Research Article

Microbiology & Infectious Diseases

Integron-Associated Multidrug Resistance among Gram-Negative Bacteria: A Review

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Received: 19 August 2018; **Accepted:** 14 September 2018

Citation: Mafulul LI, Onwuliri FC, Onyimba IA, et al. Integron-Associated Multidrug Resistance among Gram-Negative Bacteria: A Review. Microbiol Infect Dis. 2018; 2(3): 1-6.

ABSTRACT

Multidrug Resistance (MDR) by Gram-negative bacteria is no longer a mystery as studies by researchers has revealed that integrons are amongst the genetic elements responsible for antimicrobial resistance. Integrons are known to consist of Integrase gene, attI site and promoter region. Integrons harbours gene cassettes containing various MDR genes. Polymerase chain reaction and Gel electrophoresis are one of the common ways of detecting integrons in Gram-negative bacteria. As at the time of this review, 4 classes of integrons have been discovered by scientists with only class 1 – 3 known to be capable of inducing antibiotic resistance in Gram-negative bacteria. However, class 4 integrons found in Vibrio cholerae does not contain gene cassettes probably making it difficult for researchers and scientists to link it to antibiotic resistance. In Nigeria, integrons survey seems to have only been carried out in the south. Thus, it is imperative to conduct studies regarding the presence and distribution of integrons in the North and other areas where the survey has not been carried out in order to create awareness and promote antimicrobial resistance vigilance. The search for novel anti-integron genes compounds should be encouraged in order to tackle the antimicrobial resistance problems in pathogenic Gram-negative bacteria.

Keywords

Integron, Multidrug, Resistance, Gram negative bacteria.

Introduction

The incidence of microbial infections has increased in the last few decades. The emergence of resistance among different microbial strains has been possible as a result of the frequent use of antimicrobial drugs in treating infections. Multidrug resistance (MDR) is defined as insensitivity or resistance of infectious agents usually microbes, to the administered microbial agents or drugs despite earlier sensitivity or susceptibility to them [1].

Multidrug resistance (MDR) among bacteria of clinical importance such as members of the enterobacteriaceae is still a big healthcare challenge all over the world, with increase in morbidity and mortality [2]. Even though chromosomal mutations remain one of the mechanisms by which resistance genes are acquired and spread, resistance genes can also be disseminated by extra-chromosomal elements such as integrons, plasmids and transposons acquired from other bacteria [3,4].

Resistance to antimicrobial drugs occurs due to one or more of the following mechanisms; drug inactivating enzymes, alteration in the target molecule, decreased uptake of the drug, increased elimination of the drug, spontaneous mutation, and gene transfer [5]. With few exceptions, antibiotic resistance in bacterial pathogens was identified soon after particular drugs such as penicillins and sulfonamides were introduced into clinical practice illustrating the genetic flexibility of bacteria [6]. The emergence of multiple resistant strains could not be attributed to mutation alone. It was soon established that bacteria were acquiring genes that confer resistance, relying on this means to escape antimicrobial activity rather than on mutations arising in resident genes [6]. However, integrons, plasmids and transposons have been identified as the three (3) exchangeable genetic elements responsible for the dissemination of antibiotic resistance in many bacteria. Although integrons are not self mobilizeable, they are usually found in association with transposons and are often located on plasmids, facilitating their mobility [7]. This clearly means that integrons are non-mobile genetic elements, which depend on the other two exchangeable elements (plasmids and transposons) for dissemination of the resistance genes, which they harbour.

Integrons are usually, if not completely, associated with Gram-negative bacteria especially members of the enterobacteriaceae. Several studies have been conducted by researchers including the use of molecular detection techniques to reveal the presence of integrons in Enterobacteriaceae [7-11].

This review is aimed at emphasizing and creating further understanding on the role of integrons in multidrug resistance in Gram-negative bacteria of clinical and environmental importance.

Integrons

Integrons were defined as DNA elements that function as gene - capture and expression systems [12,13]. This element contains three necessary components located within the 5'-conserved segment. These components include an integrase gene (Int I), which encodes a site - specific recombinase enzyme; an att I site [12]. which is recognized by the integrase and acts as an acceptor for gene cassettes; and a promoter region (PC) which promotes the expression of any suitably integrated gene [14,4,15]. Gene cassettes become a part of the integron when integrated [16-18]. Gene cassettes are discrete genetic elements that may exist as free, circular, non- replicating DNA molecules when moving from one genetic site to another [16] but which are normally found as linear sequences that constitute part of a larger DNA molecule, such as plasmid or bacterial chromosome. Gene cassettes normally contain a single gene and an additional short sequence, called a 59 base element that functions as a specific recombination site [16].

Although integrons are not mobile, they can be transferred between bacteria by transposons or plasmids in which they are present. Accordingly, integrons are a major mechanism for the spread of multidrug resistance (MDR) [19]. El-Rahman et al. defined integrons as genetic structures capable of capturing and exercising gene cassettes, which usually encode antimicrobial drug resistance determinants. Their study was on the role of integrons in multidrug resistant extended beta-lactamase-producing enterobacteriaceae. They were able determine an association between integrons and MDR especially to aminoglycosides and tetracyclines. The same study revealed high incidence of class I integron in enterobacteriaceae isolates, especially *Klebsiella sp.* and *Eschericia coli*. Integrons are natural genetic engineering platforms that can incorporate open reading frames and convert them to functional genes by ensuring correct expression thereby playing a major role in MDR among Gram-negative species [6]. Integrons harbour gene cassettes containing various MDR genes. Thus, gene cassettes are inserted into integrons thereby favouring the dissemination of resistance genes even among nosocomial bacteria [20]. Leon et al attributed the cause of multiple-antibiotic resistance to the presence of integrons among clinical enterobacteriaceae isolates. The unrecognized widespread presence of integrons-containing Gram-negative bacteria, both within hospitals and in the community, poses a serious threat of the spread of antibiotic resistance [21]. Data from the U.S. National Health Care Safety Network indicated that Gram-negative bacteria were responsible for more than 30% of hospital-acquired infections and these bacteria predominate in cases of ventilator-associated pneumonia (47%) and urinary tract infections (45%) [22].

A number of studies have investigated the prevalence of integrons in hospital isolates of Gram - negative bacilli and somewhat conflicting results were obtained [10]. Some studies of selected clinical bacterial populations have shown a high integron prevalence ranging from 28.5 to 89.2% [8,23]. Other studies, in contrast, reported significantly lower prevalence's (e.g. 13%) of integrons in hospital isolates of enteric bacteria [24,25]. Only a few studies-investigated the occurrence of integrons in enterobacteriaceae that cause community-acquired infections. The studies reported prevalences ranging from 48.3 to 70% in *E. coli* isolates causing community - acquired Urinary Tract Infections (UTIs) [26,27].

Classes of Integrons

At present, four classes of integrons are known to have a role in the dissemination of antibiotic resistance genes [28]. These classes are distinguished by their respective integrase, Int I, genes [28,29]. Three classes, 1, 2, and 3, are strongly associated with the multiple antibiotic resistance phenotype, with the class 1 integrons being the most prevalent [28]. The wide distribution of the class 1integron has mainly been attributed to the spread of the integron-containing transposon, Tn21 [17,25,30].

Class 1 Integrons

Class 1 integron is composed of a 5' - conserved segment (5CS) including the intl and attI genes, and pant promoter, also and 3' - conserved segment (3CS) which encodes resistance to sulfonamides (SULl) and disinfectants [31,32]. As stated previously, the class I integrons are still the most common integrons found in members of the enterobacteriaceae such as *Enterobacter sp.*, *Klebsiella pneumoniae*, *E.coli* and *Proteous sp.*, as well as other clinically important Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [17,33].

A study in Nigeria by Odumosu et al. revealed a high prevalence of aadA gene, which confers resistance to streptomycin and spectinomycin [34] in *P. aerugiriosa* strains positive for class I, integron. They however warned that the rapid and unabated spread of the detected class 1 integron-positive *P. aeruginosa* multidrug resistant strain in South West Nigeria might reduce successful treatment of infections caused by such strains. They advocated for the creation of rapid antimicrobial resistance surveillance programme in Nigeria.

The dissemination of class 1 integrons has been attributed to the spread of an integron-containing transposon, Tn21 [17,25,30]. Tn21, a large (19.7kb) class II replicative transposon, carries a mercury resistance (mer) operon, an integron (In2) and a transposition module. In addition to drug resistance, Tn21 confers mercury resistance through its mercuric reductase genes, merA [35].

Furthermore, among the different integron families, class I integrons are found to be most prevalent in drug - resistant bacteria [36].

Class I integrons are mobile DNA elements with a specific structure consisting of two (2) conserved segments flanking a central region containing "cassettes" that usually code for resistance to specific antimicrobials. The 5' - conserved segment contains the integrase gene (In + II), a promoter region and the In+II-specific integration site att II. The 3'-conserved segment usually contains a combination of the three genes qac EΔ1 (antiseptic resistance), sull (resistance to sulfonamides), and an open reading frame (Orf5) of unknown function. Between the two conserved segments, the central variable region can contain from zero to multiple cassettes [37].

However, the big question as to whether integrons are generally mobile or not is an issue that needs to be critically resolved. This is due to the divergent statements attributed to Farshad et al. and Wu et al. As earlier stated in this review, Farshad et al. asserted that integrons are not mobile but can be transferred between bacteria by transposons or plasmids in which they are present. Wu et al. on the other hand stated that class I integrons are mobile DNA elements. Therefore, if class I integrons are mobile, what about the remaining classes?

Class 2 Integrons

A study in Southern China found class 2 integron-carrying Tn 7-like dfrAl -sat 1- aadAl conferring resistance to trimethoprim, streptothricin and streptomycin/spectinomycin respectively in clinical strains of *P. aeruginosa* [38]. Class 2 integrons have been discovered to exhibit decreased diversity primarily because of the presence of a stop codon at amino acid 179 in the class 2 integrase (Intl2) [39]. It is believed that the stop codon results in the production of a shorter and probably inactive polypeptide that is unable to catalyze the recombination reaction observed in other classes of integrons [40]. Class 2 integrons are mostly found on transposon Tn7 and its relatives and commonly carry the three antibiotic resistance genes dfrAl, Sat2, and aadAl. However, studies into the variability of class 2 integrons have identified a number of novel rearrangements within class 2 integrons [41,42]. Antibiotic resistance genes previously not associated with class 2 integrons such as ereA [43] and estX (GenBank accession no. AB161462) have been shown to be associated with Tn7- related class 2 integrons [38].

Furthermore, Ramirez et al. recently described a novel rearrangement of a class 2 integron from non - epidemiologically related *Acinetobacter baumannii* isolates. This class 2 integron have the genes sat2, aadB and CatB2 inserted upstream of the three conventional antibiotic resistance genes of Tn7 class 2 integrons. The resulting structure is a class 2 integron with a variable region comprising six antibiotic resistance genes and represents the first description of aadB and CaIB2 within a class 2 integron [38]. Class 2 integrons are embedded in the Tn7 family of transposons and have been found in S*almonella, Acinetobacter, Escherichia, Shigella, Aeromonas,* and *Morganella* [17].

Class 3 Integrons

Xu et al. detected two environmental strains of *Delftia species*, which were found to carry class 3 integrons from aquatic samples in Canada. The species are *Delftia acidovorans* C17 and *Delftia tsuruhatensis* A90. These strains have rarely been reported and then onlyfrom pathogens in which they are associated with antibiotic resistance genes. *Delftia* species are rod -shaped, nitrate -reducing, Gram negative bacteria with G + C content in the range of 66% that are widely distributed in the environment and capable of degrading a variety of xenobiotic compounds including chlorinated aromatic compounds [44]. They were formerly considered members of the genus *Comamonas* or *Pseudomonas* [45]. There have been no previous reports of integrons in Delftia; however, super integrons are commonly found in related bacterial genera such as *Pseudomonas* [46]. It is noteworthy that the study by Xu and other researchers became the first to reveal the presence of integrons in *Delftia*. The detailed genetic organization of the two *Delftia* integrons was determined by cloning and nucleotide sequencing and revealed two closely related chromosomal elements with gene cassettes but no known antibiotic resistance determinants [47].

Earlier, lntI3 sequences were known only from *Serratia marcescens*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Pseudomonas putida*, and *Alcaligenes xylosoxidans* [48-51]. This brought about the decision to examine available strains of *Delftia* and related genera in the Comamonadaceae to determine the prevalence of IntI3 in this new host group [47]. The only one of 14 strains tested that was positive for lntI3 by a PCR assay was another *Delftia* strain. *Delftia acidovorans* C17 was confirmed by Southern blot analysis to possess intl3 sequences. Strain C17 also originated from an activated-sludge community [44] but the source was a wastewater treatment facility in Idaho that was geographically distant from the source of A90 in Canada.

Since class 3 integrons [48,50] or portions thereof [49,51] have previously been reported only from Japan and Portugal, strains A90 and C17 represent the first intI3 - bearing strains from North America. The lack of association of intI3 with a phylogenetic lineage among the representatives of the group and the geographic separation of the source habitats suggest that the IntI3 sequences were introduced into the two *Delftia* strains horizontally, on 2 occasions [47].

Integrons often have associated gene cassettes encoding resistance to a variety of antibiotics and play a role in dissemination of resistance in hospitals. The two class 3 integrons characterized, one from *S. marcescens* Ak 9373 [48] designated In3-1 here for convenience, and one from K. pneumonia FFUL 22K [50] referred to as In 3-2 were both isolated from clinical strains and were associated with gene cassettes for resistance to broad spectrum beta-lactams and other antibiotics. *Delftia* strains A90 and C17 are distinct from these in originating from an environmental rather than a clinical setting; it was therefore of interest to the researchers [47] to examine the organization of integrons associated with the IntI3 sequences in *Delftia*.

Class 4 Integron

This is a special or unique class of integrons found in the genome

of *Vibrio cholerae*. Its association with antibiotic resistance remains unknown [52]. It also lacks gene cassettes [9]. It can be hypothetically deduced as a probability that the absence of gene cassettes is the reason for the little knowledge of the antimicrobial resistance of this integron. Since the gene cassettes that are harboured by integrons are not present, it therefore means that the resistance genes with respect to integrons may not be found. Hence the inability of scientists to associate any antimicrobial resistance to the class 4 integrons. More studies needs to be done in resolving the mystery surrounding the integrons' link to antimicrobial resistance.

Super Integrons

According to Mazel Super Integrons (SIs) were discovered in the late 1990s following studies examining the relationship between Resistant Integrons (Rls) gene cassette arrays and a cluster of repeated sequences identified in the genome of *Vibrio cholerae*, known as *Vibrio cholera* repeats (VCRs). The super integron is now known to be an integral component of many g-proteobacterial genomes. The SI shares some similarities with the RI such as: possessing a specific integrase (Int IA) and also is responsible for inserting coding sequences (ORFs) into a unique chromosomsal attl site. The SI has two structural features that differentiate it from other Rls:

- The large number of cassettes that it gathers and
- The high homology observed among the attC sites of these cassettes, which in the case of *V. cholerae* are known as VCRs.

Furthermore, a super integron is defined by these key features enumerated above including the sedentary nature of the functional platform (TntIA + attl site). Such SI structures are found among the Vibrionaceae and their close relatives, the Xanthomonads, and a branch of the Pseudomonads. They share the same general characteristics such as their large number of more than 20 cassettes and a high homology between their endogenous cassette attC sites. More importantly, they predate the antibiotic era, and have been identified in isolates collected from the 19lh century [6].

Integrons Detected in Nigeria

Odumosu et al. detected integrons and associated gene cassettes in multidrug resistance *P. aeruginosa* in South West Nigeria. The study revealed an incidence rate of 57.4% for class 1 integrons with no record of class 2 and 3 integrons. This study conducted in South West Nigeria is in concordance with other surveys carried out by Labuschagne et al. in South Africa and Hammani et al. in Tunisia which suggested strongly the association of class 1 integrons and multidrug resistance, and the detection of class 1 integrons among *P. aeruginosa* isolates of clinical importance. However, Odumosu et al. recommended effective surveillance of antimicrobial resistance and correct strategies targeted towards preventing indiscriminate and unregulated antibiotics use as ways to urgently stop urgently multidrug resistant outbreaks in Southwest Nigerian hospitals.

In addition, the prevalence of antibiotic resistant cell-detaching *E. coli* (CDEC) strains from Nigerian children was studied including the detection of integrons [53]. CDEC were originally defined by their capacity to detach tissue culture cells from solid supports in adherence assays or in a cell-detaching assay [54]. CDEC strains possess pyelonephritis - associated pilli (P -pilli) and produced alpha-haemolysin including cytotoxic necrotizing factor I (CNF1) [55]. Diarrhoea was also elicited by CDEC strains in the reversible intestinal tie adult rabbit diarrhoea model in animal studies [58]. Okeke et al. however reported a higher prevalence among pathogenic *E. coli* than among normal flora, with the highest prevalence among CDEC strains. The authors also reported that multiple - resistant cell detaching *E. coli* strains could be important reservoirs for genes that are responsible for antibiotic resistance such as integrons.

Conclusion

In this review, four classes of integrons (1–4) are known to exist. However, classes 1–3 confer antimicrobial resistance in Gram-negative bacteria whereas class 4 integrons' association to antimicrobial resistance is still unknown. It can also be said that class 1, 2 and 3 represents the resistant integrons whereas class 4 are the super integrons. Thus, the unavailability of gene cassettes in class 4 integrons which are found in *Vibrio cholerae* may be the reason for the inability of scientists to understand whether antibiotic resistance (if any) is caused by the integrons or not. Therefore, more research is needed towards understanding the link between antibiotic resistance and class 4 integrons including the absence or lack of gene cassettes.

There is need for constant monitoring and investigation of multiple drug resistance genes by local, state, federal, and global health and research bodies in order to prevent and/or mitigate the effects of MDR in human and animal population. In addition, awareness campaigns via public enlightenment on the dangers of antibiotic (antimicrobial substances) use and misuse by individuals should be strengthened and sustained. It should focus on discouraging; self-medication and prescription of drugs, wrong administration of required dosage, unnecessary incorporation of antimicrobial agents in animal feeds, and the manufacture of fake and counterfeit antimicrobial drugs.

Use of molecular techniques should be strengthened in Nigeria and other developing countries towards resolving the challenges associated with the genetic mystery of antimicrobial drug resistance in microorganisms. This can be achieved through provision of modern molecular technique equipment and regular training of academicians, technologists, scientists and researchers in all relevant institutions.

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