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Opinion

The Role of Integrative and Conjugative Elements in Antibiotic Resistance Evolution

João Botelho[1,2,*] and Hinrich Schulenburg[1,2]

Mobile genetic elements (MGEs), such as plasmids and integrative and conjugative elements (ICEs), are main drivers for the spread of antibiotic resistance (AR). Coevolution between bacteria and plasmids shapes the transfer and stability of plasmids across bacteria. Although ICEs outnumber conjugative plasmids, the dynamics of ICE–bacterium coevolution, ICE transfer rates, and fitness costs are as yet largely unexplored. Conjugative plasmids and ICEs are both transferred by type IV secretion systems, but ICEs are typically immune to segregational loss, suggesting that the evolution of ICE–bacterium associations varies from that of plasmid–bacterium associations. Considering the high abundance of ICEs among bacteria, ICE–bacterium dynamics represent a promising challenge for future research that will enhance our understanding of AR spread in human pathogens.

Highlights

Integrative and conjugative elements (ICEs) and plasmids can both promote the spread of antibiotic resistance (AR), but they vary in important characteristics, including transmission dynamics and, most likely, fitness costs and their compensation.

ICEs outnumber conjugative plasmids, suggesting an important role during bacterial evolution, yet they still have been largely overlooked as vectors of AR.

Overall, ICE–bacterium coevolution appears to vary from plasmid–bacterium coevolution.

ICE–bacterium dynamics thus represents a promising focus for future research on bacterial evolution and AR spread.

Antibiotic resistance and the Importance of Horizontal Gene Transfer

Antibiotics are powerful medicines used not only for the direct treatment of bacterial infections but also as prophylactics during cancer chemotherapy and surgery. The inappropriate use of these drugs has promoted a widespread rise in antibiotic resistance (AR) in bacteria [1–4]. The emergence of AR is driven by two processes: (i) chromosomal mutations altering the cellular targets of antibiotics or decreasing intracellular antibiotic concentrations, and (ii) **horizontal gene transfer (HGT)** (see Glossary) of AR genes encoded on mobile genetic elements (MGEs), mostly **plasmids** and **integrative and conjugative elements (ICEs)** (Box 1) [5–8].

MGEs include a large array of elements that mediate the mobility of DNA chunks, either intracellularly (e.g., **transposons**) or between cells. Intercellular mobility can be achieved through transformation (i.e., the uptake of extracellular DNA), transduction (promoted by bacteriophages), or **conjugation** [7,8]. The latter mechanism of genetic exchange frequently involves plasmids or ICEs. The concerted activities of MGEs play a vital role in promoting the HGT of beneficial traits, such as genes encoding AR [7,8]. These genes are frequently stockpiled in genetic entities called **integrons**, which can be transferred intracellularly and/or intercellularly with the help of transposons, plasmids, or ICEs. These elements are often arranged at multiple nested levels, similar, in principle, to Matryoshka dolls [9].

Several studies have explored plasmid–bacterium **coevolution** [10–12], as well as the link between conjugative plasmids and the spread of AR genes in multiple bacterial families (e.g., Enterobacteriaceae) [7]. Less than 20 years ago, the potential of ICEs (popularly known as conjugative transposons up to the last decade [13]) to shape bacterial evolution began to attract more attention mostly due to the advent of whole-genome sequencing (WGS) and the resulting improvements in reconstructing HGT events [14]. However, the role of ICEs as vectors for the spread of AR is largely unexplored (Box 1) [7,8]. Our opinion article puts the spotlight on these neglected elements and their importance for the dissemination of AR genes.

[1]Antibiotic Resistance Evolution Group, Max-Planck-Institute for Evolutionary Biology, Plön, Germany
[2]Department of Evolutionary Ecology and Genetics, Zoological Institute, Christian-Albrechts University, Kiel, Germany

*Correspondence: botelho@evolbio.mpg.de (J. Botelho).

Box 1. ICEs as Drivers of AR

Together with plasmids, ICEs have been recognized as key vectors for the spread of AR genes in Proteobacteria and Firmicutes [7,83]. The highly abundant MPF_T class [25] of conjugative plasmids carries more AR genes, and exchange genes, more frequently than do ICEs of the same class [27]. Still, AR genes are common within ICEs, as highlighted by the examples below. In *Pseudomonas aeruginosa*, ICEs often carry cassette-borne AR genes, including genes conferring resistance to carbapenems; they occur in class I integrons [8,18,84] or are flanked by insertion sequences [85,86]. The large ICE SXT/R391 family disseminates AR genes between many enteric pathogens, such as *V. cholerae* [49,87] and *Proteus* spp. [88]. In Firmicutes, and specifically in *Streptococcus*, *Staphylococcus*, and *Enterococcus*, Tn*916*-like ICEs harbor genes encoding resistance to tetracycline and vancomycin [89,90]. ICE-*emm*12, encoding genes for tetracycline and macrolide resistance, were linked to the emergence of scarlet fever *Streptococcus pyogenes* clones in Hong Kong [91]. Also in *S. pyogenes*, a macrolide resistance *erm*(TR)-carrying ICE was identified in outbreak isolates in New Zealand [92]. The acquisition of *tet*(M)-harboring ICE led to the expansion of tetracycline-resistant *Streptococcus agalactiae* clones and an increase in neonatal infections [93]. Interestingly, the *tet*(W) tetracycline-resistance gene was identified within ICEs in several ruminal bacterial genomes [74]. Recently, the oxazolidinone/phenicol resistance gene *optrA* was identified within an ICE*Sa*2603-family ICE in *Streptococcus suis* [94]. More examples have been covered in excellent reviews of ICEs elsewhere [17,22].

ICEs are widespread mobile units carrying modules responsible for the excision, maintenance, conjugative transfer, and integration within the new host genome [15]. As the name implies, these elements are transmitted both vertically to daughter cells (in an integrative state) and horizontally through excision and transfer to other cells (in a conjugative state) [16]. ICEs integrate in the chromosome by site-specific recombination between direct repeats located in the host and the ICE, a reaction mediated by an integrase. The integrase is also involved in excision and the formation of the circular intermediate that will be available for conjugative transfer [15,17]. The horizontal transfer of these elements can result in abrupt changes in niche preferences. ICEs may cargo genes that are not linked to their life cycle, such as AR genes [7,18]. Even though our focus is on the role of these elements in the spread of AR, ICEs can also contain genes for a variety of other functions, such as virulence-associated genes (e.g., the yersiniabactin-encoding ICE*Kp* in *Klebsiella pneumoniae* populations [19] and the pathogenicity islands found in *Pseudomonas aeruginosa* [20]) and symbiosis genes, as reported for the unique group of ICEs identified in *Mesorhizobium* spp. [21]. More examples have been covered in excellent reviews of ICEs elsewhere [17,22].

ICE excision and subsequent transfer promotes the horizontal spread of the ICE in the bacterial population [23,24]. Even though ICEs are widely distributed in bacterial genomes, and outnumber conjugative plasmids [25], by comparison with plasmid studies we still have only little knowledge of their exact evolutionary dynamics and their adaptation to bacterial hosts. Several studies have focused on the evolutionary dynamics in plasmid–bacterium coevolution. This discrepancy, when compared with ICE–bacterium dynamics (Table 1), may be explained by the fact that plasmids are easier to manipulate experimentally and the study of ICEs has gained momentum only recently, due to the advent of WGS. The comparatively large number of studies focusing on MGE evolution, highlighted in Table 1, may be misleading as these are not necessarily analyzing their evolutionary dynamics, and their specific adaptation to the bacterial host, but rather some aspect of the evolution of bacterial host cells. Importantly, the inferred numbers highlight the huge disproportion observed in studies focusing on plasmids and ICEs or conjugative transposons.

Table 1. PubMed Search Performed on the 15 April 2020, Including 'Plasmid', 'Integrative Conjugative Element', or the Former Designation 'Conjugative Transposon' and a Combination of Relevant Keywords

	Evolution	Antibiotic resistance	Fitness	Compensatory
Plasmid	7550	18 720	738	220
Integrative conjugative element	278	403	36	3
Conjugative transposon	441	1343	54	4

Glossary

Bet-hedging strategy: a strategy for maximizing the geometric mean (and thus long-term) fitness across different environmental conditions at the cost of suboptimal fitness in individual environments.
Coevolution: a process involving reciprocal adaptive changes between two or more genetic entities.
Compensatory mutation: secondary-site mutations that ameliorate the fitness cost of beneficial mutations, such as those encoding antibiotic resistance.
Conjugation: contact-dependent transfer of genetic material between cells through a type IV secretion system.
Fitness cost: the trade-off observed when a mutation/gene leads to a selective advantage in one fitness-associated trait (e.g., antibiotic resistance) yet simultaneously a disadvantage in another fitness-associated trait (e.g., reduced growth rate).
Horizontal gene transfer (HGT): also called lateral gene transfer, HGT involves the movement of genetic material between genomes.
Integrative and conjugative element (ICE): previously known as conjugative transposons, ICEs are chromosomally integrated mobile elements that can be transferred horizontally between cells by conjugation.
Integron: a genetic element that stockpiles and shuffles gene cassettes through site-specific recombination.
Plasmid: an autonomous self-replicating extrachromosomal element that can be transferred horizontally between cells by conjugation.
Transposons: also called jumping genes, transposons are intracellular mobile elements that can 'jump' to different regions of the genome.
Tripartite ICE: when integrated in the host, this ICE exists as three separate chromosomal regions that recombine to form a single region before excision and conjugative transfer.

We argue that there are three main reasons for studying the evolution of ICE-mediated AR in bacteria: (i) AR represents one of the most concerning threats to hospitalized patients; (ii) AR is often encoded on ICEs, and (iii) ICE–bacterium coevolution may result in outcomes that differ from those documented for plasmid–bacterium associations. Here, we explore the different lifestyles of ICEs and conjugative plasmids, and we present evidence supporting our hypothesis that the evolution of ICE–bacterium associations differs from that of plasmid–bacterium associations. We also propose an integrative approach based on experimental and computational methods to study ICE–bacterium coevolution. In this opinion article we focus on the role of ICEs in the spread of AR and the associated evolutionary dynamics by comparison with conjugative plasmids. It is important to emphasize that ICEs (as plasmids) may similarly contribute to the dissemination of virulence genes and other genes beneficial for the host cells [17,22].

ICE Biology

ICEs contribute to genome plasticity and acquisition of novel traits, such as AR, pathogenicity, and metabolism [14,15]. Like plasmids and many other MGEs, ICEs owe their evolutionary success in part to these adaptive phenotypes. Phylogenetic analyses of type IV secretion system (T4SS) suggested that ICEs and conjugative plasmids have exchanged conjugation modules along their evolutionary history, whereby T4SS associated with the two elements are only distinguishable at short evolutionary distances [25]. These analyses allowed the classification of the secretion machinery involved in bacterial conjugation into eight mating-pair formation (MPF) classes [25,26]. Many important aspects of ICE biology are unknown, such as the burden that its acquisition imposes on the new host and the traits involved in ICE–bacterium coevolution (Table 1). At the same time, certain properties have been assessed, as exemplified in the following, generally highlighting that ICEs are distinct from plasmids in their characteristics and evolutionary dynamics: (i) ICEs combine features of transposons and prophages since they can integrate into and excise from the chromosome using tyrosine/serine recombinases or DDE transposases [15]; (ii) ICEs are more frequently transferred between distant taxa than are conjugative plasmids [27]; and (iii) ICEs have a dual lifestyle including both vertical and horizontal transmission and are

Table 2. Specific Features Typically Associated with ICEs and Conjugative Plasmids

	ICEs	Conjugative plasmids
What separates them		
Location	Integrated in the chromosome[a]	Extrachromosomal
Signature modules[b]	Integration/excision	Replication
GC content (by comparison with that of the host genome)[c]	Closer	More distinct
Size[c]	Less variable	More variable
Density of DNA repeats[c]	Lower	Higher
What brings them together		
Type of mobility	Intercellular	
Mobility mechanism	Conjugation	
Shared modules	Maintenance, conjugative transfer	

[a]ICEs can also exist as circular extrachromosomal elements, formed upon excision and transfer to a new host.
[b]Even though the integration/excision module is classically associated with ICEs and the replication module with plasmids, ICEs may carry genes coding for replicases, while some plasmids may also carry genes encoding integrases [27].
[c]Data retrieved from the comparison between conjugative plasmids and ICEs belonging to a specific mating-pair formation class, the MPF_T [27].

typically immune to segregational loss, whereas plasmids are affected by segregation during cell division (Table 2) [16,28,29]. These differences can translate into different coevolutionary paths.

Upon transfer to a new recipient cell, the ICE can integrate into the chromosome or can potentially evolve to become an extrachromosomal element (Figure 1, Key Figure). Integration can occur at a single attachment site or can be random (Table 3) [30,31]. ICEs can share the same integration site in the genome [32,33], potentially leading to strong competition for the limited integration sites among different coinfecting ICEs and inducing high levels of selection for an ICE's competitive ability. Because plasmids use a very small proportion of cellular resources [34], competition between coresident plasmids is expected to be weaker.

Key Figure

Evolutionary Dynamics of Conjugative Plasmid–Bacterium (A) and ICE–Bacterium (B) Associations

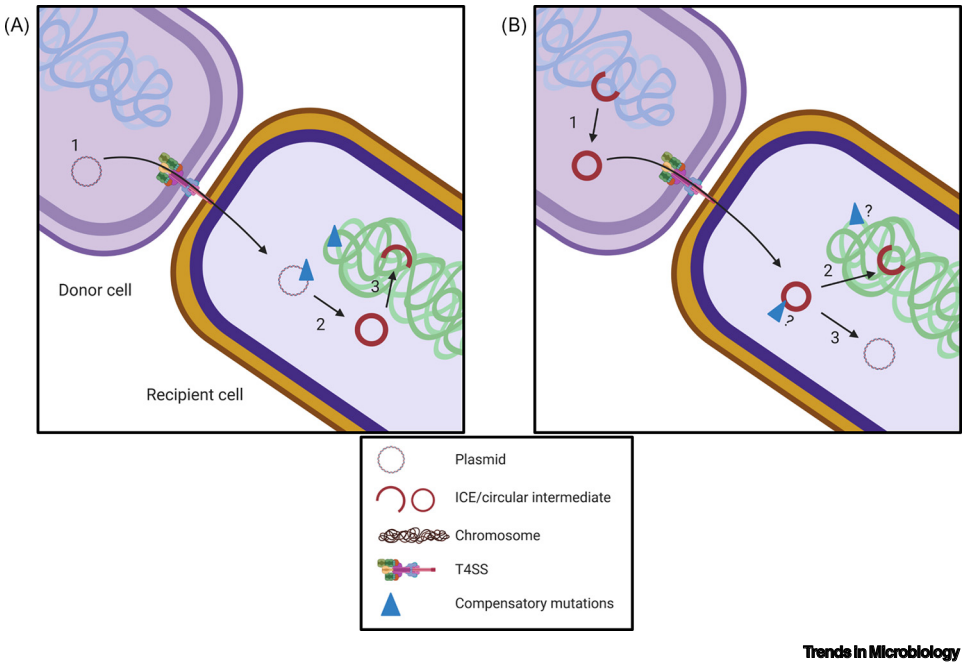

Figure 1. (A) A plasmid is transferred to recipient cells by a conjugative T4SS (1) and may impose a fitness cost upon arrival in a new host. Compensatory mutations may arise in the plasmid, the host, or both (blue triangles) to circumvent the burden of carrying a new plasmid. Plasmid–bacterium coevolution enables plasmid fixation with or without positive selection. Exclusion systems may prevent the acquisition of the new plasmid. Under certain conditions, plasmids may evolve to become an ICE (2), for example by acquisition of a module responsible for integration/excision, after recombination with a coresident MGE, and integrate into the chromosome of the new host (3). (B) An ICE is excised from the donor chromosome and forms a circular intermediate (1). This circular intermediate is then transferred to a new host by conjugative T4SS. The integration of an ICE within the chromosome of a new host may impose a burden on the cell. However, currently there are no studies assessing the strategies used to minimize or even eliminate the cost of ICE carriage and the putative emergence of compensatory mutations in ICEs, in the host chromosome, or in both (represented by question marks next to the blue triangles). As for conjugative plasmids, exclusion systems may block the acquisition of the new ICE. It is still unknown which selective pressures promote the integration of the circular intermediate (2). It is possible that this intermediate is maintained as an extrachromosomal element (3) that will then evolve to become a plasmid (for example, by acquisition of a replication module after recombination with a coresident MGE). The figure was created with BioRender. Abbreviations: ICE, integrative and conjugative element; MGE, mobile genetic element; T4SS, type IV secretion system.

Table 3. Summary of Features Observed in Model ICE Families

ICE family prototypes	Originally described in	Common hotspot for integration	Experimentally determined systems[a]	Transfer rate (per donor)	Number of experimentally tested family members[c]	Refs
SXT/R391	*Vibrio cholerae*	Into the 5′ end of a *prfC* gene	Eex, Par, Rep, Rmo, T/At	1×10^{-4}	81	[42,95–99]
Tn*916*	*Enterococcus faecalis*	Many different chromosomal regions	Rep	10^{-4} to 10^{-7}	58	[100–102]
ICE*clc*	*Pseudomonas knackmussii*	Into the 3′ end of a $tRNA^{Gly}$ gene	Par, Rep	1×10^{-2}	9	[43,103–105]
ICE*St1*/ICE*St3*	*Streptococcus thermophilus*	Into the 3′ end of a *fda* gene	Rmo	3.4×10^{-6}	2	[106–108]
ICE*Bs1*	*Bacillus subtilis*	Into the 3′ end of a $tRNA^{Leu}$ gene	Eex, Rep	1×10^{-2}[b]	1	[48,109,110]

[a]Eex, entry exclusion; Par, partition; Rep, replication; Rmo, restriction–modification; T/At, toxin–antitoxin.
[b]Transfer rate drops by about 50-fold when the ICE is transferred into recipient cells that already contain ICE*Bs1* [48].
[c]Data retrieved from ICEberg, accessed on the 15 April 2020[i].

Once integrated into the host chromosome, ICEs are replicated as part of it, likely making ICEs more stably maintained than plasmids in bacterial lineages, even though their stability still awaits a critical and comparative analysis. ICEs can be excised and transferred to other cells, either stochastically or under specific conditions (e.g., in the presence of recipient cells lacking SXT/R391 family ICEs [35] or host cell damage leading to ICE*clc* induction [36]). A single DNA strand is transferred to the new host, where a DNA polymerase regenerates the double-stranded element. A copy of the ICEs is maintained in the donor cell, and a recombination event may reintegrate the ICE into the chromosome. Detecting replicating intermediates in population-based assays is challenging, as the observed small fraction of ICE*clc* cells induced for transfer [37]. Besides ICE*clc*, replication was also observed for SXT/R391 and ICE*Bs1* [38–40], and this guarantees stability and propagation in the dividing cell lines. Partition systems are widespread in bacteria and their role in stable inheritance of plasmids has been extensively studied [41]. These systems were reported in SXT/R391 and in the ICE*clc* family of ICEs and they help to reduce the loss of excised ICEs [42,43]. Toxin–antitoxin and restriction–modification systems, encoded within ICEs, can trigger postsegregational killing of daughter cells that have lost the respective system, thereby eliminating possible competitors [44] and enhancing ICE maintenance. Superinfection exclusion systems were identified in SXT/R391 family ICEs; they prevent redundant transfers, promoting recombination between ICEs of different exclusion groups and the formation of tandem arrays [35].

Two model elements have been used to study transfer events: ICE*clc* and ICE*Bs1*, identified in *Pseudomonas knackmussii* and *Bacillus subtilis*, respectively. The former is prevalent in Proteobacteria (a list of strains carrying ICEs belonging to the ICE*clc* family can be browsed online[ii] [45]) and its transfer occurs only at low frequencies during the stationary phase from specialized transfer-competent cells and requires the addition of fresh nutrients [23,40]. Transfer-competent cells are characterized by reduced cell division or growth. Recently, a four-step regulatory cascade was proposed to activate ICE*clc* transfer competence in *Pseudomonas* [46]. The second model ICE, ICE*Bs1*, is widespread in Gram-positive bacteria and its transfer can be stimulated by the presence of recipient cells lacking the ICE. As seen for many ICEs (Table 3), ICE*Bs1* is site-specific and tends to integrate into a single attachment site in the chromosome. Integration of this ICE into alternative attachment sites can be detrimental to both ICE*Bs1* and the host and leads to reduced or absent excision and also low cell proliferation and viability [47]. Conditions that induce DNA damage of the host cell can also trigger ICE*Bs1* transfer and SXT-related ICEs from *Vibrio cholerae* [48,49].

The Missing Information on ICE–Bacterium Evolutionary Dynamics

ICEs and plasmids can be regarded as biological entities with ecologies and evolutionary trajectories relatively independent from their host cells [50]. Unlike plasmids, the genes and trait functions involved in ICE adaptation to their host cells – and how bacteria respond to the acquisition of foreign DNA – are currently unknown. Also, a direct comparison between the cost of carriage of ICEs and conjugative plasmids encoding similar traits is currently missing. However, theory predicts that ICEs should impose lower **fitness costs** than conjugative plasmids. In detail, as the mobility of MGEs increases, they should become increasingly costly for two main reasons. First, the increased capacity for HGT observed in conjugative plasmids, when compared with ICEs [51], should impose direct energy costs for expressing the conjugation genes. Second, mobile elements with a high rate of HGT move between multiple hosts, and the acquisition of these elements introduces foreign DNA (the frequency of gene exchange is expected to be higher in plasmids [27]) into the genome of a bacterium that tends to be deleterious [51,52].

The evolutionary dynamics between plasmids and bacteria dictate the evolution of AR in many species [53–58]. These dynamics represent a simple case of adaptation through natural selection. Plasmids may cause a fitness cost when they arrive in a new bacterial host, but these costs are eased over time by **compensatory mutations** during plasmid–bacterium coevolution [55,59–62]. Importantly, these dynamics of cost and compensation shape the evolution of plasmid-mediated resistance, producing successful combinations of AR plasmids and clinical bacteria, which thrive in clinical settings [53]. ICE's bistable lifestyle (as explained below) may also impact the fitness effects of these elements and the evolution of ICE-mediated AR. On the one hand, when ICEs are in the OFF or basal state, transfer genes are not expressed and the elements are stably maintained while integrated in the chromosome. The ICEs are not lost during cell division (segregational loss, which affects plasmids), ensuring vertical transmission. Moreover, ICEs tend to downregulate their core functions (but not their potentially beneficial accessory genes) [16], which may partially alleviate the fitness cost imposed on the host. On the other hand, when ICEs are in the ON state, for example, under certain external triggers, the expression of conjugation genes can be activated in a small subpopulation of transfer-competent, usually slowly growing cells (as a **bet-hedging strategy** to minimize the metabolic burden for the total population, while preserving the capacity to transfer DNA) [16,63]. Taken together, these characteristics strongly suggest that the evolution of ICE–bacterium associations may not be completely analogous to plasmid–bacterium coevolution.

Despite the great relevance of ICEs to the evolution of AR, there is little information available on the fitness effects of ICEs, and none related to the presence/absence of compensatory mutations (either in the chromosome and/or in the ICEs) that may ameliorate the cost of carriage (Figure 1). In the presence of tetracycline, a *tet*(M)-carrying ICE from *Enterococcus faecalis* was beneficial to the host, but in the absence of selection these ICEs tend to reduce host growth [64]. Similar results were observed for the integration of a multiple AR gene-carrying ICE named ICE*Mh1*^{PM22} in *Pasteurella multocida* and *Mannheimia haemolytica* [65]. Interestingly, the latter ICE was retained in transconjugants following extended passage without antibiotic selection. Transfer of ICE*clc* to *Pseudomonas putida* improved fitness on 3-chlorobenzoate (exclusively metabolizable due to the ICE), but impairs fitness on other carbon substrates [66]. ICE*clc* transfer to *P. aeruginosa* PAO1 did not cause significant fitness reductions in the bacteria [67], which might explain the broad distribution of this family among Proteobacteria. A copper resistance-encoding ICE did not incur any measurable fitness costs in *Pseudomonas syringae* pv. *actinidiae*, even in the absence of copper [68]. Thus, AR genes and genes encoding other beneficial traits carried on ICEs and plasmids are likely to persist in bacterial populations even in the absence of selective pressure.

New Approaches to Dissect ICE–Bacterium Coevolution and AR Spread

We propose an integrative approach to help improve our understanding of different aspects of ICE evolution and biology: competition experiments followed by experimental evolution, genomics, and transcriptomics (Figure 2). We also briefly discuss the importance of studying ICEs in relevant reservoirs and the need for better bioinformatics resources to trace the presence of these elements.

The evolutionary dynamics of ICE–bacterium associations can be assessed by experimental evolution [69]. Mating experiments should be conducted to allow ICE conjugation from wild-type strains to ICE-free recipient strains. Using different markers for donors and transconjugants allows us to distinguish them in the laboratory and to track the transfer of the ICE into ICE-free

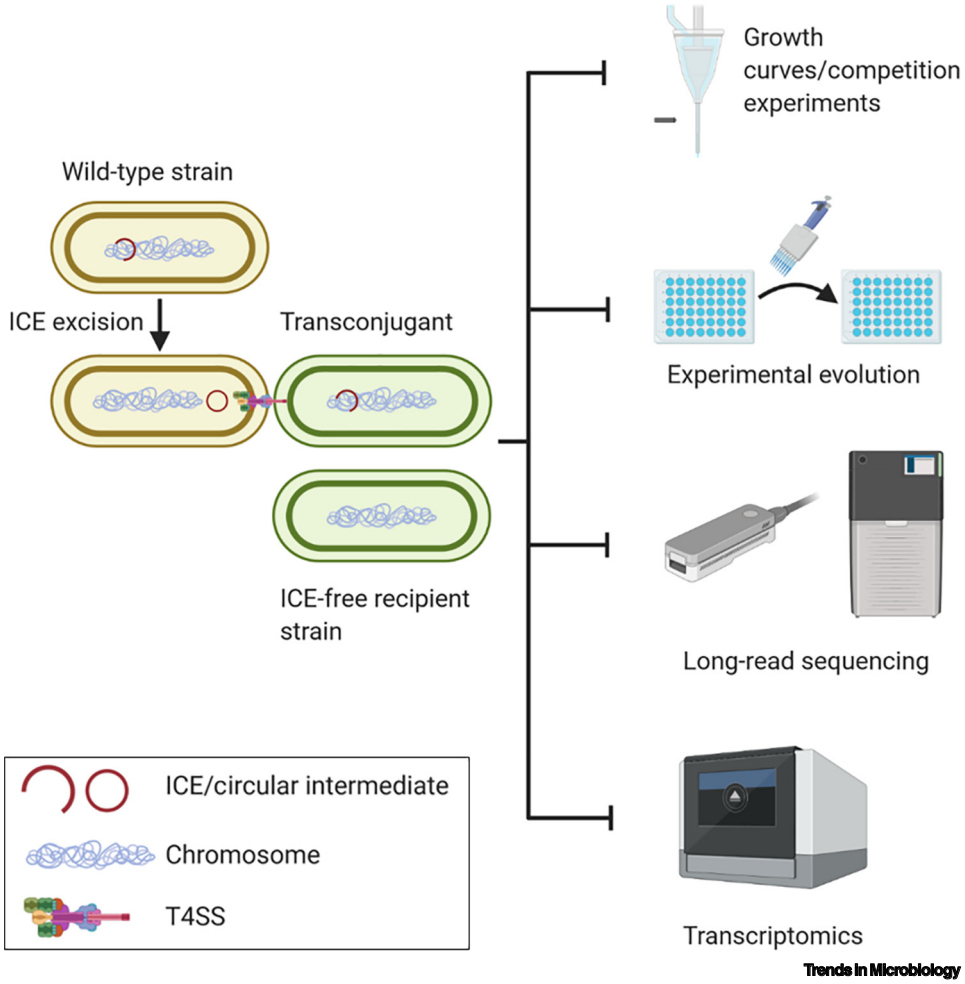

Figure 2. Proposed strategies for Studying ICE–Bacterium Coevolution. Growth curves and competition experiments between transconjugants and ICE-free recipient strains using high-throughput flow cytometry should be used to estimate the fitness cost of ICE carriage. Experimental evolution will then follow to characterize the putative emergence of compensation mechanisms associated with ICE carriage. These mechanisms, as well as the ICE structure and integration site, can be traced in evolved populations by WGS. RNA sequencing of naïve and compensated transconjugants is likely to help in exploring the global transcription profile of ICE-carrying recipient strains and studying the nature of compensatory adaptation. The figure was created with BioRender. Abbreviations: ICE, integrative and conjugative elements; T4SS, type IV secretory system; WGS, whole-genome sequencing.

cells. To estimate the fitness cost of ICE carriage, growth curves and competition experiments between the different transconjugants and ICE-free recipient strains should be performed. Experimental evolution of transconjugants and ICE-free recipient strains, followed by fitness measurements, should allow identification of the putative emergence of compensatory mechanisms associated with ICE carriage. Compensatory mutations can be traced in evolved populations by WGS of naïve and compensated ICE-carrying recipient strains. Using transcriptomics helps to explore changes in the global transcriptional profile of the recipient host as a consequence of harboring the ICE and thus to understand the nature of compensatory adaptation [34,70,71]. This integrative approach, combining fitness assays, experimental evolution, genomics, and transcriptomics, would allow the identification of compensatory mutations and differentially expressed genes related to the evolutionary adaptations between ICEs and bacteria (Figure 2). It will also help to explore the contribution of ICEs in bacteria where plasmids do not seem to play a significant role in the spread of AR genes [8]. These signatures could then be used to predict which ICE–host associations are likely to be selected in the future from the ICEs and bacteria present in a given environment. Controlled evolution and/or microcosm experiments are an additional approach that allows us to assess to what extent AR genes on ICEs are important for AR spread in either the presence or absence of antibiotic pressure. We predict that the interplay between fitness cost, selection, HGT, and compensatory evolution will determine the fate of ICEs in bacterial populations and therefore the onset of ICE-mediated AR.

A further challenge will be to study the distribution of ICEs in relevant reservoirs, such as the human microbiome and the resistome of soil bacteria [72,73]), and assess the contribution of these elements to the acquisition of AR genes in complex communities. Most recently, ICEs were demonstrated to shape the resistome of the rumen microbiome [74], with further implications for human health, while AR genes appear to be more likely disseminated by ICEs than by prophages in the human gut microbiome [75]. Moreover, understanding how ICEs disseminate within populations can assist in selecting which antibiotics and antibiotic combinations can be used to prevent the HGT of AR determinants [76,77]. Shedding new light on the evolutionary basis of ICE-mediated AR could make a significant contribution to the development of innovative therapeutic approaches and intervention strategies.

New strategies for improving the detection and characterization of ICEs within bacterial genomes should also be explored. Currently available tools – ICEfinder [45] and CONJscan [26,78] – have important limitations, such as the inaccurate prediction of ICEs in draft genomes and the boundaries of the attachment sites, as well as the inability to track **tripartite ICEs** [79]. A recently developed tool, MGEfinder, tracks the integration site of mobile elements, such as ICEs, by using short-read sequencing data [80]. However, looking for MGEs on genomes sequenced with short-read approaches is challenging since these elements are usually fragmented due to the presence of repetitive regions. Also, the identification of ICE blocks on fragmented genomes requires reference-based alignments, which would bias the analysis. The best solution to retrieve contiguous MGEs is to use hybrid assembly of Illumina plus PacBio or Illumina and Nanopore sequencing data [81,82].

Concluding Remarks and Future Perspectives

Even though ICEs are abundant and important for the spread of AR genes, the transmission and evolutionary dynamics of these elements are still poorly characterized. Future experiments will help to explain the evolutionary and molecular basis of ICE–bacterium dynamics and to predict the epidemiology of ICE-mediated resistance. From a more general point of view, these new studies will offer significant information regarding the evolution and adaptation of bacteria by HGT. Ideally, these results will be extremely relevant in the field of microbiology and will create

Outstanding Questions

How often do ICEs exchange AR genes in complex microbial communities?

Does ICE–bacterium coevolution differ from plasmid–bacterium coevolution?

Do compensatory mechanisms emerge to overcome the burden imposed by ICE carriage?

Upon arrival in a new host, is ICE integration in the chromosome a mandatory step, or is it possible that the circular intermediate evolves to become an extrachromosomal element with plasmid-like features?

the perfect scenario for the development of translational research that may ultimately lead to the development of new intervention strategies aimed at counteracting the spread of AR (see Outstanding Questions).

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Resources

[i]https://db-mml.sjtu.edu.cn/ICEberg2/browse_fam.php

[ii]https://db-mml.sjtu.edu.cn/ICEberg2/browse_result.php?type=fam&fam_id=5

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