversible. (Xu et al. 2011).

Gene cassettes express the gene cassettes coupled (Tansirichaiya et al. 2019) with a third-party promoter (*Pc*) within the integron structure. This is because their structures lack promoter sequences. Integrons serve as both the gene's expression vector and a natural cloning system since they possess promoter regions that allow them to make gene cassettes that express the genes (Ke et al. 2011).

The mobility of mobile DNA elements like transposons and plasmids contributes significantly to antibiotic resistance in clinical disorders (Ghaly et al. 2019). Despite not being mobile themselves, integrons, especially Class I integrons, commonly reside on transferable plasmids. This facilitates the transmission of gene cassettes carried by these plasmids to other integrons or even the entire genome. Through the integron mechanism, bacteria can integrate gene cassettes and express the genes to produce functional proteins (Sütterlin et al. 2020). Different bacteria share integrons, which can store a vast amount of information in secretory sequences and genomic islands. Integrons and gene cassette motility both play a significant role in the dispersion and proliferation of resistance genes. Numerous studies on the presence of integrons in ecological microbes show their great variety of roles in addition to clinical issues. This fully explains the function of integrons, ancient genetic components of the genome critical for evolution and adaptation (Tansirichaiya et al. 2019). Our study aims to isolate environmental DNA of bacteria from soil in Iraq and detect resistant integrons for antibiotics.

Materials and Methods

We randomly collected soil samples from various regions of Iraq and isolated DNA from these samples using the Favor Prep™ Soil DNA Isolation Mini Kit according to the manufacturer instructions according to Zielińska et al.(2017). The primers of genes under investigation were mentioned in table (1) and thermocycler parameters in table (2). Denaturation of the DNA strand, primer association with annealing, and DNA chain lengthening were followed Garibyan and Avashia (2013).

Table 1: Primers used in this study

Primers	The sequence of nitrogenous bases
Int1	F:TCTCGGGTAACATCAAGG R:AGGAGATCCGAAGACCTC
Int2	F:CACGGATATGCGACAAAAGG R:TGTAGCAAACGAGTGACGAAATG
Int3	F:AGTGGGTGGCGAATGAGTG R:TGTTCTTGTATCGGCAGGTG

Table 2: Ideal conditions for the PCR thermal cycles

PCR Step	Temperature (°C)	Cycle	Time (min)
Initial denaturation	94	1	5
Denaturation	94	40	1
Annealing	48		1
Extension	72		3
Final Extension	72	1	10
Hold	4	-	Forever

Using Sambrook and Green's (2012) method, we made the agarose gel by melting a certain amount of agarose powder in 100 ml of Tris-borate-EDTA (TBE) solution and heating the mixture with an electric heater until it boiled. To make pits in the gel, add the sterile comb to the mixture, cool it to 65 °C, and then pour 4 µl of the DNA staining dye ethidium bromide into the desired location. 30 to 40 minutes should pass for the mixture to solidify at room temperature. We coated the gel with 1 µl of TBE solution, removed the comb, added 7 µl of ladder to the first hole, and then added the extracted samples to the remaining holes. We covered the gel with 1 µl of TBE solution, removed the comb, added 7 l of ladder to the first hole containing the standard DNA fragments, and then added the extracted samples to the remaining holes. We investigated the gel using a UV source (transilluminator) with a wavelength of 260–280 nm and took photographs of the gel using a digital camera.

Results and Discussion

The DNA was amplified and transferred onto an agarose gel using the 16S rRNA gene for a purpose of detecting bacteria using the ethidium bromide dye and examining it with ultraviolet light. The results showed that the soil samples contained bacteria with clear bands, as the DNA bands appeared with a size of 1300 bp, while the concentration of the DNA was between 25-50 nanograms, as shown in the figure (1).

The 16S rRNA gene was used to study the evolution of bacteria and classification as a genetic measure, which was used for several reasons, including its presence as a multi-gene family or operons in all bacteria, and its structure is of little change and random changes in the sequence are more accurate to measure evolution and the 16S rRNA gene composed of 1500 bp is sufficient for complete information (Janda et al. 2007). High throughput 16S rRNA gene surveys have revealed new information about soil bacterial diversity and enhanced understanding of the environmental variables that drive abundances across geographies. However, because often proprietary

to individual investigations derived taxonomic units and the databases of sequence identification only characterize taxa, current analytical tools are of limited utility in formalizing synthesis of the environmental characteristics of newly discovered species (Jones et al. 2021). The results show that the soil samples contain bacterial DNA and after PCR and detection about integrons, the result show that the bacterial DNA contain the three types of integrons 1,2,3 as shown in the (Fig. 2).

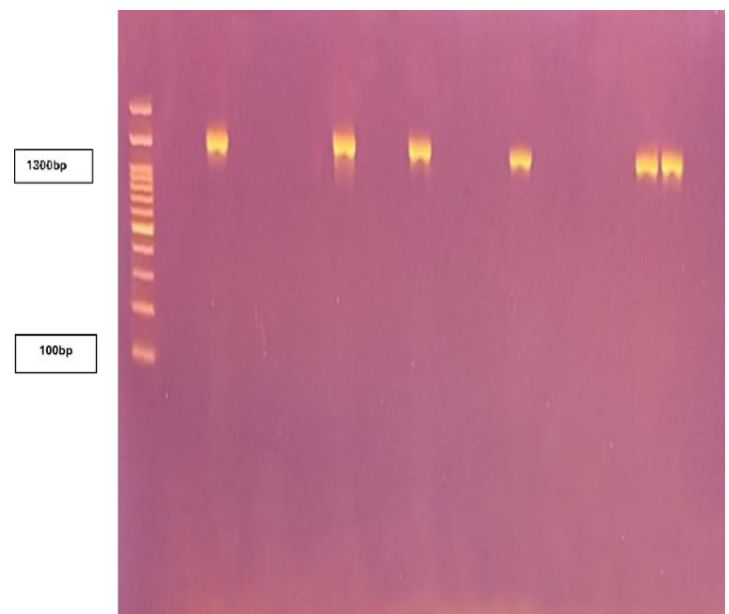


Fig 2. Agarose Gel Electrophoresis (1.5% agarose, 100V, 80mA, 1h) showing PCR results of 16S rRNA from soil bacteria. M: Marker Ladder (100-1500 bp).

Integrons frequently have two characteristics: a proximal main recombination site and an integron gene (*attI*) (Partridge et al. 2009; Xu et al. 2011). Integrons have already been classified into classes Depending on the amino acid sequence of the (*Int I*) integron, those with (*IntI1*) are classified as class 1, (*IntI2*) and (*IntI3*) as class 2, class 3 respectively, etc. Among others, chromosomal integrases (*IntI1*), (*IntI2*) and (*IntI3*) were first discovered in connection with mobile genetic elements. The *IntI* gene codes for the integrase enzyme (*IntI*) that belongs to

the tyrosine recombinase family. Its presence is characterized by a constant RHRY (where Y is the catalytic tyrosine). It is the target chosen most often for integration detection.

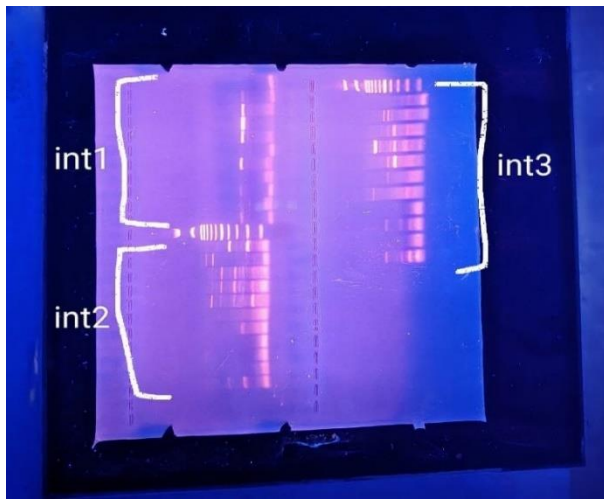


Fig 3. The soil bacteria integrons.

Integrons have already been categorized and divided into a number of groups based on the alterations and divergence in the *intI* sequences. There are four main classes of integrons, or classes (1-4) have so far been recognized and differentiated. The ability of classes (1-3) integrons to acquire the same gene cassettes through a comparable recombine platform is characterized as a multi-resistant integrons (RI) and was supported by recombination sites from such integrons that were used for in vitro excision and integration (Hall et al. 1999). The majority of the studies on integrons that are currently accessible focused on class (1) integrons and Gram-negative bacteria. Class (4) integron was first discovered as a separate kind of integron on the tiny chromosome of *V. cholerae* and was later discovered to be a crucial part of several proteobacterial genomes (Barker et al. 2002; Martn et al. 2008) which, despite the limited number of findings related to *Vibrio* species, had also been regarded as a major worry about antibiotic resistance and bacterial genome evolution. Antibiotic resistance gene cassettes may also be present in the remaining kinds of integrons, but this is still uncommon globally (Nield et al. 2001). These results agreed with (Tomar et al. 2024), who used direct DNA isolation from soil and identified the microbial species carried by bacteria and fungi, as they found that the soil contained Gram-positive and Gram-negative (Sadiqi et al. 2022; Jayaraj et al. 2023) isolated DNA directly from soil samples and identified the bacteria contained in the soil. Genome sequencing

provides valuable information on a large scale for inferring the evolutionary relationship between different species and genes as well as inferring the lineages of species based on paleontological information or morphological traits (Nei 2003).

Environmental isolates have frequently been shown to have integron-related mutations (Ozgumus et al. 2009; Laroche et al. 2009). Antimicrobial resistance is disseminated via integrons not only within a species but also across genera and species (Mokracka et al. 2012).

Conclusion

Scientists worldwide need to urgently focus specifically on the impacts of human activities, including agriculture, industry, and wastewater. Today, DNA sequencing and metagenomic data from environmental samples are useful alternatives to the old ways of isolating and growing bacteria in labs. They can also be used for microscopic identification and biocharacterization to get a good idea of the soil's biodiversity.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Ethical Approval

Ethical approval to perform this study was obtained by Department of Ecology, Faculty of Science, Al-Qadisiyah University, Iraq.

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