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Antimicrobial resistance acquisition via natural transformation: context is everything

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Natural transformation is a process where bacterial cells actively take up free DNA from the environment and recombine it into their genome or reconvert it into extra-chromosomal genetic elements. Although this mechanism is known to mediate the uptake of antibiotic resistance determinants in a range of human pathogens, its importance in the spread of antimicrobial resistance is not always appreciated. This review highlights the context in which transformation takes place: in diverse microbiomes, in interaction with other forms of horizontal gene transfer and in increasingly polluted environments. This examination of the abiotic and biotic drivers of transformation reveals that it could be more important in the dissemination of resistance genes than is often recognised.

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Introduction

The widespread selection for antibiotic resistance genes (ARGs) through over and misuse of antimicrobials and exposure to pollutants that co-select for antimicrobial resistance (AMR) is one of the most pressing concerns in healthcare globally [1]. To mitigate the spread of ARGs within and between reservoirs of environmental bacteria and human pathogens, it is vital to gain a detailed understanding of the mechanisms that drive their transmission. One mechanism of horizontal gene transfer (HGT) is natural transformation, a process wherein cells take up DNA from the extracellular environment and incorporate it into their chromosome or reassemble it as part of the self-replicating episome [2]. Transformation is evolutionary conserved, phylogenetically widespread [3] and capable of mediating the acquisition of large tracts of DNA (7–50 kb) [4].

Importantly, transformation has been shown to result in the transfer of clinically relevant ARGs in a variety of human pathogens (Table 1). These examples notwithstanding, transformation is generally considered to not be as important in the transfer of ARGs compared to HGT mechanisms based on Mobile Genetic Elements (MGEs), particularly conjugation (e.g. Refs. [5,6]). Although this may be true, it is likely that the role of transformation in ARG dissemination is underappreciated. This could be due to two main reasons. First, transformation-mediated gene transfer is more difficult to quantify than most other HGT mechanisms. Unlike MGEs such as prophages or integrative conjugative elements, transformation does not leave distinct traces in the DNA of the recipient. Transformation can also result in the deletion, rather than the addition of DNA, which is undistinguishable from mutational loss. In addition, the presence of plasmids might often automatically be attributed to conjugation, whereas it is possible that some were taken up via transformation. Second, only a relatively small number of bacteria have been shown to take up DNA under laboratory conditions, likely because the specific physiological requirements to initiate the competence state required for transformation were not met [2,3]. Furthermore, in those species amenable to lab experiments, transformation rates could frequently be underestimated using standard antibiotic marker-based assays [7].

In this short review, we will focus on a host of exciting new studies that indicate that transformation could be more important than often is assumed, particularly in the context of AMR spread. Specifically, we will focus on the role of inter-strain and inter-species interactions, the interaction of transformation with MGEs and the effect of pollution on rates of transformation, specifically in the light of ARG spread.

Table 1

Examples of inter-species ARG transfer in human pathogens mediated by natural transformation.

Recipient species	Donor Species	ARGs	Antibiotics	Description	Reference
A. baumannii	*K. pneumoniae* CRKp, *Providencia rettgeri* M15758 (bla_{NDM-1}) and methicillin-resistant *Staphylococcus aureus* 'Cordobes' clone (SAC) (*mecA*)	bla_{TEM-1}, bla_{KPC-2}, bla_{SHV-11}, bla_{SHV-12}, bla_{KPC}, bla_{OXA-23}, bla_{NDM-1}, *mecA*	Meropenem, cefotaxime, ampicillin	Interspecies cell-free DNA was added to *A. baumannii* cultures and successfully transformed. Resulting transformants were resistant to multiple antibiotics	[46]
Enterococcus faecalis	*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella aberdeen*	Not specified	Kanamycin, ampicillin, tetracycline	Transfer of RP4 from chlorine-killed antibiotic-resistant bacteria to chlorine-injured bacteria via natural transformation.	[41]
Haemophilus influenzae, H. suis, H. parainfluenzae	*H. influenzae, H. suis, H. parainfluenzae*	Not specified	Streptomycin	DNA conferring streptomycin resistance from *Haemophilus* species was used to transform different species of Haemophilus	[49]
S. pneumoniae, S. oralis, S. mitis	*S. oralis, S. mitis, S. sanguis* and *S. constellatus, S. pneumoniae*	*parC* and *gyrA*	Fluoroquinolones (Pefloxacin and Sparfloxacin)	Cell-free DNA conferring fluoroquinolone resistance from *Streptococcus* species was used to transform multiple other *Streptococcus* species	[50]
Neisseria meningitidis	*N. cinerea and N. flavescens*	*penA*	Penicillin	Cell-free DNA conferring penicillin resistance from *Neisseria* species was used to transform *N. meningitidis*	[51]

Transformation and interactions between strains and species

Recent years have seen somewhat of a paradigm shift where it is no longer automatically assumed that transformation-mediated recombination is at the mercy of random death of donor bacteria that happen to be in the vicinity of recipient cells. Instead, it has become clear that recipients often take an active role in acquiring DNA [8]. For instance, *Vibrio cholerae* [9] and *Acinetobacter baylyi* [10] employ Type-VI secretion system (T6SS) to lyse other strains and species, freeing DNA which subsequently is taken up by transformation. Transformation efficiency is predicted to decrease with increasing genetic divergence because of the constraints imposed by homology-based recombination [11]. However, when strains obtain DNA for recombination by lysing other strains, increased divergence in toxin gene content facilitates lysis, making transformation-mediated recombination with more diverged donor strains more efficient than that with more closely related strains [12••]. Around half of all *V. cholerae* strains harbour temperate kappa phage K139 which is able to kill neighbouring non-lysogenic strains; genomic DNA thus released could be shown to be incorporated by the lysogenic host [13]. In this case, the lysogen acts at least partly in the interest of its host and thus could be hypothesised to function akin to T6SS. Both genetic mechanisms provide the host strain with an advantage because competitors are lysed (interference competition, a form of natural selection) but also because they may facilitate the targeted uptake and recombination of foreign DNA (akin to mate choice, a form of sexual selection [14•]).

Both phylogenetic distance and ecological specialisation generally form strong barriers to HGT [15–17] and transformation is most effective between related strains inhabiting the same (micro)niche. However, the vast number of bacterial cells means that HGT is a 'numbers game' and ARGs occasionally cross deep ecological and taxonomic divides [15,18]. Proteobacteria are prevalent in soils but also represent an important group of human pathogens and are thought to be an important conduit through which ARGs move between lineages and niches [16]. A recent genomic study on soil Actinomycetes and Proteobacteria builds a convincing case of gene transfer from Proteobacteria to Actinomycetes and back again, in what the authors term the 'carryback model' [19••]. Here, a Proteobacterium transfers a plasmid to an Actinomycete via conjugation, after which an actinobacterial transposon inserts into the proteobacterial plasmid. After cell death of the actinobacterial host, this DNA is released into the environment where it can be taken up by Proteobacteria through natural transformation, with the flanking proteobacterial sequence mediating efficient recombination of actinomycete ARGs into the chromosome. Good *in silico* evidence exists for all steps in this process and this study furthermore experimentally demonstrated the uptake of recombinant proteobacterial-actinomycete DNA by *A. baylyi* via natural transformation [19••].

Transformation and mobile genetic elements

Textbook explanations of HGT customarily list transformation, transduction and conjugation as distinct processes, but it is clear from the example above that HGT can progress via combinations of transformation

and MGE-based mechanisms as well. A good example of the blurred boundaries between HGT mechanisms are plasmids, which can be transferred via conjugation, but also via transformation, transduction [20] and vesicle transfer [21] (the latter of which is rarely listed among HGT mechanisms). As is the case for conjugation, transformation can facilitate plasmid spread to phylogenetically unrelated recipients [22], but the relative importance of conjugation versus transformation in plasmid spread has not received much attention. Distinct advantages and disadvantages could be listed for each mechanism. Disadvantages for conjugation include: a live donor cell and close cell–cell proximity are required, resident plasmids could suppress transfer of compatible plasmids, plasmid transfer rates can be slow [23], and some (smaller) plasmids require mobilization by co-resident conjugable elements. A main disadvantage of transformation relative to conjugation is that it is sensitive to the action of DNases and extracellular DNA degradation (although it has been argued that transformation is less sensitive to disinfection than conjugation in that it is not dependent on live cells [4]). Since transformation is based on the uptake of single strands of DNA, at least two complementary strands need to be taken up for successful plasmid reassembly in the new host. Therefore, plasmid transformation efficiency is expected to drop with the square of the plasmid size [24]. Indeed, plasmid transformation has been shown to be less effective than chromosomal DNA (e.g. Ref. [25]). When comparing transformation efficiency of genomic DNA versus plasmid DNA, plasmids have the advantage that there are no barriers of sequence homology (since no recombination is involved) and relative uptake efficiency could be increased when plasmid copy numbers in donor cells are high.

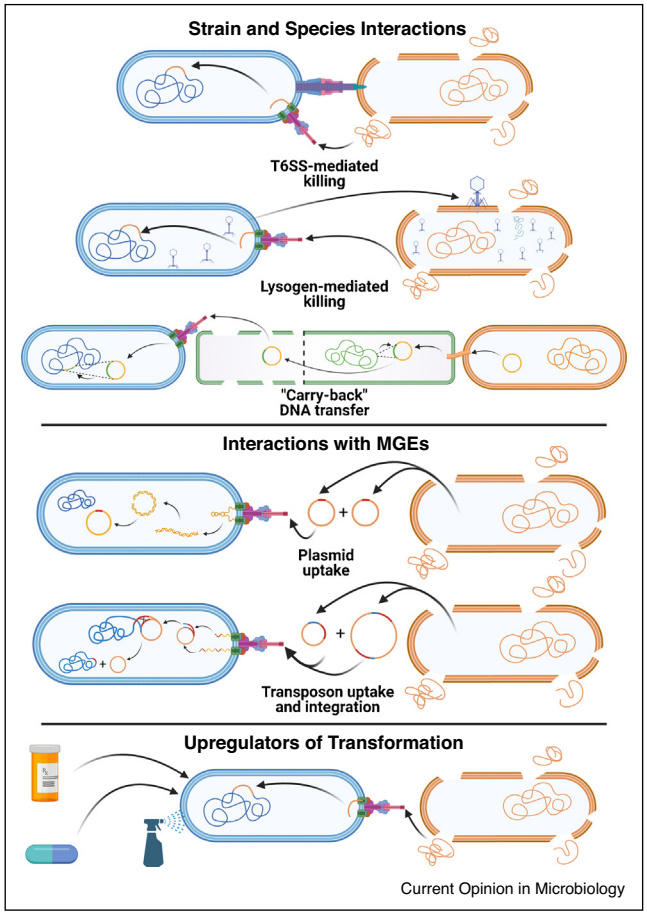
The transfer of genetic elements via transformation is not limited to plasmids. Recent experimental studies have shown that genetic elements harbouring ARGs that are not capable of their own mobilisation can be transferred via transformation as well. Experiments using *A. baylyi* have demonstrated that integrons on transposons or on IS elements [26], or ARGs located in fully heterologous DNA [27] can insert chromosomally. Efficient incorporation of the DNA requires sections of DNA with sequence identity or similarity and therefore likely depends on the cellular homologous recombination machinery. Genomic insertion efficiency decreases with increasing sequence heterology due to DNA mismatch repair [28] or other recombination obstacles such as disruption of synteny [29]. One-sided sequence homology may be sufficient in some prokaryotes to allow integration, by using a second recombination event between heterologous DNA molecules (homology-facilitated illegitimate recombination) [30,31]. In the absence of any homology, genomic integration of genes occurs rarely by mechanisms still poorly understood (double illegitimate recombination) [32]. As an interesting exception, transposable elements taken-up by a cell can insert themselves into the chromosome even without homology requirements [26], indicating that transformation of ARGs associated with transposons could be especially efficient. A recent study in *A. baylyi* demonstrated that the genes conferring the transposition are expressed when the foreign DNA containing these genes is temporarily protected from degradation in the cytoplasm [33•].

Finally, diverse conjugative elements have been shown to disable transformation in a range of species through a variety of pathways, including insertion directly into competence-related genes [34], sRNA-mediated silencing [35] or nuclease production [36]. It has been proposed that transformation could be selectively advantageous because of its tendency to remove MGEs from the chromosome via recombination of flanking sequences of donor DNA that lack the same MGEs [34]. Any MGE able to disrupt processes that could result in their removal thus is expected to be selectively favoured. Such interactions between transformation and MGE-based mechanisms of HGT seem pervasive across many bacterial species and are likely important drivers of bacterial genome evolution [4,34].

Transformation and anthropogenic pollution

Unprecedented, human-driven global changes increasingly affect the distribution, activity and diversity of microbial communities, and could even change the mode and rate of bacterial evolution itself [37]. It has been known for some time that stress imposed by antibiotics can upregulate competence [38,39]. Several recent studies have provided evidence that even disinfectants such as chlorine [40] and triclosan [41] found in low concentrations in contaminated environments can upregulate transformation, thereby enhancing the potential for ARGs to spread. One study found that a by-product of drinking water disinfection, bromoacetic acid, increased transformation frequency in *A. baylyi* in a dose-dependent manner [42]. This was found to be mediated via oxidative stress leading to upregulation of RecA. Mixtures of water disinfection by-products at environmentally relevant concentrations resulted in a low but significant increase in transformation frequency. Several non-antimicrobial drugs, including the commonly prescribed anti-inflammatory drug Diclofenac, were found to elevate the transformation rate of a plasmid containing ampicillin and tetracycline resistance genes in *A. baylyi* to a similar extent (1−3-fold) as in the afore-mentioned study [43•]. Again, this effect was mediated through an increase in reactive oxygen species, but in addition genes involved in the transformation process were found to be upregulated [43•]. As any type of cell damage can result in the stress responses linked to the upregulation of transformation, it is likely that other anthropogenic stressors, for example, metal pollution or increased solar irradiation, could also be important [42]. Finally, stressors can co-occur and

Figure 1



A summary of some the processes influencing transformation, with recipient cells in blue and donor cells in yellow. Mechanisms involving conspecific cells and other species (top panel) include T6SS-mediated killing, lysogen-mediated killing and 'back transfer' in conjunction with a third partner and conjugation. Mechanisms involving Mobile Genetic Elements (middle panel) include the uptake of plasmids and transposons. Finally, anthropogenic pollutants (bottom panel) can upregulate transformation. Mechanisms are explained in more detail in the main text.

additively or synergistically interact and apart from increasing transformation frequencies could simultaneously increase ARG transfer through the killing of donor cells [40].

Conclusions

This short review highlighted some of the diverse biotic and abiotic drivers of ARG spread by natural transformation (Figure 1). ARG transformation takes place in complex microbial communities, including in host microbiomes [44•], and both donor and recipient cells are influenced by myriad other strains, species and genetic elements. For instance, transformation can be enhanced through the action of lytic phages [45] releasing donor DNA, and at the same time can mediate the transfer of genetic elements such as lysogens [46]. Natural transformation is an underappreciated mechanism of gene transfer, which is demonstrated by the fact it was only very recently discovered to operate in the important opportunistic pathogen *Pseudomonas aeruginosa* [47•]. Transformation might be especially important in the transfer of plasmids between unrelated species [10,19••,22], playing an important role in paving 'highways of gene transfer' [17]. It is also increasingly clear that antimicrobials can result in increased transformation rates, as has been shown before for conjugation (e.g. Ref. [48]).

Can interventions to prevent ARG spread by transformation be imagined? Scope for this seems very limited due to the diverse and interconnected ways transformation governs gene transfer. Some have even argued the opposite: the deletion of ARG-containing MGEs from chromosomes could in theory be promoted by upregulating transformation [4]. It is possible that disinfection measures designed to reduce the exposure to AMR pathogens could be counterproductive by selecting for transformation-mediated ARG transfer. The same goes for phage therapy, which could result in (plasmid-borne) ARG release through cell lysis, enhancing transformation efficiency [45]. Transformation was the first mechanism of horizontal gene transfer to be uncovered, but new discoveries continue to be made at a rapid pace. A better understanding of the contexts in which natural transformation takes places will provide valuable insights into bacterial evolution in general and the conditions under which AMR is disseminated specifically.

Conflict of interest statement

Nothing declared.

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