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Integrons

Vehicles and pathways for horizontal dissemination in bacteria

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Integrons are genetic elements first described at the end of the 1980s. 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. Integrons are present in ≈10% of the sequenced bacterial genomes and are frequently linked to mobile genetic elements (MGEs); particularly the class 1 integrons. Genetic linkage to a diverse set of MGEs facilitates horizontal transfer of class 1 integrons within and between bacterial populations and species. The mechanistic aspects limiting transfer of MGEs will therefore limit the transfer of class 1 integrons. However, horizontal movement due to genes provided in trans and homologous recombination can result in class 1 integron dynamics independent of MGEs. A key determinant for continued dissemination of class 1 integrons is the probability that transferred MGEs will be vertically inherited in the recipient bacterial population. Heritability depends both on genetic stability as well as the fitness costs conferred to the host. Here we review the factors known to govern the dissemination of class 1 integrons in bacteria.

Introduction

Horizontal gene transfer (HGT) is the transfer of genetic material between bacteria from the same generation. A successful HGT event does not rely only on the introduction of DNA into a recipient cell's cytoplasm, but also on the heritability of the transferred sequences in the recipient microorganism.[1] HGT contributes to the genetic diversity and evolutionary trajectories of bacterial populations. For instance, HGT of mobile genetic elements (MGEs) is the major contributor to emergence, recombination and dissemination of multidrug resistance among bacterial pathogens.[2,3] Due to their capacity to physically move between host genomes, MGEs are common elements in bacterial communities. A variety of MGEs have been described to date such as plasmids, bacteriophages, genomic islands (GIs), integrative and conjugative elements (ICEs), insertion sequences (ISs), transposons (Tns), integrons and miniature inverted repeat transposable elements (MITEs).[4] Integrons are genetic elements that contain a site-specific recombination system able to integrate, express and exchange specific DNA elements, called gene cassettes.[5] The complete integron is not considered to be a mobile element as such as it lacks functions for self-mobility. In contrast, the gene cassettes present in integrons are considered mobile, although the natural exchange of gene cassettes are rarely experimentally observed.[6,7] Nevertheless, sequence similar integrons appear to be widespread among bacterial species and genetic backgrounds, suggesting that they are frequently exposed to mechanisms that allow them to disseminate horizontally through bacterial populations.[8] The aim of this review is to examine how different vehicles and mechanisms of HGT enable the dissemination of integrons. We focus on the main type of integron involved in the spread of antibiotic resistance, the class 1 (C1) integrons.[9]

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Integrons

Integrons were initially described at the end of the 1980s.[8] Bioinformatics based analysis of partially or fully sequenced bacterial genomes show that integrons or integrase genes are present in approx. 10% and 17% of them, respectively.[9,10] Most of the descriptions of integrons have focused on their presence in human clinical isolates,[9,11,12] but they are also found in many non-clinical environments, such as in aquatic environment[13-15] and soil.[16,17]

Integrons consists of three elements: the gene that encodes a tyrosine recombinase (integrase, encoded by the *intI* gene), needed for site-specific recombination within the integron; the adjacent recombination site (*attI*) that is recognized by the integrase; and the promoter (P_c), located upstream of the integration site, necessary for efficient transcription and expression of gene cassettes present in the integron. Although the P_c promoter is assumed to be part of all classes of integrons, its presence and activity has not been shown for all of them.[10] Many of the integrase genes present in integrons carry LexA binding sites close to their promoter regions and can be controlled by the host LexA protein, which is a transcriptional repressor of the SOS response;[6] as shown for a class 1 integrase and in an integrase present in a CI (chromosomal integron) of *Vibrio cholorae*.[6,7] These studies

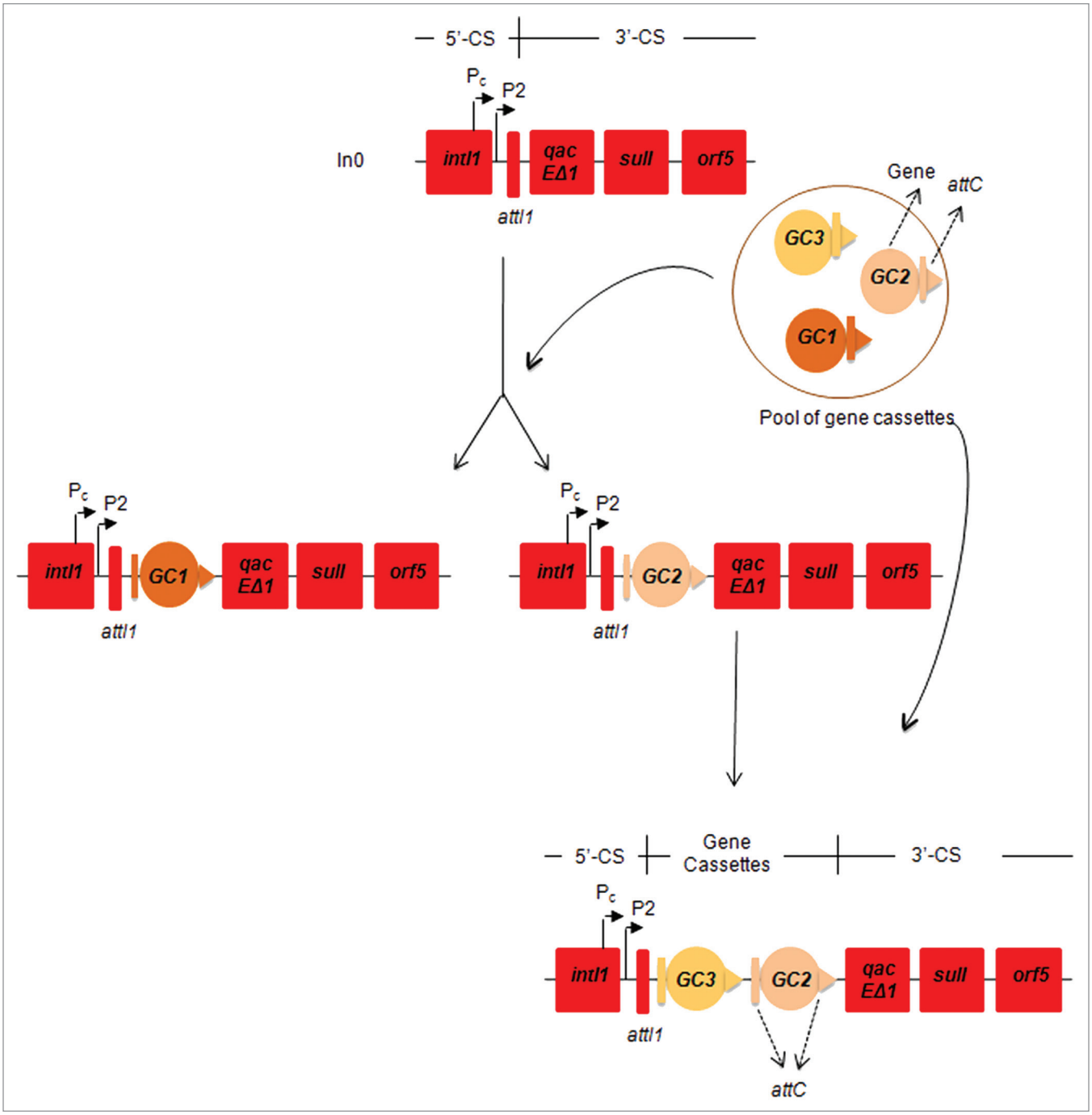

Figure 1. Schematic representation of class 1 (C1) integrons. In0 is the most basic integron that does not contain any gene cassettes. Different cassette arrays can be integrated in the variable region of the integron. Abbreviations: *intI1*, class 1 integrase gene; P_c and P2, gene cassette promoters; *attI1*, integron-associated recombination site; *qacEΔ1*, truncated version of a quaternary ammonium resistance gene; *sulI*, sulphonamide resistance gene; *orf5*, open reading frame; *attC*, recombination site of the gene cassette; GC, gene cassette; 5'-CS,5' conserved segment of the integron; 3'-CS,3' conserved segment of the integron.

suggest that SOS induction can lead to increased transcription of the integrase gene and increased integrase activity, including cassette rearrangements, in organisms harboring *lexA* alleles.[6,7] The regulation of the integrase transcription in species where a LexA ortholog is absent is unknown.

Integrons can incorporate one or more gene cassettes (**Fig. 1**).[5] Usually integrons present in clinical bacterial strains carry less than five cassettes,[18] although integrons with up to nine antibiotic resistance genes have been described.[19] Numerous combinations of gene cassettes have been reported so far;[20] a list of numbered C1 integrons is available from the INTEGRALL database (http://integrall.bio.ua.pt/).[21]

Based on the genetic relatedness of the integrase *intI* gene sequence, the integrons were initially classified into a few classes,

of which class 1, 2 and 3 have received most attention.[10,16] More extensive DNA sequencing efforts have however now revealed more of the genetic diversity of the integrase gene and identified > 90 different gene variants, questioning the initial classification scheme.[10,22] Phylogenetic analyses of sequenced *intI* genes suggest integrons belong to 3 broad groups, the soil/freshwater proteobacteria group, the marine γ-proteobacteria group and the inverted integrase group.[10] C1 integrons have been reported as the most common and widespread, especially in clinical settings and are one of the main contributors to the problem of antibiotic resistance dissemination.[9]

The origin of C1 integrons is not clear, but it is assumed that they were present in bacteria before the "antibiotic era."[23] C1 integrons without antibiotic resistance genes found in environmental Betaproteobacteria have been suggested to be the original source of these genetic structures. These environmental integrons lack the Tn*402* (also called Tn*5090*) features,[22,23] and it is proposed that they were incorporated into a plasmid-borne Tn*402* transposon, carrying a gene cassette that conferred a host advantage.[22] For instance, the *qac* gene, which codes resistance to quaternary ammonium compounds, as such biocides have a long history of use in clinical practices;[24] and the *sulI* gene, which confers resistance to sulphonamides, one of the first antibiotics introduced in the clinical practice.[25] Another possible origin of the C1 integrons is the early association between the ancestor of the Tn*402* transposon with a class 1 integrase and an *attI1* site. This early association is supported by the observation that most C1 integrons contain the 5'-CS region at the same location.[25] The 3'-CS region has been suggested to be the result of a fusion of the *qacE* gene of the Tn*402* transposon with the *sulI* gene, with consequent partial deletion of the *qacE* gene; that occurred at the same time as a deletion event in the transposition functions of the Tn*402* transposon, resulting in a structure unable of self-mobilization.[25] In fact, most C1 integrons are defective transposons;[26] though a few C1 integrons with a complete transposition module have been identified.[27,28] After initial formation of C1 integrons, strong selection for resistance to antimicrobials has favored events of capture of antibiotic resistance gene cassettes resulting in the compositions of C1 integron as we know them today.[4,18] There is also evidence that C1 integrons can capture gene cassettes from CIs (see description of CIs below).[29]

Integrons are also classified based on their context, which includes two different types of integrons, mobile integrons (MIs) and chromosomal integrons (CIs).[9] CIs can carry a variable number of gene cassettes, ranging from zero to hundreds, which are usually not involved in antimicrobial resistance. CIs are considered to be sedentary, though movement of gene cassettes from CIs has been reported.[9,10] In contrast, MIs, such as C1 integrons, carry a limited number of gene cassettes and are often involved in dissemination of antimicrobial resistance.[9] This review focuses on mobile C1 integrons.

Gene cassettes are small mobile elements composed by a single and most often promoterless gene and a recombination site (*attC*). Gene cassettes are linear when integrated in the C1 integron and circular in the free form, after excision or before site-specific insertion.[30] The *attC* site (previous called 59-base element or 59-be) is recognized by the integrase and recombination between this site and the *attI1* site of the C1 integron backbone leads to the addition of the gene cassette into the integron structure.[20,31,32] Due to the nature of the site-specific recombination, part of the *attC* site is located at the start of the gene cassette and part at the end.[31,33] The majority of the 194 known gene cassettes encode antibiotic resistance; including resistance to frequently used aminoglycosides, β-lactams, quinolones, chloramphenicol and trimethoprim, among others.[20] Transcription and posterior expression of gene cassettes depends on the promoter of the integron, as the gene cassettes themselves rarely possess a promoter;[5] though a few exceptions have been reported.[20] Thirteen different variants of the gene cassette promoter P_c, with different expression levels, have been described. As the P_c promoter is located within the *intI1* coding sequence, the occurrence of different P_c leads to the existence of 10 different variants of the class 1 integrase IntI1.[34] The P_c can be associated or not with a second gene cassette promoter, P2.[35] The level of transcription and expression of gene cassettes present in the integron is also influenced by the distance to the promoter, where cassettes closer to the promoter are expressed at higher levels than more distal ones.[35]

Usually, C1 integrons have three distinct genetic regions of which two are highly conserved, the 5'-conserved segment (5'-CS) and the 3'-conserved segment (3'-CS), that flank the central variable region where the gene cassettes are located.[8] The 5'-CS includes the integrase *intI1* gene, the recombination site *attI1* and the promoter P_c and P2 when present. The 3'-CS consists of the *qacE*Δ1 gene, which encodes an incomplete version of a protein that mediates resistance to certain detergents, the *sulI* gene, encoding resistance to sulphonamides and an open reading frame *orf5*, of unknown function.[33] Although most of the C1 integrons have this classic structure, an increasing number of C1 integrons with different structure have been reported;[36-38] these C1 integrons are associated with IS*CR1* and therefore will be mentioned as IS*CR1*-linked C1 integrons throughout this review. These integrons are often referred to, in the scientific literature, as complex class 1 integrons. The IS*CR1*-linked C1 integrons frequently have non-cassette type resistance genes, a truncated version of 3'-CS region followed by an IS*CR1* element and another variable region and or 3'-CS duplications.[38,39]

C1 integrons are mainly found in Gram-negative bacteria, although several studies have reported the presence of these mobile elements also in Gram-positive bacteria. In fact, Nandi and collaborators[40] suggested that Gram-positives represent a larger reservoir of C1 integrons than Gram-negatives. Reports of C1 integrons in the Gram-positive genera *Corynebacterium*,[40,41] *Enterococcus*,[42] *Staphylococcus*[42-44] and *Streptococcus*,[42] are available. Nonetheless, the majority of the reports of C1 integrons concerns Gram-negative genera, such as *Acinetobacter*,[45,46] *Aeromonas*,[13] *Burkholderia*,[47] *Enterobacter*,[46] *Escherichia*,[13,46,48] *Klebsiella*,[46,48] *Morganella*,[13] *Proteus*,[48] *Pseudomonas*,[45,46] *Salmonella*[49,50] and *Vibrio*.[51] It should also be noted that C1 integron-carrying Gram-positive bacteria have mainly been isolated from human clinical samples, while C1 integron-carrying Gram-negative isolates have been collected from a wider range of environments,

including hospitals,[11,12,52,53] healthy humans,[52,54] animals,[52,55,56] food-products,[53,57] soil[17,58] and aquatic environments.[13-15]

The Genomic Locations of C1 integrons, Context and Mobility

Most of the descriptive studies of C1 integrons focus on the identification and characterization of various C1 integrons and their gene cassette arrays; fewer studies also aim to unravel the genetic context where they are inserted.[57] When investigated, most of the C1 integrons are found genetically linked to and embedded in MGEs, such as MITEs, ISs, Tns, GIs and plasmids. Some of these MGEs contain self-mobility determinants, whereas others can only be mobilised by other elements or when some genes/elements are provided in trans (further information below). These elements confer mobility to the C1 integrons when part of the larger MGE. Thus, genetic linkage to various and sometimes several MGEs facilitates both intra and intergenomic transfers of integrons at frequencies determined by the mobility functions and transfer dynamics of the element(s).

Evidence of mobility of C1 integrons. There are many studies that suggest horizontal dissemination of C1 integrons, as the same C1 integron structure is found in different species, in different geographical places and isolated in different periods of time. For example, C1 integron In76, carrying a bla_{IMP-5} gene cassette, was isolated in *Acinetobacter baumannii* 65FFC from Coimbra University Hospital in 1998,[59] and also in eight *Pseudomonas aeruginosa* isolates collected between 2001 and 2003 from São Bernardo Hospital, Setúbal, Santa Maria Hospital and Dona Estefânia Hospital, both from Lisbon;[60] these latter isolates were not epidemiologically related.[59,60] Similar is the case of 13 epidemiologically unrelated *Acinetobacter baumannii* isolates collected between 1989 and 2000 in six different Italian hospitals that carried a C1 integron with the cassette array *aacC1*-orfX-orfX'-*aadA1a*.[12] The same C1 integron was described in an *Acinetobacter baumannii* collected in Spain in 2000.[61] Interestingly, the same cassette array *dfrA1-aadA1* was found in C1 integron in the Gram-negatives *Escherichia coli* and *Aeromonas* sp.,[13] and in the Gram-positives *Corynebacterium ammoniagenes* and *Corynebacterium casei*.[40]

Genetic linkage to MGEs with consequences for mobility. The genetic linkage between C1 integrons and MGEs increases the potential of C1 integrons for dissemination by HGT. C1 integrons are frequently observed in larger MGEs with several layers of horizontal mobility and such combination of MGEs confers a broad horizontal dissemination potential to C1 integrons. Importantly, the mobility of C1 integrons embedded in MGEs emerges mainly from a complex interplay between various modes of transposition and plasmid-based conjugative processes. The fate of newly transferred integrons is further modulated by functional and selective constraints to the stable maintenance of the elements in new hosts. MGEs vary in their ability to enable intra- vs. inter-cellular movements of integrons. For instance GIs, MITEs, ISs and MTns (mobilisable Tns) allow transfer and genomic integration of C1 integrons **within** cells (see section 3.2.1 to 3.2.4) whereas conjugative plasmids, transduction and transformation contribute to the transfer of C1 integrons **between** cells (see section 3.2.5 to 3.2.7). Some bacteriophages, ICEs and some GIs can enable both intra- and intercellular transfers. In many other cases, horizontal dissemination of integrons should be viewed as a two-step process, the first step being dependent on mechanism allowing intercellular transfer. The second step can however draw on a much wider set of mechanisms allowing intracellular transfer and genetic relocalization.

Below we provide further considerations and examples of the horizontal mobility caused by the genetic linkage of C1 integrons with diverse MGEs; including combination of MGEs. Some of the studies referred to directly demonstrate HGT of the integron-associated MGE whereas others infer horizontal acquisition of C1 integron-containing elements, usually by transposition, due to the presence of target site duplications (TSDs). TSDs are short direct nucleotide repeats that can be found on both ends of the inserted mobile element. **Table 1** shows some examples of the various MGEs associated with C1 integrons.

Genetic linkage to MITEs. MITEs are non-autonomous mobile elements consisting of small repeat sequences, which do not encode proteins and are found in random locations in the genome of various bacteria.[62,63] MITEs are quite abundant[62,64,65] and their capacity of transposition has been shown in the genomes of archaea,[66] eukaryotes[67] and bacteria.[63] Bacterial MITEs do not carry a transposase gene, but are suggested to transpose when a transposase is provided in trans. The movement of MITEs generates a TSD (**Fig. 2A**).[63] To our awareness, there are only four reports of presence of MITE-like structures flanking C1 integrons. Identical copies of the same 439 bp MITE-like structure have been found flanking the C1 integron of a clinical *Acinetobacter baumannii*,[68] a prawn-associated *Acinetobacter johnsonii*[69] and in a clinical *Acinetobacter bereziniae*[70] (GenBank accession number JX235356). TSDs were detected on both sides of the MITEs in these three isolates, indicating that the C1 integrons flanked by the MITEs were mobilized by transposition promoted by these genetic elements; in addition to the TSD, the fact that the host gene is interrupted by the single insertion of the MITE-integron-MITE structure in *Acinetobacter baumannii*[68] and in *Acinetobacter bereziniae* (GenBank accession number JX235356) contribute to the assumption that it was acquired by transposition promoted by the MITEs. Transposition of the entire MITE-integron-MITE structures presented in the *Acinetobacter baumannii*[71] and in the *Acinetobacter johnsonii*[69] was not experimentally observed, suggesting that the transposase required for the movement is not present in the respective strains.

In the fourth study, two copies of a MITE-like structure, a 288 bp IMU (Integron Mobilization Unit) were found to flank a defective C1 integron in the plasmid of a clinical *Enterobacter cloacae*.[72] In this case, TSD was not present, but the entire structure (IMU-integron-IMU) was shown to transpose and create a TSD when a transposase was experimentally provided in trans (**Fig. 2A**).[72]

Genetic linkage to ISs. ISs are one of the most basic and smallest mobile elements, consisting of a central region encoding a transposase, responsible for mobility that is flanked by terminal inverted repeats (IRs). Transposition of ISs also produce TSD.[73]

Table 1. Examples of mobile genetic elements (MGEs) associated with class 1 integrons

MGE	Species	Isolation source	Isolation country	Reference
MITE				
MITE-like	*Acinetobacter johnsonii*	Prawn	Australia	69
	Acinetobacter baumannii	Clinical	Portugal	68
	Acinetobacter bereziniae	Clinical	Portugal	70
IMU	*Enterobacter cloacae*	Clinical	Canada	72
IS				
IS*6* family	*Escherichia coli*	Clinical	Poland	76
	Salmonella enterica serovar Newport	Clinical	France	75
	Escherichia coli	Clinical	France	19
	Escherichia coli	Clinical	Greece	74
	Salmonella enterica serovar Typhimurium	Clinical	Albania	74
IS*CR1*	*Aeromonas punctata*	Wastewater	China	80
	Citrobacter freundii	Clinical	Portugal	78
	Klebsiella pneumoniae	Clinical	Argentina	36
	Enterobacter cloacae	Clinical	Argentina	36
	Escherichia coli	Clinical	Argentina	36
IS*Ecp1*	*Klebsiella pneumoniae*	Clinical	Israel	81
	Escherichia coli	Clinical	Israel	81
	Enterobacter cloacae	Clinical	Israel	81
	Proteus mirabilis	Clinical	Israel	81
	Escherichia coli	Clinical	Tunisia	82
MTn				
Tn*21*-like	*Aeromonas salmonicida* subsp. *salmonicida*	Brown trout	France	93
	Escherichia coli	Dead chicks and turkey poults	China	92
	Salmonella enterica serovar Brandenburg	Clinical and foodborne	Spain	91
	Klebsiella oxytoca	Clinical	Spain	90
	Enterobacter cloacae	Clinical	Spain	90
Tn*5045*	*Pseudomonas aeruginosa*	Permafrost	Russia	96
Tn*6001*	*Pseudomonas aeruginosa*	Clinical	Taiwan	98
ICE				
SXT	*Vibrio cholerae*	Clinical	India, Sri Lanka, Bangladesh, Hong Kong	151
GI				
n.n.	*Pseudomonas aeruginosa*	Clinical	Australia	112
n.n.	*Shigella flexneri*	Primate	Japan	111
AbaR	*Acinetobacter baumannii*	Clinical	Australia	105
	Acinetobacter baumannii	Clinical	Belgium, Bulgaria, Czech Republic, Spain, Ireland, Italy, Netherlands, Poland, United Kingdom	107
SGI	*Salmonella enterica* serovar Haifa and Newport	Clinical	France	75
	Proteus mirabilis	Clinical	Palestine	110
Plasmid				
n.i.	*Enterococcus faecalis*	Clinical	United States of America	114
n.i.	*Salmonella enterica* serovar Typhimurium	Foodborne	Portugal	53
n.i.	*Acinetobacter baumannii*	Clinical	Taiwan	115

Abbreviations: n.n., without attributed name; n.i., without Inc group determined.

Table 1. Examples of mobile genetic elements (MGEs) associated with class 1 integrons (continued)

n.i.	*Aeromononas* sp.	Wastewater	Portugal	13
n.i.	*Escherichia coli*	Wastewater	Portugal	13
n.i.	*Enterobacter cloacae*	Clinical	Spain	90
n.i.	*Klebsiella oxytoca*	Clinical	Spain	90
n.i.	*Pseudomonas aeruginosa*	Clinical	Spain	90
IncHI2	*Enterobacter cloacae*	Clinical	Spain	90
IncI1	*Klebsiella pneumoniae*	Clinical	Spain	90
	Escherichia coli	Clinical	Spain	90

Abbreviations: n.n., without attributed name; n.i., without Inc group determined.

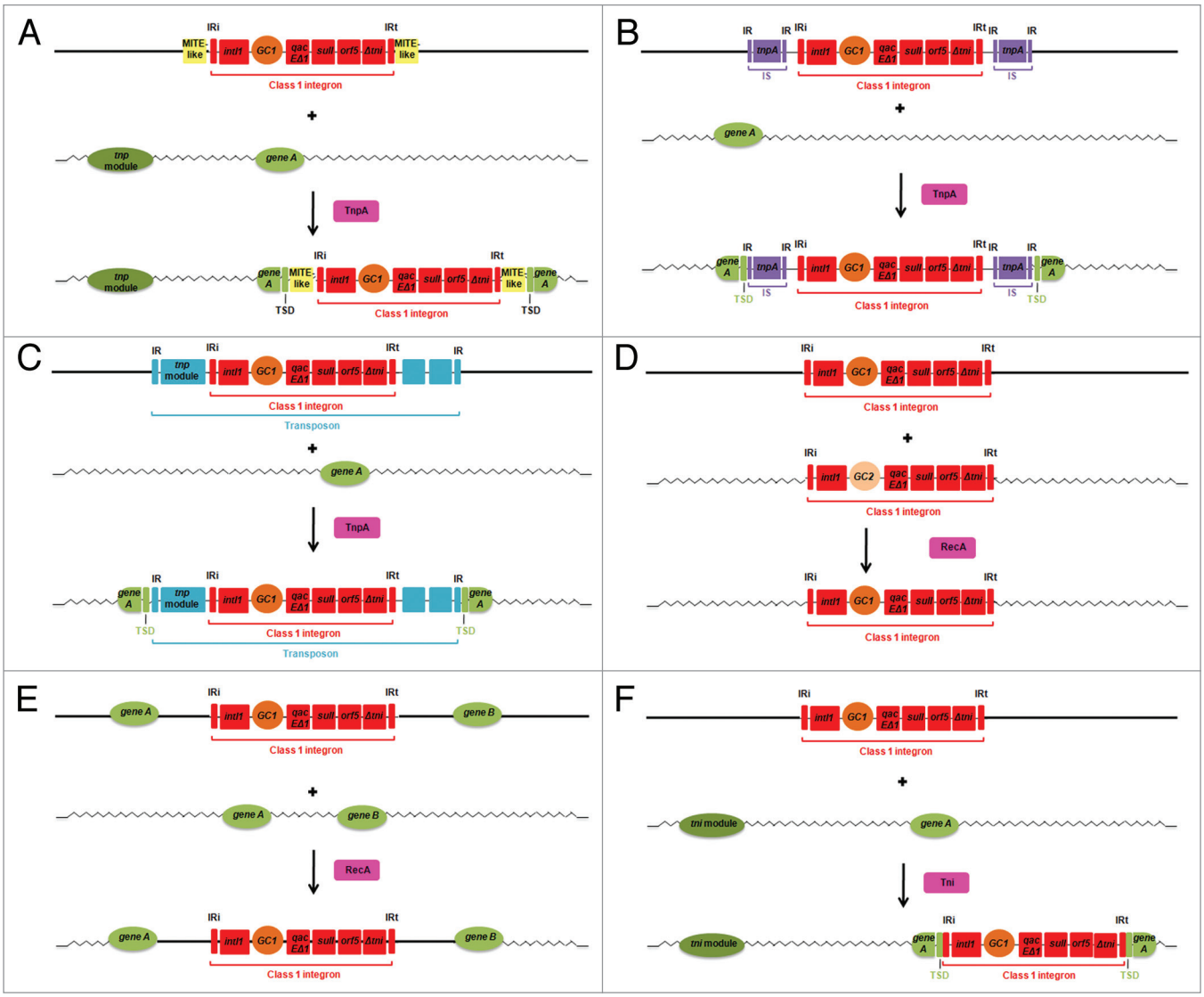

Figure 2. Representation of the possible mechanisms contributing to mobility of class 1 (C1) integrons (in red). (**A**) Transposition of C1 integrons due to linkage with MITEs and a transposase acting *in trans*; (**B**) Transposition of C1 integrons due to linkage with a composite transposon with IS elements; (**C**) Transposition of C1 integrons due to linkage with a transposon; (**D**) Exchange of gene cassettes by double homologous recombination between the conserved end regions of C1 integrons; (**E**) Integration of C1 integrons/gene cassettes by homologous recombination between two adjacent DNA regions in the chromosome; (**F**) Transposition-like movement of C1 integrons containing two Inverted Repeats and a *tni* module provided in trans. Abbreviations: IRi, inverted repeat at the 5'-CS outer end of the C1 integron; IRt, inverted repeat at the 3'-CS outer end of the C1 integron; IR, inverted repeat; *tnp* module, transposition module including the *tnpA*, *tnpR* and *tnpM* genes; TnpA, transposase enzyme; TSD, target site duplication; RecA, enzyme responsible for homologous recombination; *tni* module, transposition module including the *tniA*, *tniB*, *tniQ* and *tniR* genes; Tni, TniA, TniB and TniQ enzymes.

ISs are important for dissemination of antibiotic resistance genes. A DNA segment flanked on each side by the same IS is called a composite transposon, where at least one of the two IS copies must be intact to confer mobility.[38] Only a few reports of C1 integrons flanked on each side by an IS can be found in the literature and these include mainly elements of the IS*6* family.[19,74-76] In some cases C1 integrons flanked by ISs are incorporated into even larger composite transposons.[74,75]

The C1 integron In53, which carry an unusual number of gene cassettes (9), is located in the pNLT-1 plasmid of *Escherichia coli* and is flanked on each side by IS*26*; the IS*26*-composite transposon, named Tn*2000*, is flanked by TSDs, suggesting acquisition of this element by transposition.[19] The C1 integron carried by the p1658/97 plasmid of *Escherichia coli* is flanked by one complete and one incomplete IS*26*, followed by another intact IS*26*; the C1 integron is inserted in a 48 kb region flanked by different IS*26* and similar TSDs, suggesting the acquisition of the entire region by transposition.[76] One C1 integron of a *Salmonella enterica* serovar Newport isolate is flanked by one IS*26* and one IS*6100* and it is inserted in a larger IS*26*-composite transposon that was inserted into the genomic island SGI1 backbone by transposition, as suggested by the presence of TSDs.[75]

IS*CR*s (insertion sequence common regions) are an unusual group of IS that belongs to the IS*91* family and are characterized by the lack of the inverted repeats and by dissimilar ends, an *ori*IS and a *ter*IS. IS*CR* also transpose but by a mechanism different from other ISs. The mechanism is called rolling-circle (RC) and result in "one-ended transposition." IS*CR*s do not generate TSD, which makes it challenging to identify DNA moved by this IS in sequencing analysis. The presence of a single copy in the genome is sufficient to mobilize adjacent DNA.[38,39,77] IS*CR1* is always found in IS*CR1*-linked C1 integrons,[38,39] and have been reported in diverse bacterial species.[36,37,78-80] Another IS conferring "one-ended transposition" is IS*Ecp1*, which has been associated mainly with the dissemination of the bla_{CTX} genes, which encode extended-spectrum β-lactamase (ESBL) enzymes, linked with C1 integrons.[81,82]

Genetic linkage to Tns. Tns are distinguished on the basis of transferability: some are mobilisable and others conjugative. Mobilisable Tns (MTns) do not encode functions for self-transfer, but can hitchhike with other MGEs. Conjugative Tns, also designated ICEs, have the ability to excise from the chromosome where they are located and promote their own transfer to a new host, where they integrate.[83]

Transposition of MTns relies on the action of a transposase, usually part of the MTn, which recognizes the IRs of the MTn. The insertion of a MTn in a new location also generates TSDs. Transposition of Tn*402*, a C1 integron that is also an active transposon has been observed.[84,85] Another C1 integron containing a complete transposition module, named Tn*6007*, was detected in a human commensal strain of *Enterobacter cloacae*; Tn*6007* with a single base pair mutation in the *tniA* gene, present in a fosmid clone, was shown to transpose in the presence of another *tniA* gene in trans—TSDs flanked both ends of the Tn*6007* insert.[86]

With the exception of the Tn*402*[84,87] and a few Tn*402*-like transposons,[86,88] most C1 integrons are considered defective transposons, due to deletions present in DNA regions involved in transposition.[22,26] Nevertheless, many C1 integrons are present in other functional and mobilisable Tns. For instance, C1 integron In2 is incorporated in Tn*21*;[89] and various bacterial species carry Tn*21*-like transposons with different C1 integrons.[90-93] Transposition of Tn*21* and Tn*21*-like transposons has been widely observed, which contributes to the broad dissemination of C1 integrons.[71,94,95] There are also reports of other MTns carrying C1 integrons, such as Tn*5045*,[96] Tn*5086*[97] and Tn*6001*.[98] The TSD flanking the C1 integron InC* and the TSD flanking the integron-carrying transposon Tn*5045* suggest two transposition events in the generation and acquisition of this transposon by the chromosome of permafrost isolate *Pseudomonas* sp. Tik3.[96] Another example of mobility of C1 integrons due to the linkage with a MTn includes the experimentally observed transposition of a C1 integron-carrying transposon, Tn*5086*, carried by an *Escherichia coli* isolate.[97]

Unlike MTns, ICEs do not generate TSD when inserted in a new genome,[99] so HGT of these elements might be only inferred when comparing their nucleotide composition with the core genome. To date, there are no reports of C1 integrons carried in ICEs.

Genetic linkage to GIs. GIs are large chromosomal regions containing genes encoding a diverse range of functions and that display evidence of acquisition by HGT, such as a different GC content and codon usage from the core genome. When these islands encode resistance to antibiotics and heavy metals, they are denominated resistance islands.[100] Similar GIs are usually inserted in defined sites on the chromosome of different host chromosomes.[100,101] Most of the GIs are not self-mobile and transfer of GIs is supposed to occur by hitchhiking with other MGEs;[100,102] however, self-transfer of a few GIs has recently been demonstrated.[103]

Several resistance islands containing C1 integrons have been described in diverse strains of *Acinetobacter baumannii* (AbaR islands),[104-107] in several serovars of *Salmonella enterica*,[108,109] in *Proteus mirabilis*,[110] in *Shigella flexneri*[111] and in *Pseudomonas aeruginosa*.[112]

A recent study demonstrated the mobilization *in trans* of the SGI1 island, containing the C1 integron In104, from two strains of *Salmonella enterica* serovar Agona to *Escherichia coli* by conjugation; only plasmids from the IncA/C incompatibility group were able to mobilise this GI.[109]

Genetic linkage to plasmids and horizontal transfer of C1 integrons by conjugation. Plasmids are ubiquitous vectors for HGT and often carry a variety of antimicrobial resistance genes; they have been identified in most bacterial species investigated and carriage of multiple plasmids is also common.[18] Plasmids are termed conjugative, when they encode the functions needed for self-mobilization or mobilisable, when they rely on the help of other conjugative elements or functions to move between cells.[18] Plasmids are the main vector for antibiotic resistance dissemination, including those resistances coded by C1 integrons.[18,113] There are a high number of reports of plasmids carrying C1 integron in a wide range of bacterial species.[13,41,52,53,90,114,115] These reports do not usually report on the incompatibility (Inc) group of the plasmid,

which have important implications for the broader potential for dissemination of these elements. Plasmids that belong to the same Inc group utilize common mechanisms for replication or transfer and cannot co-exist in the same cell.[113] One of the few studies that determined the Inc group of integron-carrying plasmids was done by Tato and collaborators.[90] Plasmids belonging to the IncHI2 and IncI1 groups were detected in transconjugants, showing the capacity of these plasmids to horizontally move and disseminate C1 integrons.

Several other studies have reported on the transfer of C1 integrons by conjugation, due to the linkage with plasmids. These latter studies were done in several different species and genera.[57,116-118] For instance, Meng and colleagues[57] demonstrated a plasmid-mediated transfer of two C1 integrons, from *Salmonella enterica* serovar Derby and *Salmonella enterica* serovar Indiana strains, isolated from pork and chicken food, respectively, to *Escherichia coli* by conjugation. The conjugative plasmid transfer occurred at a frequency of 10^{-6} to 10^{-5}. In another study, clinical isolates of *Serratia marcescens* were shown to carry C1 integron-borne plasmids; three different plasmids and respective C1 integron, were transferred to *Escherichia coli* by conjugation.[117]

A possible limitation in the interpretation of some of these conjugation studies is that the description of the genomic location of C1 integrons is usually limited to the plasmid; without further information being provided on the immediate flanking regions of the resistance determinants such as flanking ISs or Tns. The latter will likely also be a determinant for the mobility and exact genetic location of C1 integrons.

Conjugation has recently been shown to induce the SOS system and consequently to induce the integrase activity of the *intI1* and *intIA* in integrons present in *Escherichia coli* and *Vibrio cholerae*, respectively, leading to recombination of gene cassettes.[7]

Horizontal transfer of C1 integrons by transduction. Viruses that infect bacteria, bacteriophages, are abundant forms of life and co-reside in environments with bacteria.[119] The DNA of bacteriophages can exist in the host cell either in an extrachromosomal form or integrated in the host DNA. Bacteriophages can, during transduction, transfer fragments of the host DNA to the new infected bacterial cell.[120] Therefore is expected that also DNA fragments with C1 integrons can be horizontally transferred to other cells by this mechanism. The amount of DNA that can be transferred during transduction depends on the size of the phage capsid, but can range from tens to hundreds of kbs.[121] The ability to transduce host DNA seems to be limited to relatively large double-stranded DNA phages.[120] Both chromosomal and plasmids are known to be transferrable by transduction.[122]

To our awareness there is only one report on the role of transduction as a mechanism of horizontal transfer of resistance genes embedded in C1 integrons; in *Salmonella enterica* serovar Typhimurium.[123] It should however be noted that retrospective studies might not be able to identify transduction as a causal mechanism for intercellular DNA transfer as there is no specific genetic signature for events of DNA acquisition by transduction or events of natural transformation.

Horizontal transfer of C1 integrons by natural transformation. Natural transformation is the uptake and stabilization of extracellular DNA by a competent cell.[3,124] Any fragment of DNA can be taken up over the bacterial membrane of competent cells, except in species with sequence dependent uptake.[125] However lack of DNA sequence similarity between recipient and donor DNA limits the probability of recombination-based integration of chromosomal DNA in the new host.[3] Uptake of self-replicating plasmids by natural transformation has the potential to disseminate linked C1 integrons.[57,126] The stable uptake of plasmid DNA by transformation is however less efficient than uptake of chromosomal DNA due to the complex steps necessary to reassemble a circular duplex molecule in the bacterial cytoplasm.[3] For example, Meng and collaborators[57] demonstrated the transfer of two integron-borne plasmids, one from a *Salmonella enterica* serovar Derby and one from a *Salmonella enterica* serovar Indiana strains, isolated from pork and chicken food, respectively, to the oral bacterium *Streptococcus mutans* UA159, via natural transformation; the transformation frequency was in the order of 10^{-6}–10^{-7}. Less is known about the transformation potential of integrons present in plasmids incompatible with the new host or when present in chromosomal locations. A recent study by Domingues et al.[71] showed that natural transformation can contribute to the uptake of C1 integrons, gene cassettes and transposons in an *Acinetobacter baylyi* strain. Three different mechanisms were found to be involved in the incorporation of C1 integrons in the chromosome of the recipient *Acinetobacter baylyi* BD413; transposition promoted by IS*26* (**Fig. 2B**) or Tn*21* (**Fig. 2C**) and homologous recombination (**Fig. 2D and E**). The acquisition of C1 integrons and transposons was shown to occur from related as well as unrelated host species. A previous study with synthetic circular cassettes and linear gene cassette arrays also demonstrated acquisition of gene cassettes by natural transformation in *Pseudomonas stutzeri*, which were integrated by site-specific recombination rather than by homologous or illegitimate recombination.[127]

Mobility of C1 integrons independent of genetic linkage to MGE. *Movement of C1 integrons supported by elements in trans.* Some authors suggest that C1 integrons can move independently under appropriate circumstances, such as in the presence of a complete transposition (*tni*) module provided *in trans* and the presence of the two inverted repeats IRi and IRt flanking the C1 integron (**Fig. 2F**).[26,128] However, only few studies have reported experimental evidence of horizontal transfer of C1 integrons without the genetic linkage to other MGEs. Transposon Tn*2521*, identified in clinical strains of *Pseudomonas aeruginosa*, has been shown to have the ability to transpose.[129] This transposon was however later described to be a C1 integron, renamed In*33* and lacking transposition genes.[130] Further analysis of the recipient revealed the presence of a 5 bp direct TSD, which confirmed the transfer of In*33* by transposition. It was suggested that the transposition event was supported by *tni* genes from another transposon present in the same cell, although this was not shown.[130] In another study, experimental transposition of C1 integrons lacking a transposition module, In0 and In2, was observed when assisted by *tni* genes from transposon Tn*502* provided in trans. Transposition was further confirmed by the presence of a 5 bp direct TSD flanking the C1 integrons in their new location.[131]

Movement of C1 integrons and gene cassettes by homologous recombination. As the conserved regions of C1 integrons provide sufficient stretches of DNA similarity for recombinational exchange between two C1 integron-containing bacteria, homologous recombination has been suggested to contribute to the replacement of the gene cassette arrays in C1 integrons.[5] For example, such recombination could explain the loss of the gene cassette array of In3 when Tn*1405* was acquired by the In3-containing R388 plasmid.[130] The role of homologous recombination in the replacement of gene cassettes (**Fig. 2D**) and in the acquisition of C1 integrons (**Fig. 2E**) in new locations has been recently demonstrated.[71] Although it has only been experimentally shown to occur after natural transformation, it is expected that stable integration of C1 integrons by homologous recombination also can occur after the uptake of DNA by conjugation or transduction.[113]

Limitations to the dissemination of C1 integrons. Genetic linkage to one or more MGEs enables C1 integrons to transfer horizontally within and between bacterial populations and species. The limiting factors to dissemination of C1 integrons will therefore in most cases be similar to those governing the dissemination of the MGE they are linked to. Typically, transfer by conjugation will be limited by mechanistic aspects of the conjugal machinery, including surface receptors of the recipient cell[18] as well as compatibility of the plasmid with the new host cytoplasm and its restriction modification system.[3] For transduction, key limiting factors are the host range of the transducing bacteriophages as well as the host restriction modification system.[132] Moreover, not all bacterial species are known to undergo transduction. The host range of bacteriophages depends on the specificity of the interaction between the bacteriophage and bacterial receptor sites.[1,132] For natural transformation, key limiting factors are the ability to develop competence for DNA uptake as well as the ability of incoming DNA to recombine with the genome of the transformed cell.[3] In order to be vertically inherited, non-replicative DNA with C1 integrons transferred, taken up or injected into the bacterial cytoplasm must be able to recombine with the host genome. Such recombinational barriers are the same as those preventing HGT of any chromosomal DNA between the genomes of non-related bacteria.[3,120]

A general limiting factor, beyond the mechanistic aspects defining the probability of transfer to new hosts, is the likelihood that the acquired MGE will become vertically inherited in the larger bacterial population. It is known that initially rare events of HGT are unlikely to establish in a larger bacterial population if they do not confer a benefit to the host.[133,134]

Thus, in cases where the acquisition of C1 integron-carrying MGE will negatively affect the relative or absolute growth rate of the new host, rare HGT events will be lost from the larger population as they will be outgrown by more fit members of the population. At high transfer rates, a temporal equilibrium of C1 integron-carrying MGEs in the population can be established when the frequency of new successful HGT events equals the loss of non-fit members.[135] However, several studies have shown that both the MGEs and the bacterial host can rapidly co-adapt; so that the initial high fitness costs to the host can, within weeks, be reduced or lost. Eventually, the MGE carrying hosts can be equally or more fit than the remaining bacterial population.[135-137] This amelioration of fitness costs is due to compensatory mutations.[138,139]

In cases where the C1 integron-carrying MGE does not result in an initial fitness change in the new host,[140] the frequency of successful transfer and random fluctuations among genotypes in the bacterial population (genetic drift) will determine the short-term-fate of the new C1 integron carrying host cells. C1 integrons present in such cells are not expected to remain in the bacterial population over evolutionary time in the absence of purifying selection, as spontaneous deletions will occur. However, in cases where co-adaptation takes place between the MGE/C1 integrons and the host genome, more complex population trajectories can be expected.[141,142] Whereas the bacterial mutation rate is well understood,[143] less is known about the various mechanisms leading to deletions of acquired DNA segments in the bacterial genome.

In cases where the C1 integron-containing MGE confers a growth advantage to the new host, even very few HGT events can be sufficient for establishing the C1 integron in the new host.[140] For instance, the use of antimicrobial drugs in treatment of bacterial infections can effectively remove drug sensitive cells, so that even exceedingly low initial proportions of C1 integron-containing cells can rapidly expand their population sizes to become the dominant one. Dominant populations of resistance-carrying bacterial pathogens are a major public health issue.

Prohibiting the use of antibiotics is often considered as a strategy to reduce antimicrobial resistance, with the assumption that resistance genes impose a fitness cost to the host and the absence of the antibiotic pressure will lead to their loss. However, reversing resistance development is challenging,[144,145] particularly because not all resistance genes confer initial or continual costs to their hosts.[146,147] A recent study by Starikova and colleagues shows that this also applies to C1 integrons; although a C1 integron introduced in *Acinetobacter baylyi* by natural transformation conferred an initial fitness cost of 7%, the experimental evolution assay showed that the fitness of the transformant was completely restored.[152]

Other selection pressures on the MGE carrying host population can in some cases allow the maintenance of genes with negative host effects; selection on one resistance gene/trait will contribute to maintain adjacent and linked genes, even when those genes provide no beneficial effect to the host.[148,149] This means that C1 integrons conferring a fitness cost to the host can be maintained when carried, for example, on a plasmid that confers other selective advantages to the host.

General Considerations and Summary

C1 integrons are usually found linked to various types of MGEs. C1 integrons will therefore move within and between bacterial genomes as part of the MGE(s) they reside in;[150] a process called hitchhiking. Although C1 integrons embedded in transposons, IS or other replicating genetic elements are considered to have intragenomic mobility only, such elements can be transferred

between cells when part of plasmids during conjugation or through direct DNA transfer by transduction or natural transformation. The conserved regions of C1 integrons also provide sufficient DNA sequence similarity for homologous recombination with the host genome to occur. Such recombination can exchange the gene cassettes present in the recombining parts of the C1 integrons also when present in unrelated bacterial species.[71]

The potential for dissemination of C1 integrons should be seen as product of the factors governing the linkage of C1 integrons to MGEs, the supply of MGEs into a given bacterial population, their transfer potential and heritable stability in new bacterial hosts and the larger opportunity for MGE carrying hosts to co-exist with or outcompete non-carrying members of the larger population. Thus, considerations of genetic linkage, dissemination rates and absolute and relative host fitness effects are essential to understand the fate of C1 integrons. It is further important to recognize that fitness will be a variable depending on both host and environmental factors; including continual changes in the genetic composition of the host and C1 integron-containing MGE. Further complicating factors in the understanding of dissemination potential are bacterial population structure and transmission dynamics, spatial and temporal variability in transfer rates (exposure dependency), variability in directional selection (e.g., temporal antibiotic usage) and randomness in most processes including host survival and dissemination; leading to non-uniform outcomes.

Most environmental factors can be controlled in experimental laboratory model systems providing an opportunity to conduct quantitative descriptions of C1 integrons and their dissemination characteristics. However, in clinical and other environments, variability and random fluctuations in the governing factors considered above rarely leads to opportunities for a precise quantitative understanding of the transfer dynamics of C1 integrons and the factors that determine current dissemination patterns.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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