REVIEW
Dimitriu, *Microbiology* 2022;168:001214
DOI 10.1099/mic.0.001214

Evolution of horizontal transmission in antimicrobial resistance plasmids

Tatiana Dimitriu*

Abstract

Mobile genetic elements (MGEs) are one of the main vectors for the spread of antimicrobial resistance (AMR) across bacteria, due to their ability to move horizontally between bacterial lineages. Horizontal transmission of AMR can increase AMR prevalence at multiple scales, from increasing the prevalence of infections by resistant bacteria to pathogen epidemics and worldwide spread of AMR across species. Among MGEs, conjugative plasmids are the main contributors to the spread of AMR. This review discusses the selective pressures acting on MGEs and their hosts to promote or limit the horizontal transmission of MGEs, the mechanisms by which transmission rates can evolve, and their implications for limiting the spread of AMR, with a focus on AMR plasmids.

INTRODUCTION

The spread of antimicrobial resistance (AMR) threatens the use of antibiotics in modern medicine, with antibiotic-resistant infections predicted to be the primary cause of death by 2050 [1], and already causing over one million deaths per year [2]. This spread of AMR is fuelled by horizontal transmission of AMR genes between bacteria, which increases AMR abundance within species and allows cross-species dissemination. Three main mechanisms can transfer genes horizontally [3]. In natural transformation, cells competent for transformation uptake naked extracellular DNA directly from the environment. Genes can also be transmitted between cells by transduction, in which they are packaged into bacteriophage capsids together with, or instead of, phage DNA. Finally, conjugation transfers DNA after cell–cell contact, via conjugative pili encoded by conjugative elements. Phages and conjugative elements are mobile genetic elements (MGEs) which use transduction and conjugation to transfer themselves horizontally. Conjugative elements include plasmids, extrachromosomal DNA molecules that replicate autonomously, and integrative and conjugative elements (ICEs), which are integrated into the host chromosome. Conjugative elements are self-transmissible as they encode their own conjugation machinery; mobilizable elements are not, but they can be mobilized by the transfer machinery of a conjugative element present in the same cell [4]. Phages can also encapsulate full plasmids [5]. At lower frequency, phages also package bacterial DNA, which can transduce chromosomal AMR genes [6]. It is not clear how important this is for AMR spread, although lateral transduction, a mechanism by which *in situ* replication of prophages amplifies the amount of surrounding host DNA packaged in phage capsids, can lead to very high rates of chromosomal gene mobilization [7]. Still, horizontal transmission of AMR will be most efficient when AMR genes are directly carried on MGEs.

MGEs frequently carry AMR genes, which is promoted by association with transposable elements allowing gene movement between genomic locations and accumulation of AMR genes on multi-resistant elements [8]. AMR genes can be carried on phages, particularly P1-like phage-plasmids [9, 10]. ICEs also carry AMR genes, but their role in AMR spread is still little understood [11]. By contrast, plasmids are clearly enriched in AMR genes: they have disseminated AMR genes across species since the start of the antibiotic era [12–14], with a crucial role played by a few major AMR plasmid families [15, 16].

Horizontal transmission of MGEs can govern the transmission of AMR within patients [17], and facilitate epidemics [18, 19] and worldwide dissemination of AMR genes [20]. Thus, it is crucial to understand which factors promote, or limit, the evolution of horizontal transmission in bacterial populations. Horizontal transmission rates are extremely variable, due to both MGE- and

Received 05 April 2022; Accepted 07 June 2022; Published 18 July 2022
Author affiliations: [1]ESI, Biosciences, University of Exeter, TR10 9FE Penryn, UK.
***Correspondence:** Tatiana Dimitriu, t.dimitriu@exeter.ac.uk
Keywords: AMR plasmids; antimicrobial resistance; conjugation rate; plasmid transfer.
Abbreviations: AMR, antimicrobial resistance; ICE, integrative and conjugative element; MGE, mobile genetic element; RM, restriction-modification; T6SS, type 6 secretion system.

host cell-encoded variation [21, 22]. Here, I review the selective pressures and molecular mechanisms shaping the evolution of horizontal transmission in MGEs, with a particular focus on plasmids as the major vectors of AMR genes.

Evolution of MGE-controlled transmission

MGEs are subject to natural selection, which favours MGEs which maximize their fitness, defined as the average contribution to the population of MGEs in future generations [23]. One key characteristic of the life cycle of MGEs is that they can transmit within populations via two routes: vertical transmission together with host cell division, and horizontal transmission from a donor to a recipient bacterium. If horizontal transmission conferred no costs, it would evolve towards always increasing rates [24]. However, an MGE with high rates of horizontal transmission will impose a higher cost to its bacterial host, leading to reduced host growth or competitive ability. In turn, this cost to the host will reduce MGE vertical transmission, creating a trade-off between horizontal and vertical transmission [25, 26]. Thus, the horizontal transmission rate that maximizes an MGE's fitness depends on the trade-off between its direct benefits to fitness (through horizontal transmission) and its indirect cost to fitness (through reduced vertical transmission).

The cost of horizontal transmission varies with the mechanism of transmission. Horizontal transmission through phage lysis of host cells is most costly; conjugation does not require host cell lysis, but still entails metabolic costs linked to pili production and DNA translocation. In some cases, cells stop dividing when conjugating [27]. Moreover, the expression of conjugative pili can lead to cell envelope damage and induction of stress responses [28]. Overall, plasmid variants with a higher conjugation rate tend to have a higher cost [26, 29, 30]. In addition to these intrinsic costs, conjugation also puts donor cells at risk of infection by male-specific phages, which specifically infect cells expressing conjugative pili [31]; and conjugation can lead to attack by recipients with type 6 secretion systems (T6SS) because membrane perturbations caused by conjugative pili are detected as a threat [32].

The benefits of investing in horizontal transmission mechanisms mainly depend on the availability of susceptible hosts [33]. Modelling and experiments show that when few hosts are available, it is beneficial for temperate phages to not lyse their host cells [34, 35], and for conjugative plasmids to not conjugate at high rates [26, 30]. Over evolutionary times, MGEs might thus evolve towards some optimal transmission rate that depends on the costs of transmission and the opportunities for transfer they experience. However, models have shown that evolution does not necessarily converge to a single transfer rate, but low-frequency genetic diversity in transfer rates can be stable [24]. Moreover, when host availability varies over time, plasticity mechanisms that induce transmission only when a high density of potential hosts is detected can benefit both phages [36] and plasmids [37].

Experimental evolution approaches allow us to test in controlled conditions how MGEs respond to a given selective pressure. Many plasmid–host coevolution studies are done in the absence of horizontal transmission, because all hosts initially carry plasmids (which block the entry of plasmids from other cells), and plasmid loss is usually negligible. As expected, this leads to a reduction of transfer rate and plasmid cost, after a few hundred generations [38–41], or even faster when male-specific phages are present [42]. When conjugation is enforced experimentally by selecting for alternating hosts, increased transmission can instead evolve [43, 44]. Finally, a few studies have explicitly manipulated the number of new hosts available for horizontal transmission. With filamentous bacteriophages, evolving phages with longer periods of selection for horizontal transmission (in which the cells are killed and only virions are propagated) led to the evolution of phages with increased horizontal transmission [45]. Similarly, plasmid R1 increased its transfer rate only when confronted repeatedly to a large proportion of susceptible hosts [29]. By contrast, RP4 plasmid did not evolve, which might be due to a different cost–benefit ratio, but also to genetic constraints. In an earlier study [26], pB15 plasmid transfer rate evolved but did not correlate with host availability treatments. This might be due to an experimental protocol which favoured vertical transmission overall (selection for plasmid carriage was enforced regularly), or complicated by the complex genetic basis of conjugation in pB15, which contains a shufflon [46]. Thus, MGE response to selection will depend on the selective pressures encountered, but also on the mechanisms dictating horizontal transmission rates.

Mechanisms for MGE-encoded variation in transfer rates

Horizontal transmission of MGEs depends on the expression of genes required for horizontal transmission: this includes (at least) excision, packaging and lysis genes for phages, conjugation and mobilization genes for plasmids. The level of expression of these genes can vary constitutively across MGEs, but also vary over time for a given MGE.

Regulatory networks can modulate in which conditions horizontal transmission genes are expressed [47]: this phenotypic variation will be adaptive if expression correlates with the probability of successful transmission. When phage λ was evolved in treatments varying the cues indicating when new hosts would be available, the induction of horizontal transmission evolved to maximize transmission [48]. Regulation of transfer of natural MGEs might have evolved similarly, in order to induce transmission in conditions in which horizontal transmission is most likely to be successful. In plasmids, conjugation usually relies on a set of genes grouped in transfer operons, expression of which is regulated by multiple factors [47]. For instance, plasmid conjugation in *Salmonella* can be induced by physiological conditions present in the gut [49]. Many MGEs are regulated by quorum sensing-like systems allowing them to transfer when recipient hosts are available, repressing transfer functions when too many MGE carriers are already present [50, 51], or activating transfer in the presence of recipients, as in the case of pheromone plasmids [52].

Quorum sensing communication provides information at a range of a few micrometres only, allowing for relevant estimation of the number of surrounding uninfected hosts [53]. Some MGEs even induce transfer only upon physical contact between the donor and recipient cells [54]. Other systems induce horizontal transmission in conditions of stress, interpreted as a signal that the host, and thus vertical transmission, are endangered. One main example of this is the SOS response, which detects DNA damage, and commonly induces phage lysis and ICE conjugation [50, 55]. As antibiotics often cause DNA damage, antibiotic treatment itself can induce horizontal transmission of AMR elements [56–58]. However, this phenomenon seems to be rare across conjugative plasmid groups, and increased spread of plasmids after antibiotic treatment can often be attributed to selection rather than transfer [21, 59]. Overall, conditions which promote induction of transmission mechanisms are not always known, but the plasticity of transmission mechanisms seems to commonly promote investment in transmission when it is most likely to provide benefits to MGEs.

Transmission rates also vary constitutively, due to genetically encoded mechanisms. In many plasmid groups, transfer gene expression is repressed by default [47, 60], and derepressed only in specific conditions. This creates the opportunity for mutations inactivating the repressor genes, leading to constitutively derepressed mutants with up to 1000-fold increased transfer rate. Derepressed plasmids include the well-known F plasmid [61], early laboratory mutants of AMR plasmids [62], but also clinical AMR plasmids, characterized by high rates of transfer [63]. Repression can also be transiently lifted due to transcriptional overshooting of conjugation genes in new plasmid recipients before the repressor is synthesized [64]. This transitory derepression leads to high rates of transfer from newly formed transconjugants and effectively ensures epidemic spread of plasmids in naïve populations [65].

Mutations not directly related to transfer gene expression also influence transfer. For the R1 plasmid, the prevalent mechanism increasing transfer rates was a change in plasmid copy number [29]. Increased copy number causes both increased transfer gene expression, due to gene dosage effects, and increased mobilization, because more plasmid copies are present in the cell. This might be a common mechanism for increasing transfer rates across plasmid types: point mutations increasing copy number are known for conjugative as well as mobilizable plasmids [66–68]. In R1, copy number mutants were less infectious but also less costly than fully derepressed mutants, possibly explaining their prevalence. However, high copy number appears to be too costly for some conjugative plasmids. For instance, IncP plasmids with high copy number cause death in some host species [69], and the cost of colistin resistance can become extremely high when amplified by high copy number [70]. In the case of mobilizable plasmids, copy number might instead be one of the few mechanisms plasmids can control, as they rely on conjugative functions encoded on other MGEs. Mobilizable plasmids commonly have high copy number [47], which in turn leads to higher expression of mobilization genes, contributing to increased transmission [71]. A high copy number should also enhance transmission through other routes, including transduction [5] or extracellular vesicles [72], because it increases the amount of DNA available to package.

Other plasmid-encoded traits can act indirectly on plasmid transfer. For instance, biofilm formation can favour horizontal transfer [73] and can be promoted by conjugative pili [74, 75], or by plasmid-encoded fimbriae [76] or clumping proteins [77]. Plasmids can also repress T6SS secretion, preventing their host from killing potential recipients [78]. Finally, transfer regulatory networks are connected with the host cell regulatory networks [47], which can induce transfer in response to environmental changes detected by the host. This probably represents an adaptation of plasmids to transfer when most beneficial. Yet, it also provides the potential for host genes to control transfer.

Effects of the host and other MGEs on transmission

For a given plasmid, large variation in transfer rates can be observed across host strains, *in vitro* [22, 79, 80], as well as *in vivo* [81]. Both the donor host and the recipient can affect transfer rates, due to different mechanisms. On the recipient side, resistance to plasmid entry is not commonly due to the loss of surface receptors (in contrast to phages), either because such receptors are not strictly required for conjugative transfer, or because they are essential to the host [82, 83]. Conjugation is instead sensitive to targeting by bacterial immune systems [84], and exclusion systems [85]. Restriction-modification (RM) systems distinguish self from non-self through chemical modifications of DNA bases, and cleave DNA recognized as foreign. They are active against plasmid transfer [86, 87], although they might act less efficiently than against phage infection because DNA enters the cell single-stranded during conjugation. CRISPR-Cas systems are adaptive immune systems which record memory of past encounters with MGEs by inserting MGE-derived sequences (spacers) into CRISPR loci. Upon reinfection, sequence-specific recognition triggers cleavage by Cas enzymes. When CRISPR arrays contain spacers targeting plasmids, CRISPR-Cas immunity is effective against plasmid conjugation [86, 88, 89]. Other MGEs present in the recipient cell also impact transfer of a focal MGE by diverse mechanisms [90]. Type IV CRISPR-Cas systems, themselves located on plasmids, appear to be involved in plasmid competition and target mostly other plasmids [91]. Finally, most plasmids carry entry exclusion systems, which prevent physical DNA transfer from isogenic or closely related plasmids by inhibiting mating pair formation or DNA injection [85].

Selective pressures acting on recipient ability to receive MGEs depend on the direct effect of the incoming MGEs on host cell fitness. In cases where plasmid carriage is costly (but see, for instance, [92]), defence against plasmids can benefit the host [93]. However, in the presence of antibiotics to which AMR plasmids provide resistance, immunity against AMR plasmids is counter-selected [94]. Some defence systems might also limit AMR elements without any direct benefit but as a side effect of their role

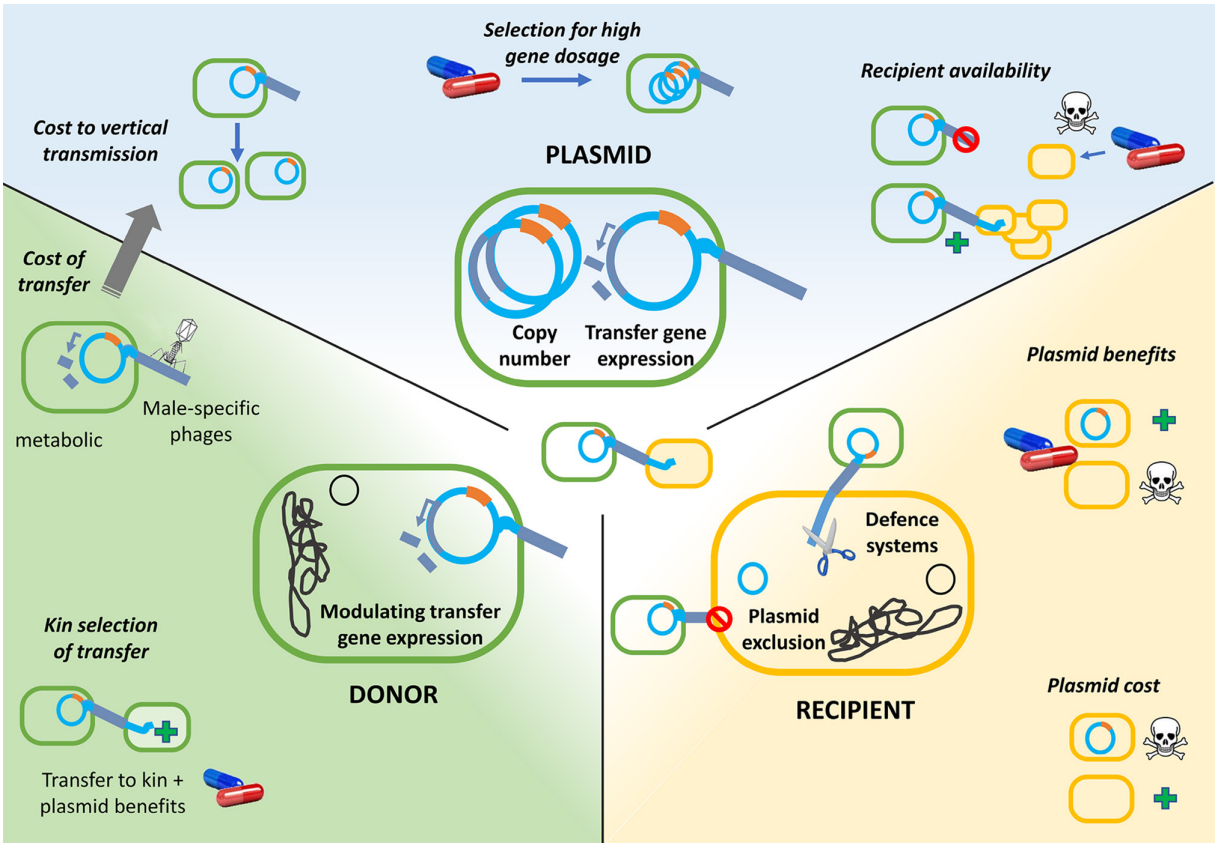

Fig. 1. Graphical summary of the forces acting on AMR plasmid transfer, through the plasmid itself, the donor cell and the recipient cell. Placed centrally, mechanisms modulating transfer are surrounded by illustrations of the selective pressures shaping them.

against lytic phages. RM systems, in particular, have little sequence specificity, and thus selection for their action against phages [95] will maintain their effect against plasmids. By contrast, CRISPR-Cas systems only target MGEs against which they carry spacers. Identified spacer targets are primarily located in phage genomes but the next most abundant targets belong to plasmid genes involved in mobility [96], suggesting a selective advantage to excluding conjugative plasmids. In addition, MGEs present in the recipient cell are at risk of displacement by incoming, incompatible MGEs, which will favour the targeting of plasmids by type IV CRISPR-Cas systems [91] and carriage of entry exclusion systems against related plasmids [85].

Donor cells act on horizontal transmission through the regulation of transfer gene expression [47]. In particular, global regulators including nucleoid-associated proteins and two-component systems play a crucial role in transfer gene expression [97, 98]. However, most of this knowledge comes from gene knock-out experiments, and it is not yet clear if more subtle variation in global regulator sequence or expression can explain transfer rate variability across natural isolates. Other plasmids present in the donor cell can also modify a focal plasmid's transfer rate by various mechanisms [99, 100]. Because the donor cell pays the cost of higher transmission, direct selection acts on genes present in donors (on the chromosome as well as on any other MGE that is not transmitted together with the focal plasmid) to decrease transmission rates [24]. Yet, plasmid transmission to recipient cells then affects the fitness of these new hosts, which indirectly affects donor fitness: if donors are related to recipients, transferring beneficial plasmids – for instance AMR plasmids in the context of antibiotic treatment – increases the inclusive fitness of donor alleles [101]. At the population level, if transfer is sufficiently biased towards recipients sharing the donor's alleles for transfer, this can in turn select for increased transfer rates [101]. Within populations, transfer might be higher between bacteria from the same genotype, because of spatially structured growth, but also because cells from the same genotype will have identical RM systems and recognize MGEs as self [79]. When MGE gene expression also benefits neighbouring cells, as can be the case for many AMR mechanisms including secreted β-lactamases but also cytoplasmic antibiotic-modifying enzymes [102], plasmid transfer even to non-related recipients will enhance antibiotic degradation and align host and plasmid fitness interests towards higher transfer rates [103].

Overall, it is unclear how much of the variation in transfer ability among bacterial hosts evolved in response to selective pressures linked to MGE carriage and transmission. Regardless, this variation will influence MGE spread and distribution in bacterial

populations. A recent model showed that effects of several orders of magnitude are required for host cells to significantly alter plasmid prevalence [24]. Thus, small effects might have little consequences, but large variations in transfer ability will impact AMR plasmid prevalence. Host immune systems, in particular, appear to exert significant selective pressures on MGEs, as these commonly encode genes to defend themselves against host defences [104–106]. In several pathogenic species, lineages with CRISPR-Cas systems carry fewer MGEs, and carrying spacers targeting MGEs vectors of AMR is associated with reduced AMR carriage [107], although this pattern can be disrupted by anti-CRISPR genes [108]. RM system presence was also shown to limit horizontal gene transfer and AMR elements [109, 110]. Overall, barriers to MGEs will impact not only the total amount of MGE transmission, but also which bacterial lineages carry MGEs [107, 111].

Consequences for the spread of AMR

Horizontal transmission is crucial to the ecology and persistence of MGE and MGE-carried genes. A high enough rate of horizontal transmission can allow persistence of an MGE conferring AMR if it compensates for MGE cost and loss [112]. Rates of transfer of most natural plasmids have long been thought to be too low for plasmid maintenance [113]. Yet, it has since been shown that measured transfer rates could be sufficient to maintain plasmids in the absence of selection [114]. Experimentally, conjugation promotes persistence of many conjugative plasmids of different incompatibility groups, at least *in vitro* [115]; and an effect of horizontal transmission on maintenance has been demonstrated *in vivo* as well [116], although correlating *in vivo* and *in vitro* dynamics can be difficult [117]. In natural and clinical environments, most of the evidence for horizontal transfer is on transmission between genetically different isolates, which can be detected much more easily (whereas within an otherwise homogeneous population, vertical and horizontal transmission cannot be distinguished). AMR plasmid transmission between species has been detected within patients after antibiotic treatment [118, 119], or in the absence of antibiotics [120]. Transmission of a derepressed AMR plasmid was also shown in the detailed study of a hospital ward [121], with plasmid transfer between *Klebsiella pneumoniae* and *Escherichia coli* detected within virtually every patient. Thus, horizontal transmission can directly increase AMR prevalence within patients as well as at a larger scale. At a community level, variability in transfer rates across hosts translates into increased overall rates of transmission, because of efficient amplification of transfer by rare efficient donors [80]. High transfer rates from one strain to another can also contribute to plasmid maintenance in a 'sink' host strain or species [122]. In addition, transfer to various hosts in which a plasmid imposes variable fitness costs could indirectly favour plasmid maintenance [123]

The contribution of horizontal transmission to AMR prevalence led to the suggestion that interventions that limit or stop horizontal transmission would help in the fight against AMR [124]. First, some chemical agents, including unsaturated fatty acids, act as specific inhibitors of plasmid conjugation [125]. Unsaturated fatty acids were shown to stop the conjugative spread of AMR plasmids first *in vitro* [115], then in conditions closer to natural environments, both water microcosms and a mouse gut model [126]. An alternative strategy to limit horizontal transmission is to promote plasmid evolution towards reduced horizontal transmission. Male-specific phages, which infect cells through conjugative pili, cause an extremely high cost of pili expression. Thus, not only are most target cells killed by phages, but the evolution of resistance to male-specific phages leads to plasmids which do not conjugate and strains which lose AMR plasmids, even in the presence of antibiotics [127].

Antibiotic treatment itself might impact the evolution of transfer rates. First, some antibiotics increase transfer gene expression, either directly [128, 129] or through induction of the SOS response [55, 130, 131]. Second, antibiotic treatment can select for plasmid variants with modified transfer rate. Exposure to high concentrations of antibiotics selects for increased copy number of plasmids that carry resistance genes to this antibiotic, because this increases the level of resistance, which in turn increases plasmid transfer rate [29]. Antibiotic treatment can also increase the expression of transfer genes more specifically: in the conjugative transposon Tn916, antibiotic treatment leads to increased transfer due to increased expression of AMR genes and of excision genes located nearby [132]. On the opposite, selection for plasmid carriage due to antibiotic treatment decreases effective transfer by suppressing recipients, which might favour variants that do not transfer, and promotes AMR gene movement to the chromosome [133]. In the long term, it has been argued that the widespread use of antimicrobials selects for higher rates of innovation and horizontal gene transfer, and against defences [134].

In conclusion, the evolution of MGE transmission rate will be shaped by opportunities for transmission, selective pressures acting on host bacteria, and molecular effects of transmission and defence mechanisms (Fig. 1). All these factors will be influenced by antibiotic treatment itself. However, most experimental evolution studies have been done *in vitro*, focusing on a few well-known plasmids and laboratory host strains. There is thus a need to understand better the evolution of transmission rates for relevant AMR plasmids and clinical strains [135], and in conditions closer to those experienced by bacteria *in vivo*, which will influence MGE transmission and its evolution, by modifying for instance population structure and antibiotic exposure. Ultimately, understanding how to influence and limit MGE spread will be crucial to fight AMR.

Funding Information
This work received no specific grant from any funding agency.

Acknowledgements
I thank Angus Buckling for critical reading of the manuscript, Andrew Matthews for discussion and input to the figure, and the reviewers for their helpful comments.

Conflicts of interest
The author declares that there are no conflicts of interest.

References
1. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. 2016.
2. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, *et al*. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022;399:629–655.
3. von Wintersdorff CJH, Penders J, van Niekerk JM, Mills ND, Majumder S, *et al*. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front Microbiol* 2016;7.
4. Smillie C, Garcillán-Barcia MP, Francia MV, Rocha EPC, de la Cruz F. Mobility of plasmids. *Microbiol Mol Biol Rev* 2010;74:434–452. ;
5. Humphrey S, San Millán Á, Toll-Riera M, Connolly J, Flor-Duro A, *et al*. Staphylococcal phages and pathogenicity islands drive plasmid evolution. *Nat Commun* 2021;12:5845.
6. Modi SR, Lee HH, Spina CS, Collins JJ. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. *Nature* 2013;499:219–222.
7. Humphrey S, Fillol-Salom A, Quiles-Puchalt N, Ibarra-Chávez R, Haag AF, *et al*. Bacterial chromosomal mobility via lateral transduction exceeds that of classical mobile genetic elements. *Nat Commun* 2021;12:6509.
8. Amábile-Cuevas CF, Chicurel ME. Bacterial plasmids and gene flux. *Cell* 1992;70:189–199.
9. Kondo K, Kawano M, Sugai M, Castanheira M. Distribution of antimicrobial resistance and virulence genes within the prophage-associated regions in nosocomial pathogens. *mSphere* 2021;6:e0045221.
10. Venturini C, Zingali T, Wyrsch ER, Bowring B, Iredell J, *et al*. Diversity of P1 phage-like elements in multidrug resistant *Escherichia coli*. *Sci Rep* 2019;9:18861.
11. Botelho J, Schulenburg H. The Role of Integrative and conjugative elements in antibiotic resistance evolution. *Trends Microbiol* 2021;29:8–18. ;
12. Baker KS, Burnett E, McGregor H, Deheer-Graham A, Boinett C, *et al*. The Murray collection of pre-antibiotic era *Enterobacteriacae*: a unique research resource. *Genome Med* 2015;7:97. ;
13. Datta N, Hughes VM. Plasmids of the same Inc groups in *Enterobacteria* before and after the medical use of antibiotics. *Nature* 1983;306:616–617.
14. Watanabe T. Infective heredity of multiple drug resistance in bacteria. *Bacteriol Rev* 1963;27:87.
15. Carattoli A. Resistance plasmid families in *Enterobacteriaceae*. *Antimicrob Agents Chemother* 2009;53:2227–2238.
16. Hawkey PM, Jones AM. The changing epidemiology of resistance. *J Antimicrob Chemother* 2009;64 Suppl 1:i3-10.
17. Kent AG, Vill AC, Shi Q, Satlin MJ, Brito IL. Widespread transfer of mobile antibiotic resistance genes within individual gut microbiomes revealed through bacterial Hi-C. *Nat Commun* 2020;11:4379.
18. Baker KS, Dallman TJ, Field N, Childs T, Mitchell H, *et al*. Horizontal antimicrobial resistance transfer drives epidemics of multiple *Shigella* species. *Nat Commun* 2018;9:1462.
19. Sheppard AE, Stoesser N, Wilson DJ, Sebra R, Kasarskis A, *et al*. Nested Russian Doll-Like Genetic Mobility Drives Rapid Dissemination of the Carbapenem Resistance Gene bla_{KPC}. *Antimicrob Agents Chemother* 2016;60:3767–3778.
20. Matamoros S, van Hattem JM, Arcilla MS, Willemse N, Melles DC, *et al*. Global phylogenetic analysis of *Escherichia coli* and plasmids carrying the *mcr-1* gene indicates bacterial diversity but plasmid restriction. *Sci Rep* 2017;7:15364.
21. Bethke JH, Davidovich A, Cheng L, Lopatkin AJ, Song W, *et al*. Environmental and genetic determinants of plasmid mobility in pathogenic *Escherichia coli*. *Sci Adv* 2020;6:eaax3173.
22. Sheppard RJ, Beddis AE, Barraclough TG. The role of hosts, plasmids and environment in determining plasmid transfer rates: A meta-analysis. *Plasmid* 2020;108:102489.
23. Hülter NF, Wein T, Effe J, Garoña A, Dagan T. Intracellular Competitions Reveal Determinants of Plasmid Evolutionary Success. *Front Microbiol* 2020;11:2062. ;
24. Sheppard RJ, Barraclough TG, Jansen VAA. The evolution of plasmid transfer rate in bacteria and its effect on plasmid persistence. *Am Nat* 2021;198:473–488.
25. Levin BR, Lenski RE. Coevolution in bacteria and their viruses and plasmids. *In Coevolution, (Futuyma and Slatkin), p* 1983.
26. Turner PE, Cooper VS, Lenski RE. Tradeoff between horizontal and vertical modes of transmission in bacterial plasmids. *Evolution* 1998;52:315–329.
27. Reinhard F, Miyazaki R, Pradervand N, van der Meer JR. Cell differentiation to "mating bodies" induced by an integrating and conjugative element in free-living bacteria. *Curr Biol* 2013;23:255–259.
28. Zahrl D, Wagner M, Bischof K, Koraimann G. Expression and assembly of a functional type IV secretion system elicit extracytoplasmic and cytoplasmic stress responses in *Escherichia coli*. *J Bacteriol* 2006;188:6611–6621.
29. Dimitriu T, Matthews AC, Buckling A. Increased copy number couples the evolution of plasmid horizontal transmission and plasmid-encoded antibiotic resistance. *Proc Natl Acad Sci U S A* 2021;118:e2107818118.
30. Haft RJF, Mittler JE, Traxler B. Competition favours reduced cost of plasmids to host bacteria. *ISME J* 2009;3:761–769.
31. Manchak J, Anthony KG, Frost LS. Mutational analysis of F-pilin reveals domains for pilus assembly, phage infection and DNA transfer. *Mol Microbiol* 2002;43:195–205.
32. Ho BT, Basler M, Mekalanos JJ. Type 6 secretion system-mediated immunity to type 4 secretion system-mediated gene transfer. *Science* 2013;342:250–253.
33. Anderson RM, May RM. Coevolution of hosts and parasites. *Parasitology* 1982;85 (Pt 2):411–426.
34. Berngruber TW, Froissart R, Choisy M, Gandon S. Evolution of virulence in emerging epidemics. *PLoS Pathog* 2013;9:e1003209.
35. Stewart FM, Levin BR. The population biology of bacterial viruses: why be temperate. *Theor Popul Biol* 1984;26:93–117.
36. Bruce JB, Lion S, Buckling A, Westra ER, Gandon S. Regulation of prophage induction and lysogenization by phage communication systems. *Curr Biol* 2021;31:5046–5051. ;
37. Banderas A, Carcano A, Sia E, Li S, Lindner AB. Ratiometric quorum sensing governs the trade-off between bacterial vertical and horizontal antibiotic resistance propagation. *PLoS Biol* 2020;18:e3000814.
38. Dahlberg C, Chao L. Amelioration of the cost of conjugative plasmid carriage in *Eschericha coli* K12. *Genetics* 2003;165:1641–1649.
39. Dionisio F, Conceição IC, Marques ACR, Fernandes L, Gordo I. The evolution of a conjugative plasmid and its ability to increase bacterial fitness. *Biol Lett* 2005;1:250–252.

40. Harrison E, Guymer D, Spiers AJ, Paterson S, Brockhurst MA. Parallel compensatory evolution stabilizes plasmids across the parasitism-mutualism continuum. *Curr Biol* 2015;25:2034–2039.
41. Porse A, Schønning K, Munck C, Sommer MOA. Survival and evolution of a large multidrug resistance plasmid in new clinical bacterial Hosts. *Mol Biol Evol* 2016;33:2860–2873.
42. Jalasvuori M, Friman V-P, Nieminen A, Bamford JKH, Buckling A. Bacteriophage selection against a plasmid-encoded sex apparatus leads to the loss of antibiotic-resistance plasmids. *Biol Lett* 2011;7:902–905. ;
43. De Gelder L, Williams JJ, Ponciano JM, Sota M, Top EM. Adaptive plasmid evolution results in host-range expansion of a broad-host-range plasmid. *Genetics* 2008;178:2179–2190.
44. Kottara A, Hall JPJ, Harrison E, Brockhurst MA. Multi-host environments select for host-generalist conjugative plasmids. *BMC Evol Biol* 2016;16:70.
45. Messenger SL, Molineux IJ, Bull JJ. Virulence evolution in a virus obeys a trade-off. *Proc Biol Sci* 1999;266:397–404.
46. Turner PE, Williams E, Okeke C, Cooper VS, Duffy S, *et al*. Antibiotic resistance correlates with transmission in plasmid evolution. *Evolution* 2014;68:3368–3380.
47. Frost LS, Koraimann G. Regulation of bacterial conjugation: balancing opportunity with adversity. *Future Microbiol* 2010;5:1057–1071.
48. Refardt D, Rainey PB. Tuning a genetic switch: experimental evolution and natural variation of prophage induction. *Evolution* 2010;64:1086–1097.
49. Aviv G, Rahav G, Gal-Mor O, Davies JE. Horizontal transfer of the *Salmonella enterica* serovar Infantis resistance and virulence plasmid pESI to the gut microbiota of warm-blooded hosts. *mBio* 2016;7:e01395-16.
50. Auchtung JM, Lee CA, Monson RE, Lehman AP, Grossman AD. Regulation of a *Bacillus subtilis* mobile genetic element by intercellular signaling and the global DNA damage response. *Proc Natl Acad Sci U S A* 2005;102:12554–12559.
51. Singh PK, Ramachandran G, Ramos-Ruiz R, Peiró-Pastor R, Abia D, *et al*. Mobility of the native *Bacillus subtilis* conjugative plasmid pLS20 is regulated by intercellular signaling. *PLoS Genet* 2013;9:e1003892. ;
52. Dunny GM. The peptide pheromone-inducible conjugation system of *Enterococcus faecalis* plasmid pCF10: cell-cell signalling, gene transfer, complexity and evolution. *Philos Trans R Soc Lond B Biol Sci* 2007;362:1185–1193.
53. van Gestel J, Bareia T, Tenennbaum B, Dal Co A, Guler P, *et al*. Short-range quorum sensing controls horizontal gene transfer at micron scale in bacterial communities. *Nat Commun* 2021;12:2324.
54. Yi L, Durand R, Grenier F, Yang J, Yu K, *et al*. PixR, a novel activator of conjugative transfer of IncX4 resistance plasmids, mitigates the fitness cost of *mcr-1* carriage in *Escherichia coli*. *mBio* 2022;13:e03209-21.
55. Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* 2004;427:72–74.
56. Liu G, Bogaj K, Bortolaia V, Olsen JE, Thomsen LE. Antibiotic-induced, increased conjugative transfer is common to diverse naturally occurring ESBL plasmids in *Escherichia coli*. *Front Microbiol* 2019;10:2119.
57. Bañuelos-Vazquez LA, Torres Tejerizo G, Brom S. Regulation of conjugative transfer of plasmids and integrative conjugative elements. *Plasmid* 2017;91:82–89.
58. Waters JL, Salyers AA. Regulation of CTnDOT conjugative transfer is a complex and highly coordinated series of events. *mBio* 2013;4:e00569-13.
59. Lopatkin AJ, Huang S, Smith RP, Srimani JK, Sysoeva TA, *et al*. Antibiotics as a selective driver for conjugation dynamics. *Nat Microbiol* 2016;1:16044.
60. Kohler V, Keller W, Grohmann E. Regulation of gram-positive conjugation. *Front Microbiol* 2019;10:1134. ;
61. Yoshioka Y, Ohtsubo H, Ohtsubo E. Repressor gene finO in plasmids R100 and F: constitutive transfer of plasmid F is caused by insertion of IS3 into F finO. *J Bacteriol* 1987;169:619–623.
62. Meynell E, Datta N. Mutant drug resistant factors of high transmissibility. *Nature* 1967;214:885–887.
63. Potron A, Poirel L, Nordmann P. Derepressed transfer properties leading to the efficient spread of the plasmid encoding carbapenemase OXA-48. *Antimicrob Agents Chemother* 2014;58:467–471.
64. Fernandez-Lopez R, Del Campo I, Revilla C, Cuevas A, de la Cruz F, *et al*. Negative feedback and transcriptional overshooting in a regulatory network for horizontal gene transfer. *PLoS Genet* 2014;10:e1004171. ;
65. Lundquist PD, Levin BR. Transitory derepression and the maintenance of conjugative plasmids. *Genetics* 1986;113:483–497.
66. Muesing M, Tamm J, Shepard HM, Polisky B. A single base-pair alteration is responsible for the DNA overproduction phenotype of a plasmid copy-number mutant. *Cell* 1981;24:235–242.
67. Persson C, Wagner EG, Nordström K. Control of replication of plasmid R1: structures and sequences of the antisense RNA, CopA, required for its binding to the target RNA, CopT. *EMBO J* 1990;9:3767–3775.
68. Santos-Lopez A, Bernabe-Balas C, Ares-Arroyo M, Ortega-Huedo R, Hoefer A, *et al*. A naturally occurring SNP in plasmid pB1000 produces a reversible increase in antibiotic resistance. *Antimicrob Agents Chemother* 2016;61:e01735-16.
69. Haugan K, Karunakaran P, Tøndervik A, Valla S. The host range of RK2 minimal replicon copy-up mutants is limited by species-specific differences in the maximum tolerable copy number. *Plasmid* 1995;33:27–39.
70. Yang J, Wang H-H, Lu Y, Yi L-X, Deng Y, *et al*. A ProQ/FinO family protein involved in plasmid copy number control favours fitness of bacteria carrying mcr-1-bearing IncI2 plasmids. *Nucleic Acids Res* 2021;49:3981–3996.
71. Lorenzo-Díaz F, Fernández-López C, Lurz R, Bravo A, Espinosa M. Crosstalk between vertical and horizontal gene transfer: plasmid replication control by a conjugative relaxase. *Nucleic Acids Res* 2017;45:7774–7785.
72. Tran F, Boedicker JQ. Genetic cargo and bacterial species set the rate of vesicle-mediated horizontal gene transfer. *Sci Rep* 2017;7:8813.
73. Madsen JS, Burmølle M, Hansen LH, Sørensen SJ. The interconnection between biofilm formation and horizontal gene transfer. *FEMS Immunol Med Microbiol* 2012;65:183–195.
74. Ghigo JM. Natural conjugative plasmids induce bacterial biofilm development. *Nature* 2001;412:442–445.
75. Reisner A, Höller BM, Molin S, Zechner EL. Synergistic effects in mixed *Escherichia coli* biofilms: conjugative plasmid transfer drives biofilm expansion. *J Bacteriol* 2006;188:3582–3588.
76. Madsen JS, Riber L, Kot W, Basfeld A, Burmølle M, *et al*. Type 3 fimbriae encoded on plasmids are expressed from a unique promoter without affecting host motility, facilitating an exceptional phenotype that enhances conjugal plasmid transfer. *PLoS ONE* 2016;11:e0162390.
77. Luo H, Wan K, Wang HH. High-frequency conjugation system facilitates biofilm formation and pAMbeta1 transmission by *Lactococcus lactis*. *Appl Environ Microbiol* 2005;71:2970–2978.
78. Di Venanzio G, Moon KH, Weber BS, Lopez J, Ly PM, *et al*. Multidrug-resistant plasmids repress chromosomally encoded T6SS to enable their dissemination. *Proc Natl Acad Sci U S A* 2019;116:1378–1383.
79. Dimitriu T, Marchant L, Buckling A, Raymond B. Bacteria from natural populations transfer plasmids mostly towards their kin. *Proc Biol Sci* 2019;286:20191110.

80. Dionisio F, Matic I, Radman M, Rodrigues OR, Taddei F. Plasmids spread very fast in heterogeneous bacterial communities. *Genetics* 2002;162:1525–1532.
81. Benz F, Huisman JS, Bakkeren E, Herter JA, Stadler T, *et al*. Plasmid- and strain-specific factors drive variation in ESBL-plasmid spread in vitro and in vivo. *ISME J* 2021;15:862–878.
82. Moriguchi K, Zoolkefli FIRM, Abe M, Kiyokawa K, Yamamoto S, *et al*. Targeting antibiotic resistance genes is a better approach to block acquisition of antibiotic resistance than blocking conjugal transfer by recipient cells: a genome-wide screening in *Escherichia coli*. *Front Microbiol* 2019;10:2939.
83. Pérez-Mendoza D, de la Cruz F. *Escherichia coli* genes affecting recipient ability in plasmid conjugation: are there any? *BMC Genomics* 2009;10:71.
84. Dimitriu T, Szczelkun MD, Westra ER. Evolutionary ecology and interplay of prokaryotic innate and adaptive immune systems. *Curr Biol* 2020;30:R1189–R1202.
85. Garcillán-Barcia MP, de la Cruz F. Why is entry exclusion an essential feature of conjugative plasmids? *Plasmid* 2008;60:1–18.
86. Price VJ, Huo W, Sharifi A, Palmer KL. CRISPR-Cas and Restriction-Modification Act Additively against Conjugative Antibiotic Resistance Plasmid Transfer in *Enterococcus faecalis*. *mSphere* 2016;1:e00064-16.
87. Roer L, Aarestrup FM, Hasman H. The EcoKI type I restriction-modification system in *Escherichia coli* affects but is not an absolute barrier for conjugation. *J Bacteriol* 2015;197:337–342.
88. Marraffini LA, Sontheimer EJ. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. *Science* 2008;322:1843–1845.
89. Price VJ, McBride SW, Hullahalli K, Chatterjee A, Duerkop BA, *et al*. *Enterococcus faecalis* CRISPR-Cas Is a Robust Barrier to Conjugative Antibiotic Resistance Dissemination in the murine intestine. *mSphere* 2019;4:11.
90. Getino M, de la Cruz F, Baquero F, Bouza E, Gutiérrez-Fuentes JA. Natural and artificial strategies to control the conjugative transmission of plasmids. *Microbiol Spectr* 2018;6:MTBP-0015.
91. Pinilla-Redondo R, Mayo-Muñoz D, Russel J, Garrett RA, Randau L, *et al*. Type IV CRISPR-cas systems are highly diverse and involved in competition between plasmids. Nucleic Acids Res 2019;13. DOI: 10.1101/780106.
92. Knight GM, Budd EL, Lindsay JA. Large mobile genetic elements carrying resistance genes that do not confer a fitness burden in healthcare-associated meticillin-resistant *Staphylococcus aureus*. *Microbiology* 2013;159:1661–1672.
93. Levin BR. Nasty viruses, costly plasmids, population dynamics, and the conditions for establishing and maintaining CRISPR-mediated adaptive immunity in bacteria. *PLoS Genet* 2010;6:e1001171.
94. Jiang W, Maniv I, Arain F, Wang Y, Levin BR, *et al*. Dealing with the evolutionary downside of CRISPR immunity: bacteria and beneficial plasmids. *PLoS Genet* 2013;9:e1003844. ;
95. Korona, R., and Levin, B.R. (1993). Phage-mediated selection and the evolution and maintenance of restriction-modification. Evolution 47, 556–575. .
96. Shmakov SA, Sitnik V, Makarova KS, Wolf YI, Severinov KV, *et al*. The CRISPR spacer space is dominated by sequences from species-specific mobilomes. *mBio* 2017;8:e01397-17.
97. Forns N, Baños RC, Balsalobre C, Juárez A, Madrid C. Temperature-dependent conjugative transfer of R27: role of chromosome- and plasmid-encoded Hha and H-NS proteins. *J Bacteriol* 2005;187:3950–3959.
98. Strohmaier H, Noiges R, Kotschan S, Sawers G, Högenauer G, *et al*. Signal transduction and bacterial conjugation: characterization of the role of ArcA in regulating conjugative transfer of the resistance plasmid R1. *J Mol Biol* 1998;277:309–316.
99. Dionisio F, Zilhão R, Gama JA. Interactions between plasmids and other mobile genetic elements affect their transmission and persistence. *Plasmid* 2019;102:29–36.
100. Gama JA, Zilhão R, Dionisio F. Conjugation efficiency depends on intra and intercellular interactions between distinct plasmids: plasmids promote the immigration of other plasmids but repress co-colonizing plasmids. *Plasmid* 2017;93:6–16. ;
101. Dimitriu T, Misevic D, Lotton C, Brown SP, Lindner AB, *et al*. Indirect fitness benefits enable the spread of host genes promoting costly transfer of beneficial plasmids. *PLoS Biol* 2016;14:e1002478.
102. Nicoloff H, Andersson DI. Indirect resistance to several classes of antibiotics in cocultures with resistant bacteria expressing antibiotic-modifying or -degrading enzymes. *J Antimicrob Chemother* 2016;71:100–110.
103. Dimitriu T, Misevic D, Capelle JB, Lindner AB, Brown SP, *et al*. Selection of horizontal gene transfer through public good production. bioRxiv 2018. DOI: 10.1101/315960.
104. Chilley PM, Wilkins BM. Distribution of the ardA family of antirestriction genes on conjugative plasmids. *Microbiology* 1995;141 (Pt 9):2157–2164.
105. Mahendra C, Christie KA, Osuna BA, Pinilla-Redondo R, Kleinstiver BP, *et al*. Broad-spectrum anti-CRISPR proteins facilitate horizontal gene transfer. *Nat Microbiol* 2020;5:620–629.
106. Roy D, Huguet KT, Grenier F, Burrus V. IncC conjugative plasmids and SXT/R391 elements repair double-strand breaks caused by CRISPR-Cas during conjugation. *Nucleic Acids Res* 2020;48:8815–8827.
107. Pursey E, Dimitriu T, Paganelli FL, Westra ER, van Houte S. CRISPR-Cas is associated with fewer antibiotic resistance genes in bacterial pathogens. *Philos Trans R Soc Lond B Biol Sci* 2021;377:20200464.
108. Shehreen S, Chyou T-Y, Fineran PC, Brown CM. Genome-wide correlation analysis suggests different roles of CRISPR-Cas systems in the acquisition of antibiotic resistance genes in diverse species. *Philos Trans R Soc Lond B Biol Sci* 2019;374:20180384.
109. Corvaglia AR, François P, Hernandez D, Perron K, Linder P, *et al*. A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical *Staphylococcus aureus* strains. *Proc Natl Acad Sci U S A* 2010;107:11954–11958.
110. Li D, Li P, Peng M, Zhao X, Jiang X, *et al*. Transmission barrier of the blaKPC plasmid mediated by type I restriction-modification systems in *Escherichia coli*. *J Antimicrob Chemother* 2022;77:952–956.
111. Oliveira PH, Touchon M, Rocha EPC. Regulation of genetic flux between bacteria by restriction-modification systems. *Proc Natl Acad Sci U S A* 2016;113:5658–5663.
112. Stewart FM, Levin BR. The population biology of bacterial plasmids: a priori conditions for the existence of conjugationally transmitted factors. *Genetics* 1977;87:209–228.
113. Simonsen L. The existence conditions for bacterial plasmids: theory and reality. *Microb Ecol* 1991;22:187–205.
114. Lili LN, Britton NF, Feil EJ. The persistence of parasitic plasmids. *Genetics* 2007;177:399–405.
115. Lopatkin AJ, Meredith HR, Srimani JK, Pfeiffer C, Durrett R, *et al*. Persistence and reversal of plasmid-mediated antibiotic resistance. *Nat Commun* 2017;8:1689.
116. Bahl MI, Hansen LH, Licht TR, Sørensen SJ. Conjugative transfer facilitates stable maintenance of IncP-1 plasmid pKJK5 in *Escherichia coli* cells colonizing the gastrointestinal tract of the germfree rat. *Appl Environ Microbiol* 2007;73:341–343.
117. Hardiman CA, Weingarten RA, Conlan S, Khil P, Dekker JP, *et al*. Horizontal transfer of carbapenemase-encoding plasmids and comparison with hospital epidemiology data. *Antimicrob Agents Chemother* 2016;60:4910–4919.
118. van Hal SJ, Wiklendt A, Espedido B, Ginn A, Iredell JR. Immediate appearance of plasmid-mediated resistance to multiple

antibiotics upon antibiotic selection: an argument for systematic resistance epidemiology. *J Clin Microbiol* 2009;47:2325–2327.

119. Sidjabat HE, Heney C, George NM, Nimmo GR, Paterson DL. Interspecies transfer of blaIMP-4 in a patient with prolonged colonization by IMP-4-producing Enterobacteriaceae. *J Clin Microbiol* 2014;52:3816–3818.
120. Gumpert H, Kubicek-Sutherland JZ, Porse A, Karami N, Munck C, *et al*. Transfer and persistence of a multi-drug resistance plasmid *in situ* of the infant gut microbiota in the absence of antibiotic treatment. *Front Microbiol* 2017;8:1852.
121. León-Sampedro R, DelaFuente J, Díaz-Agero C, Crellen T, Musicha P, *et al*. Pervasive transmission of a carbapenem resistance plasmid in the gut microbiota of hospitalized patients. *Nat Microbiol* 2021;6:606–616.
122. Hall JPJ, Wood AJ, Harrison E, Brockhurst MA. Source-sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities. *Proc Natl Acad Sci U S A* 2016;113:8260–8265.
123. Alonso-Del Valle A, León-Sampedro R, Rodríguez-Beltrán J, DelaFuente J, Hernández-García M, *et al*. Variability of plasmid fitness effects contributes to plasmid persistence in bacterial communities. *Nat Commun* 2021;12:2653.
124. Baquero F, Coque TM, de la Cruz F. Ecology and evolution as targets: the need for novel eco-evo drugs and strategies to fight antibiotic resistance. *Antimicrob Agents Chemother* 2011;55:3649–3660.
125. Getino M, Sanabria-Ríos DJ, Fernández-López R, Campos-Gómez J, Sánchez-López JM, *et al*. Synthetic fatty acids prevent plasmid-mediated horizontal gene transfer. *mBio* 2015;6:e01032-15.
126. Palencia-Gándara C, Getino M, Moyano G, Redondo S, Fernández-López R, *et al*. Conjugation inhibitors effectively prevent plasmid transmission in natural environments. *mBio* 2021;12:e0127721.
127. Ojala V, Laitalainen J, Jalasvuori M. Fight evolution with evolution: plasmid-dependent phages with a wide host range prevent the spread of antibiotic resistance. *Evol Appl* 2013;6:925–932.
128. Liu G, Olsen JE, Thomsen LE. Identification of genes essential for antibiotic-induced up-regulation of plasmid-transfer-genes in cephalosporin esistant *Escherichia coli*. *Front Microbiol* 2019;10:2203.
129. Aminov RI. Horizontal gene exchange in environmental microbiota. *Front Microbiol* 2011;2:158.
130. Liu P, Wu Z, Xue H, Zhao X. Antibiotics trigger initiation of SCCmec transfer by inducing SOS responses. *Nucleic Acids Res* 2017;45:3944–3952.
131. Showsh SA, Andrews RE. Tetracycline enhances Tn916-mediated conjugal transfer. *Plasmid* 1992;28:213–224.
132. Beabout K, Hammerstrom TG, Wang TT, Bhatty M, Christie PJ, *et al*. Rampant parasexuality evolves in a hospital pathogen during antibiotic selection. *Mol Biol Evol* 2015;32:2585–2597.
133. Hall JPJ, Williams D, Paterson S, Harrison E, Brockhurst MA. Positive selection inhibits gene mobilisation and transfer in soil bacterial communities. *Nat Ecol Evol* 2017;1:1348–1353.
134. Gillings MR, Stokes HW. Are humans increasing bacterial evolvability? *Trends Ecol Evol* 2012;27:346–352.
135. MacLean RC, San Millan A. The evolution of antibiotic resistance. *Science* 2019;365:1082–1083.