REVIEW ARTICLE

Integrons: A Mobile Genetic Element of Concern in Antimicrobial Resistance Gene Transfer: An Overview

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Abstract

Antimicrobial resistance is one of the major global public health problems. There are numerous factors which contribute to the development of antimicrobial resistance. One such factor is integrons which is a mobile genetic element. Integrons contribute to spread and distribution of antimicrobial resistance genes across diverse bacterial species through horizontal gene transfer. Integrons are made up of three components namely an integrase gene (*int*I), a primary recombination site (*att*I) and a promoter region. Integrons transfer resistance genes through transfer of plasmids or through phages. There are two types of integrons such as resistance integrons and super integrons. Resistance integrons are of 4 classes (class I-IV). Class 1 integrons are major contributors of antimicrobial resistance globally and found as contaminant in all ecosystems dominated by humans. The peculiarity of integrons is that they offer a mechanism for quick migration and exchange of various resistance genes. Bacteria with antibiotics, disinfectants and heavy metals discharged into waste streams create hot-spots where intricate interactions and selection events can take place for the interchange of genes and the creation of novel gene combinations.

Key words: Antimicrobial resistance, Antimicrobial selection pressure. Horizontal gene transfer, Integrons, Mobile genetic elements

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Introduction

Integrons play an important role in the distribution of antibiotic resistance, especially in Gram-negative pathogens. In resistance integrons, an action plan is associated with genetic moving elements such as transposons or plasmids, so interspecies and intraspecies transmission are increased. Around the world, antibiotic-resistant bacteria are a major contributor to diseases connected to healthcare, and antibiotic resistance has also been observed in infections among the general population. Morbidity, mortality, and medical expenses are all dramatically raised by infections brought on by multi-resistant pathogens. According to molecular investigations widespread multiresistance has frequently been acquired by the acquisition of pre-existing determinants known as mobile genetic elements (Patridge *et al*., 2018)

Several Mobile Genetic Elements (MGEs), including plasmids, bacteriophages, genomic islands (GIs), transposons (Tns), integrons, integrative and conjugative elements (ICEs), insertion sequences (ISs), and tiny inverted repeat transposable elements, have been described thus far by means of horizontal gene transfer (HGT), it is possible to transfer genetic material between bacteria of the same generation. Bacterial populations' evolutionary trajectories and genetic diversity are both influenced by HGT.

MGEs are ubiquitous components in bacterial communities due to their ability to travel physically between host genomes (Domingues *et al*., 2012) that significantly aided in the rapid spread of antibiotic resistance among several bacterial species with significant human and veterinary health implications. Multiple drug resistance phenotypes can be transmitted to a susceptible recipient through a single genetic event as a result of the accumulation of antimicrobial resistance genes on mobile genetic elements (Harbottle *et al*., 2006). Bacteria can acquire resistance genes through mobile elements such as integrons, which contributes to spread and distribution of antibiotic resistance genes across diverse bacterial populations (Zhao *et al*., 2020).

Integrons were first identified because of their central role in assembling and disseminating antibiotic resistance genes in commensal and pathogenic bacteria (Ghaly *et al.,* 2021). The term integron was originally coined by Stokes and Hall in 1989 (Sabbagh *et al*., 2021) to describe this specific group. Although most integrons were initially described in human clinical isolates, they have now been identified in many non-clinical environments, such as water and soil (Domingues *et al.,* 2012). Integrons are genetic elements

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that have a site-specific recombinase mechanism that allows them to express, exchange, and integrate DNA elements known as gene cassettes. Each integron is having three distinctive components: a nearby *int*I gene and an *att*I site, and a strong promoter. The *attI* recombination site is recognised by the integrase, which is a part of the tyrosine recombinase or integrase family and encodes a site-specific recombinase from the *int*I gene and a promoter site Pc. More than 60 different antibiotic-resistance genes, covering most antimicrobials presently in use, have been characterized in cassette structures (Mazel and Davies, 1999). The aim of the study was to give an overview on Integrons which is an important mobile genetic element involved in antimicrobial resistance gene transfer.

Structure of Integrons

Integrons are composed of a stable platform with the functional components needed for system operation along with a changeable array of discrete gene cassettes expressing auxiliary functions (Cambray *et al*., 2010). Open reading frames (ORFs) are incorporated by integrons, which are gene expression components, to create functional genes. All known integrons are made up of three essential components, including a central variable area between the 5′ and 3′ zone and a 5′ and 3′ conserved segment. Integrons are responsible for capturing and expressing exogenous genes that are a component of the gene cassette (Sabbagh *et al*., 2021).

Integrons has three components: an integrase gene (*int*I), a primary recombination site (*att*I), and a strong promoter. The integrase gene (*int*I) codes for a specific recombinant site and is related to the family of tyrosine recombinases. *att*I receptor site was identified by integrase and is located next to the *int*I gene. Integron integrases integrate distinct gene cassettes, also known as discrete circularised DNA units, at the proximal *att*I site, downstream of the resident promoter, allowing the production of the encoded proteins (Rowe-Magnus *et al*., 1999). Most of the cassettes are without promoters so the expression of the genes they carry is ensured by a dedicated Pc promoter which is embedded either in the *int*I site or the *att*I site and oriented toward the integration point (Cambray *et al*., 2010).

Mobility

Antibiotic resistance is a natural phenomenon that occurs when microorganisms are exposed to antibiotic drugs. Under the selective pressure of antibiotics, susceptible bacteria are killed or inhibited, whereas bacteria that are inherently (or intrinsically) resistant or that have developed antibiotic-resistant traits are more likely to live and multiply (Prestinaci *et al*., 2015).

Antibiotic resistance is being associated with mobile DNA elements (transposons or plasmids), and the mobilisation of antibiotic-resistance genes (Deng *et al*., 2015). The mobility of integrons has been considered to be a major concern of clinically antibiotic resistance. It is well established that the primary mechanism for the horizontal transfer of resistance genes is the transfer of plasmids (through transformation or conjugation) or of phages by transduction. Additionally, the primary mechanisms involved in their acquisition include recombination, the mobilisation of gene cassettes by integrons, conventional transposition, and rolling-circle transposition mediated by the recently discovered common regions (CRs) (Poirel *et al*., 2009).

Classification

Integrons can currently be classified into two main groups: the super-integrons (SI) and the resistance integrons (RI). Majority of the genes carried by RI are found on chromosomes or plasmids, and they encode resistance to antibiotics and disinfectants. Several classes of RI integrons have been identified and categorised. Four general kinds of integrons, known as class I, II, III and IV integrons, have been recognised and distinguished to date. Classes I-III integrons are known as multi-resistant integrons (RIs), and they are able to acquire the same gene cassettes using a comparable recombination platform. This ability was made possible by the *in vitro* excision and integration that occurred via recombination sites from such integrons (Deng *et al.,* 2015).

A new type of integron, a super-integron (SI) has been identified in the *Vibrio cholerae* genome harbouring hundreds of cassettes and differing in several ways. Super integrons are large integrons that are positioned on the chromosome and comprise gene cassettes that have a range of different activities. Super integrons are species-specific and can drastically alter the genetic make-up and evolution of the host bacteria. In contrast, resistance integrons can be found in a wide range of species (Rowe-Magnus *et al*., 1999).

Class I Integrons

Corynebacterium glutamicum, a Gram-positive microbe, was the first to contain a class I integron in 1998 (Sabbagh *et al*., 2021). Because they have the ability to capture and express a variety of resistance genes, class I integrons are major contributors to the global problem of antibiotic resistance. Additionally, they are frequently incorporated in transposons and promiscuous plasmids, which make it easier for them to spread laterally into a wide range of pathogens (Akrami *et al*., 2019). They are now discovered in between 40 and 70 % of Gram-negative bacteria isolated from clinical settings (Gillings *et al*., 2008). Class I is extensively spread and frequently linked to transposons similar to TN402 (Xu *et al*., 2007). Although environmental isolates containing Mobile integrons (MIs) exhibit diversity, the *int*I1 gene sequence is substantially conserved among MIs detected in clinical isolates (Stalder *et al*., 2012). The assembly of DNA components from various environmental sources was one of the major processes that produced the direct ancestor

of the clinical class I integron. The biomass of domesticated animals and people is currently 35 times greater than that of wild terrestrial mammals. Every day, a considerable fraction of this massive animal and human biomass excretes a large number of faeces, which frequently contain more than 10^8, and occasionally even 10^{11}, copies of the class I integron per gram. As a result, the clinical class I integron has contaminated all ecosystems dominated by humans, which is being spread by application of manure or sewage sludge (Gillings, 2018). Many Gram-negative taxa, including *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Burkholderia, Campylobacter*, *Citrobacter*, *Enterobacter, Escherichia, Klebsiella, Pseudomonas, Salmonella, Serratia, Shigella* and *Vibrio* have been identified to harbour RI, the majority of which belong to class I integrons. They have also been identified in other bacteria, including *Mycobacterium fortuitum* and *Corynebacterium glutamicum* (Fluit and Schmitz, 2004).

Class II Integrons

Class II integron is commonly found to be associated with the Tn7 transposon family (Tn7 and its derivatives, such as Tn*1825*, Tn*1826* and Tn*4132*), carrying both of its recombination site *attI2* and promoter Pc found within such transposons (Xu *et al*., 2009). It carries three classic gene cassettes dfrA1, sat1 and aadA1a which confer resistance to trimethoprim, streptothricin, and streptomycin respectively (Ozgumus *et al.,* 2009; Sunde, 2005). Tn7 can be recognised as a complex mobile element with a transposition module made up of the two recombinational pathway-coding genes tnsA, tnsB, tnsC, tnsD, and tnsE. The main transposition pathway is controlled by tnsD, which controls transposition by recognising a particular sequence called *att*Tn7 that is present in the chromosomes of many bacteria (Ramirez *et al.,* 2010). Class II integrons exhibit decreased diversity, primarily because of the presence of a stop codon at amino acid 179 in the class II integrase (*int*I2) (Barlow and Gobius, 2006). Gene cassettes in class III integrons are expressed by the Pc2A and Pc2B promoters, with Pc2A being stronger (Mendes Moreira *et al*., 2019).

Class III Integrons

Class III integrons are uncommon. Class III integrons that have been characterised are both resistant integrons (Class I and II integrons), because they both encode metallo-lactamases and aminoglycoside acetyl transferases, they are fundamentally organised similarly to class I and II integrons (Xu *et al*., 2007). Since they seem to be significantly less common, class III integrons also seem to play a smaller role in the spread of multidrug resistance. *Acinetobacter Pseudomonas aeruginosa*, *Escherichia coli, Serratia marcescens, Alcaligenes xylosoxidans, Pseudomonas putida, Salmonella spp., Klebsiella pneumonia* and *Delftia* spp. have all been reported to possess class III integrons (Kargar *et al*., 2014).

Class IV Integrons

Class IV integrons were first identified in 1993 on chromosome of *Vibrio cholerae* super integron (SI). This family has at least 178 gene cassettes and is 126 kb in size (Akrami *et al*., 2019). Class IV integrons have only been identified in a small number of microbes, including the Vibrionaceae, Shewanella, Xanthomonas, Pseudomonad, and other proteobacteria. To date, class IV integrons have been found to carry gene cassettes imparting resistance to the Fosfomycin and Chloramphenicol (Deng *et al*., 2015)

Integrons in Anthrapocene

When bacteria with resistance genes are discharged into waste streams at the same time antibiotics, disinfectants, and heavy metals, hotspots are created where intricate interactions and selection events can take place (Gaze *et al.,* 2011). Wastewater treatment facilities serve as large-scale reaction chambers for the interchange of genes and the creation of novel gene combinations (Gillings and Paulsen, 2014). Strong selective pressures are applied to bacteria to develop genes for antimicrobial resistance due to extensive use and the ensuing environmental contamination with antimicrobial substances.

In response to shifting antimicrobial selection pressures, integrons offer a mechanism for quick migration and exchange of various resistance genes (Ghaly *et al*., 2021). Sub-inhibitory levels of antimicrobial drugs in the environment have an impact on the distribution and abundance of non-target bacteria and encourage the introduction of mobile DNA elements and a variety of resistance genes into new species.

Such antimicrobial pollution is probably going to have an impact on the entire microbial biosphere by increasing the basal rates of bacterial evolution through selection for lineages with higher rates of mutation, recombination, and lateral gene transfer, as well as by increasing the abundance of resistance genes and their mobile DNA vectors (Gillings, 2013).

Prevalence of Integrons Among Common Pathogens

Salmonella spp were isolated in 18 out of 150 samples taken from diarrhoegenic humans and poultry, and class 1 integrons were found in all of the isolates with amplicon length ranging from 650 to 3000 bp (Gharieb *et al*., 2015). These amplicons' sequencing indicated the existence of gene cassettes containing the resistance genes *aac(3)-Id, aadA2, aadA4, aadA7, sat, dfrA15, lnuF* and *estX*.

Rubin *et al*. (2020) screened Gram-negative bacteria (GNB) from healthy individuals' faeces for class 1 integron cassette sequences using polymerase chain reaction. 36% of the faecal samples included integron sequences. The high prevalence of integrons and clinically prevalent ARGs carried by Gram-negative bacteria in the intestine of a healthy

population raises the possibility that the human intestines act as a significant reservoir for these mobile ARGs that are found in *E. coli* strains that cause extra-intestinal infections in the same population.

Kaushik *et al*. (2019) assessed the prevalence of antibiotic resistance and the contribution of integrons to the transmission of resistance genes in *Escherichia coli* isolated from urban Yamuna river waters. A high level of antibiotic resistance was found for Cefazolin among the 141 *E. coli* strains that were isolated and tested. In 32% of the isolates, integrons class I and class II were discovered. Different gene cassettes were carried by the class I integron's variable region, including *dfrA17-aadA5, dfrA12-orfF-aadA2* and *blaOXA-1-aadA1*. 54% of the isolates carried the "*dfrA17-aadA5*" gene cassette. The discovery of serologically and genetically varied integron-positive MDR *E. coli* isolates points to a significant role for integrons in the establishment and spread of resistance characteristics in aquatic *E. coli.*

Barns *et al*. (2021) tested 300 clinical samples from patients (urine 104, stool 87, endocervical swab 86, and high vaginal swab 83) for routine microbiology. Of these samples, 49 Enterobacteriaceae, including *E. coli* (45), *Proteusmirabilis* (2), *Shimwelliablattae* (1), and *Klebsiella pneumoniae* (1) were confirmed. Out of the 49 isolates, 20 were positive for class I integrons (40.82%). However, the isolates lacked class II and III integrons.

Conclusions

Integrons have played a significant role in the spread of antibiotic resistance across the globe. This phenomenon has been driven by the sustained selection pressures imposed by human antimicrobial use in agriculture and medicine. As pathogens become increasingly antibiotic resistant, we must consider which bacterial traits will arise from the next wave of anthropogenic selection. In particular, these might include increased virulence or pathogenicity, as well as resistance to novel antimicrobial strategies. Irrational use of antibiotics can promote the development and dissemination of antimicrobial resistance genes (ARGs) among the environment particularly polluted with antimicrobials and heavy metals.

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