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Mathematical modelling to study the horizontal transfer of antimicrobial resistance genes in bacteria: current state of the field and recommendations

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ABSTRACT

Antimicrobial resistance (AMR) is one of the greatest public health challenges we are currently facing. To develop effective interventions against this, it is essential to understand the processes behind the spread of AMR. These are partly dependent on the dynamics of horizontal transfer of resistance genes between bacteria, which can occur by conjugation (direct contact), transformation (uptake from the environment) or transduction (mediated by bacteriophages). Mathematical modelling is a powerful tool to investigate the dynamics of AMR, however its application to study the horizontal transfer of AMR genes is currently unclear. In this systematic review, we searched for mathematical modelling studies which focused on horizontal transfer of AMR genes. We compared their aims and methods using a list of predetermined criteria, and utilized our results to assess the current state of this research field. Of the 26 studies we identified, most focused on the transfer of single genes by conjugation in *Escherichia coli* in culture, and its impact on the bacterial evolutionary dynamics. Our findings highlight the existence of an important research gap on the dynamics of transformation and transduction, and the overall public health implications of horizontal transfer of AMR genes. To further develop this field and improve our ability to control AMR, it is essential that we clarify the structural complexity required to study the dynamics of horizontal gene transfer, which will require cooperation between microbiologists and modellers.

Keywords:

Antimicrobial resistance, horizontal gene transfer, mathematical modelling, epidemiology, microbiology

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INTRODUCTION

Antimicrobial resistance (AMR) is undeniably one of the greatest global public health challenges we are currently facing [1]. The recent discoveries on the spread of resistance genes for key antimicrobials such as NDM-1 for carbapenem resistance [2–4] suggest that to tackle this challenge, instead of only studying the spread of resistant bacteria, we must understand the processes by which individual resistance genes spread. The first is "vertical gene transfer", where genes are passed from parent to progeny during bacterial replication. The second, which is our focus here, is "horizontal gene transfer" (HGT). This allows bacteria to acquire genetic material, including AMR genes, from their environment or other bacteria [5–7]. There are three mechanisms of HGT. Firstly, "transformation" is the capacity of bacteria to intake genetic material from their environment. Secondly, "conjugation" occurs when two bacteria come into contact with each other and form a conjugative bridge, enabling direct exchange of genetic material. Finally, "transduction" occurs when a bacteriophage (a virus that can infect bacteria) replicates and packages a bacterial gene instead of its own genetic material, then acts as a vector and transfers this gene into another bacterium.

The consequences of HGT of AMR in a bacterial population are varied and can change depending on the setting that this process occurs in. Firstly, HGT can often be at the origin of new combinations of resistances to multiple antimicrobial in single bacteria strains [8]. This is amplified by the fact that it can occur both intra- and inter-species [9], therefore allowing for mixing between many different gene pools. Fortunately, these resistance mechanisms often impose a fitness cost which reduces the competitiveness of bacteria with AMR genes in settings where antibiotics are absent [10], thereby limiting the increase in the prevalence of these bacteria in the environment. Studying HGT of AMR can be further complicated by differences in transfer rates and importance of transfer mechanisms between bacterial species [11], with transformation for example being rare for *Staphylococcus aureus* [12] but common for *Neisseria gonorrhoea* [13], and by differences between rates estimated *in-vitro* and *in-vivo*, as was seen with transduction in *Staphylococcus aureus* [14]. Lastly, HGT dynamics appear to vary depending on the presence or absence of antibiotics in the surrounding environment [15–18], therefore requiring studies to be conducted in multiple settings to fully capture this process.

It is essential to fully understand HGT of AMR since it can impact the overall transmission of AMR and therefore the predicted effect of interventions against bacterial infections to varying degrees depending on the setting. A most striking example of this is phage therapy, where bacteriophages are proposed as antimicrobials. The risk is that therapeutic phages could perform transduction and increase the proportion of bacteria in the patient that carry a resistance gene. In that case, if the phage therapy treatment fails to clear all the bacteria this could leave the patient at a higher risk of antimicrobial-resistant bacteria infection [19,20]. In addition to the aforementioned differences between bacterial species, HGT mechanisms themselves are biologically complex. For example, the capacity to form a conjugative bridge generally requires the presence of a specific set of "*tra*" genes [21]. These can be transferred themselves, leading to an increase through time in the prevalence of bacteria that can perform conjugation. Transformation gene expression is extremely variable [6], therefore we cannot realistically assume that bacteria are able to perform transformation at all times. Finally, some phages can either undergo a "lytic cycle", where they immediately replicate upon infecting a bacterium, or a "lysogenic cycle", where they first integrate into the bacterial genome for a variable duration [12]. We therefore cannot represent transduction as a process that occurs at a constant rate through time, since this will depend on the variable phage cycles.

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HGT is therefore complex in its dynamics, and studying these requires appropriate tools. Mathematical modelling is often used to study infectious disease processes [22]. It provides a simulation environment that can be informed by real-life data, in which dynamics can be disentangled and easily studied. Mathematical models can be split into “deterministic models”, which always generate the same results for a given set of parameter values [22], and “stochastic models”, which generate variability in their results using random events [22]. Mathematical modelling is already being used to study AMR dynamics and their public health implications [23,24]. For example, it has been employed to study within-host bacterial dynamics (i.e. the bacterial processes that occur during colonisation or infection of a host) and derive conclusions on patterns of AMR seen in the population [25]. Consequently, it can provide novel insight into optimal strategies to combat AMR spread by analysing the effect that these have on the transmission dynamics [26]. However, existing models may not always capture the relevant and complex microbiological dynamics of HGT. In this systematic review, we aimed to find modelling studies which focus on HGT of AMR, to record their methods and research questions, and hence, to identify potential research gaps and areas for improvement in this field.

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METHODS

The methodology of our systematic review follows the recommended PRISMA guidelines [27].

Inclusion criteria:

In order to be included in this review, studies had to fulfil all of the following criteria:

1) Study the horizontal transfer of genes between bacteria
2) The genes studied must explicitly be identified as genes encoding antimicrobial resistance
3) Use at least one dynamic population model. A model is "dynamic" if it tracks the changes in the number of bacteria belonging to various populations (e.g. antibiotic-resistant and susceptible bacteria) over time

Screening process:

The entire screening process is summarised in Figure 1. We searched two databases using the following terms:

- PubMed search: "(antimicrobial OR antibacterial) resist* AND (horizontal transfer OR MGE OR plasmid OR transformation OR conjugation OR transduction OR phage) AND math* model*", 55 results
- Web of Science search: "TS = ((antimicrobial OR antibacterial) resist* AND (horizontal transfer OR MGE OR plasmid OR transformation OR conjugation OR transduction OR phage) AND math* model*)", 32 results

After removal of duplicates, these combined searches yielded a list of 69 studies. Both QL and GK independently screened the titles and abstracts of all 69 studies. 19 studies were retained by both authors, and two more were discussed and retained after an additional screen of the methods due to uncertainty, leading to a total of 21 studies retained after the first screening step.

The full texts of these 21 studies were then screened by QL, leading to 16 studies being retained as relevant for this review. Finally, by screening the reference lists in these 16 studies, ten more were included, leading to a total of 26 studies to discuss in this review.

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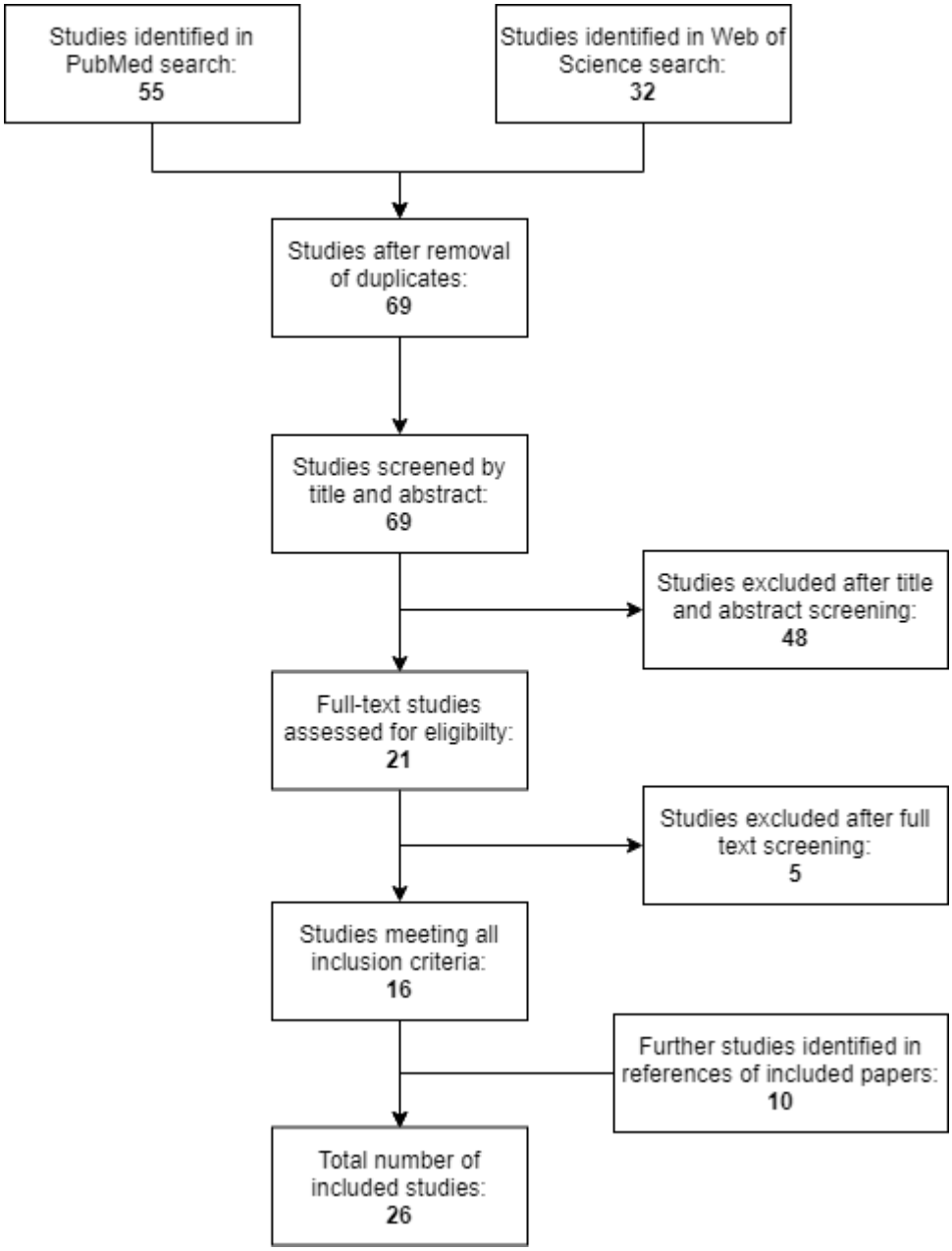

Figure 1. PRISMA flow diagram of the search and exclusion process.

Information extracted from the included studies:

To maximise comparability between studies, we devised a list of 11 elements to extract from every study. These are summarised and explained in Table 1.

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Table 1. Elements recorded from all included studies. Where no "Possible values" are given in the table, this indicates that the values were not restricted to a predetermined list.

RECORDED ELEMENT	SIGNIFICATION	POSSIBLE VALUES
Transfer mechanism	Biological mechanism of horizontal gene transfer modelled	"Conjugation" or "Transformation" or "Transduction"
Bacteria	Any species of bacteria explicitly modelled	-
Aim of the study	Whether the study looked at gene transfer to understand evolutionary trends seen in the bacterial population, or to understand its impact on public health, or both	"Evolutionary" or "Public Health" or "Both"
Bacterial environment	Any environment which contained bacteria in the model	-
Antibiotic effect considered	Whether the study quantified the effect of the presence or absence of antibiotic on its results	"Yes" or "No"
Multiple resistances considered	Whether the model(s) tracked multiple resistance genes that could be transferred separately	"Yes" or "No"
Fitness cost of resistance considered	Whether the model(s) included a fitness cost for bacteria carrying a resistance gene	"Yes" or "No"
Source of model parameters	Whether the study also generated its own experimental data to support its parameter values, or chose values informed by previous studies (which could be experimental studies or not), or assumed values.	"Experimental" and/or "External" and/or "Assumed"
Type of model	Whether the structure of the model(s) was deterministic or stochastic, or both (if the study presented more than one model)	"Deterministic" or "Stochastic" or "Both"
Type of parameter values	If the model(s) structure was deterministic, whether the parameter values were constant or were sampled from distributions before each model run (the "Sampled" option was always given to stochastic models)	"Constant" or "Sampled"
Sensitivity analysis performed	Whether the study performed any type of sensitivity analysis of the effect of model parameter values on the results	"Yes" or "No"

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RESULTS

The table showing all of the recorded elements from the 26 included studies can be found in the Supplementary Material of this paper.

Firstly, when looking at the transfer mechanism modelled by these studies, we observe that almost all exclusively focus on conjugation (24 out of 26) [28–51] (Figure 2). Of the remaining two, one focused on transformation [52], and one on transduction [53]. Additionally, more than half of the studies chose exclusively *Escherichia coli* (*E. coli*) as the bacteria in which to model the transfer processes [28,32,34,39–46,48,50,53] (Figure 2). It is also worth noting that a high number of studies do not model a specific organism, and instead indicate that they are looking at bacteria in general [29,30,35,36,47,49,51]. Finally, while four studies applied their model to more than one bacterial species [31,33,37,38], only one of these modelled two strains of bacteria simultaneously and captured inter-species transfer of resistance genes [37].

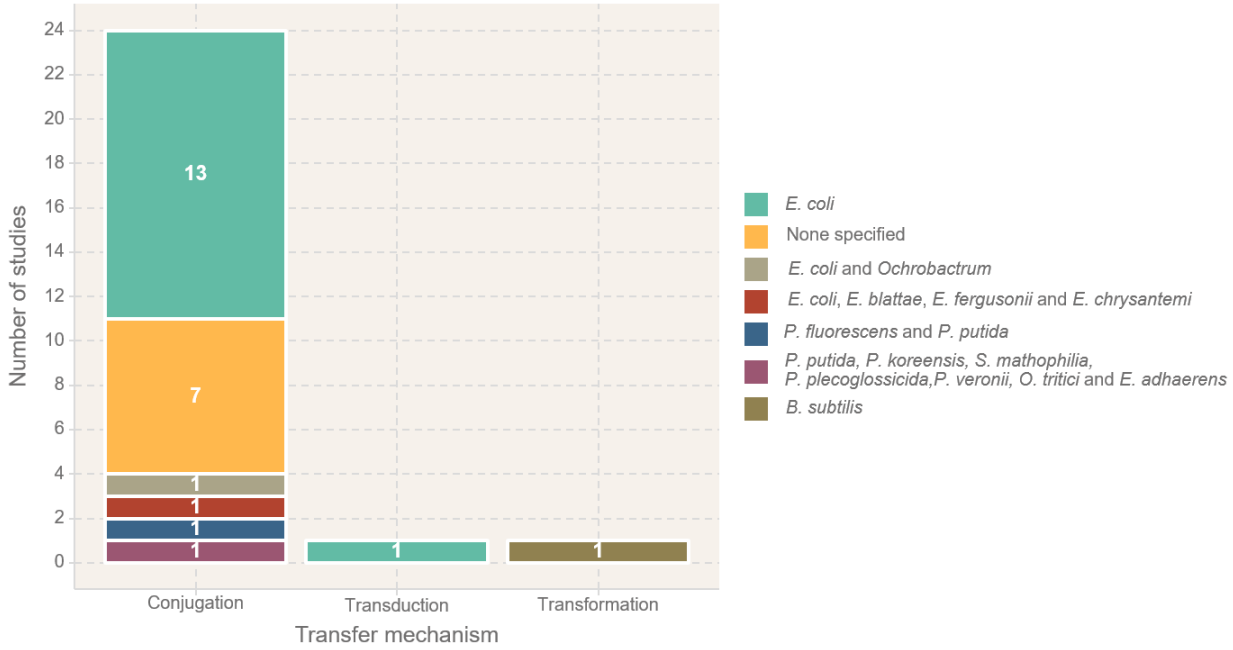

Figure 2. Transfer mechanisms and bacterial species modelled in the 26 studies included in our review.

In terms of the aims of these studies, all except four [30,46,47,51] used modelling approaches exclusively to improve the understanding of bacterial evolutionary dynamics (Figure 3). This covered questions such as how the prevalence of resistance genes in the bacterial population changes over time (as in [32] for example), or how the rise of multi-drug resistant bacteria varied under different environmental conditions (as in [28] for example). Inversely, the remaining four studies attempted to place at least some of their results in a public health setting by, for example, quantifying the impact of transfer on the incidence of multi-drug resistant bacteria infection in humans [30,51]. In accordance with this previous point, half of the studies modelled bacteria exclusively in culture [31–40,45,48,52], and only four modelled bacteria in humans [28,30,47,51] (Figure 3). Interestingly, more studies modelled bacteria in cattle-related environments than in humans [41–44,46,53]. In the remaining studies, two did not specify an environment for their bacteria [29,49].

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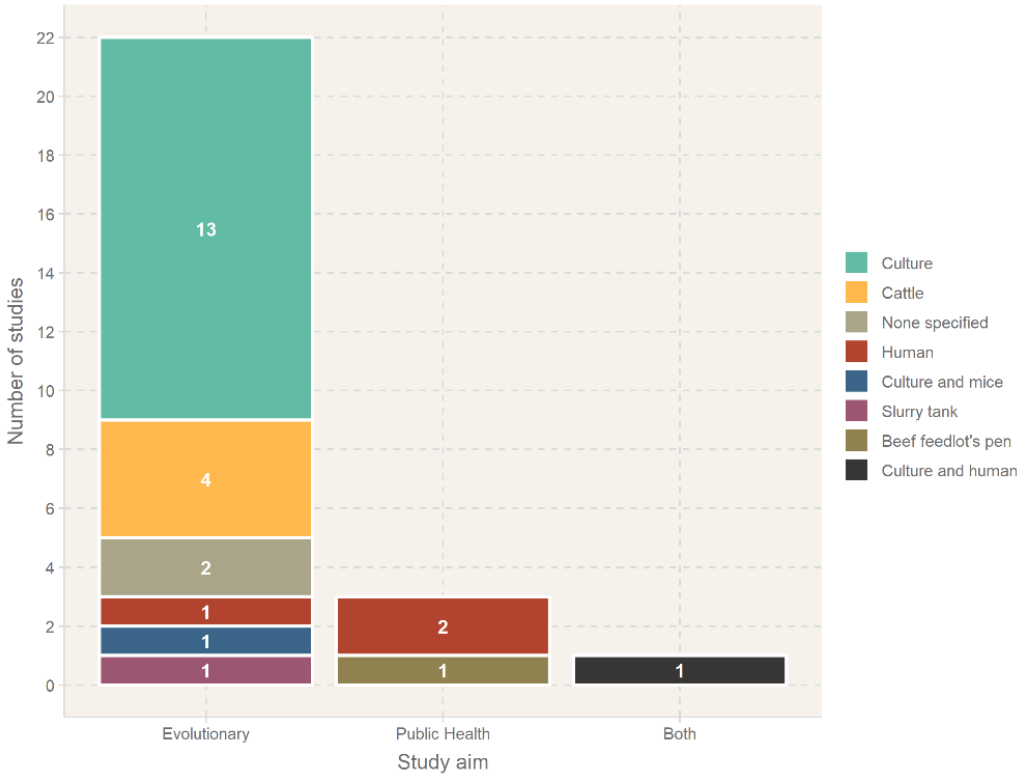

Figure 3. Aims and environments modelled in the 26 studies included in our review.

Almost all studies included a bacterial fitness cost for the carriage of a resistance gene in their models (Table 2), except for three [30,40,48]. On the other hand, despite the fact that in reality bacteria can acquire multiple AMR genes independently, only three studies included this feature [28,30,51] (Table 2). Lastly, it is important to note that approximately half of the studies did not consider the effect of antibiotics on transfer rates [31–34,37–40,45,48,50,53] (Table 2).

Table 2. Summary of the presence or absence of model characteristics in the 26 studies we reviewed.

	Include antibiotic effect	Include multiple AMR genes	Include fitness cost	Include sensitivity analysis
Yes	14	3	23	17
No	12	23	3	9

Half of these modelling studies (14/26) included their own experimental work to generate data and estimate at least some parameter values for their models [31–34,37–40,45,48,50,52] (Figure 4). On the other hand, more than half (16/26) chose to assume the values of at least some of their parameters, without explicitly citing any sources to support their choices, and almost a quarter assumed the values of all of their parameters [29,30,35,36,47,49]. As for the rest (11/26), they used previous studies to obtain at least some of their parameter values. For these, except for one study which was the direct follow-up of another one on the same topic [42], more than one previous study

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was taken to estimate the value of parameters, with a median number of studies of 8.5 and a maximum of 42.

Figure 4. Sources of parameter values in the 26 studies included in our review. “Assume” (top, green): no clear reference is given to support the choice of parameter value; “Experimental” (right, orange): the study generated its own experimental data to support the choice of parameter value; “External” (left, brown): the study references a previous study to support the choice of parameter value.

Finally, except for four [29,33,42,52], all of the studies exclusively relied on deterministic models to obtain their results. Out of these studies, only seven acknowledge variability in the parameter values by running their model multiple times and sampling parameters from distributions instead of assuming them to be constant [30,36,41,44,46,47,53]. Nevertheless, most studies performed sensitivity analyses of the effect of their parameter values on their model results (Table 2). Overall, seven studies still relied solely on a deterministic model without either sampling their parameter values or performing sensitivity analyses [28,31,34,38–40,50]. We also noted that except for the one study on transduction [53], all the studies modelled transfer as a mass-action process. This assumes that the number of transfer events is determined by multiplying the number of bacteria that can receive the gene, the number of bacteria that can transfer the gene, and the rate at which transfer occurs. This is therefore generally written as some form of $\beta*S*R/N$, where β is a rate of transfer, S is the number of bacteria that can receive the resistance gene, R is the number of bacteria that can provide the resistance gene, and N is the total bacterial population in the system.

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DISCUSSION

We used a systematic literature review of mathematical models of horizontal gene transfer (HGT) to determine our current understanding of the dynamics of HGT of AMR. The first main observation from our results is that the majority of studies assessed only focus on HGT by conjugation (24 out of 26). The likely reason for this is the simplicity in conjugation dynamics. Effectively, these are equivalent to the mass-action dynamics of an infection transmitted upon contact, such as influenza, where established modelling exists [22]. Consequently, modelling conjugation does not require much complexity to be added to these models. However, we know that transformation and transduction also heavily contribute to HGT [7] and the lack of studies on transduction and transformation is worrying [7,14]. These mechanisms fundamentally differ in their biology, making it essential to study each of them in their own modelling framework; it is unknown whether models of conjugation could be directly applied to transformation and transduction.

E. coli is the most commonly studied model organism for bacteria in general [54]. Its rapid growth and consistent behaviour in *in-vitro* settings make it perfect for experimental work, including transfer studies, therefore its overwhelming presence as the organism of choice for studies modelling HGT of AMR genes is not a surprise. However, HGT is known to occur with varying rates in multiple bacterial species, consequently it is unlikely that the rates of transfer estimated by looking at *E. coli* are equally applicable to other bacterial species [7]. In addition, HGT of AMR is also a process that can also occur between bacterial species [9,11], while most models here exclusively focused on *E. coli* alone. Some resistances in bacterial species are in fact thought to have been originally acquired following a gene transfer event with another species, such as the *mecA* resistance gene in *Staphylococcus aureus* acquired from *S. fleurettii* [55].

Despite the fact that the carriage of an AMR gene often imposes a reduction in the growth rate of the bacteria [10], a few studies did not model this (3/26), but only one argued that this element could be ignored after fitting their model to experimental data [48]. However, this was once more only based on observations *in-vitro*, which are likely to differ from the *in-vivo* reality. Including a fitness cost, while requiring the estimation of an additional parameter, does not add any particular complexity to the model structure itself, effectively only requiring a reduced growth rate value for the bacteria carrying AMR genes as opposed to bacteria susceptible to the modelled antibiotic (as can be seen in [50] for example), and should therefore be included at least for sensitivity analyses. In addition, although it is understandable that the first models of HGT of AMR should focus on tracking single genes to understand the basic dynamics of this process, in reality many bacteria carry multiple AMR genes that can move independently [8]. However, we only identified three studies in our review which included more than one AMR gene in their model [28,30,51].

Many studies did not allow for the presence of an antibiotic in their model. However, antibiotics are likely to modify HGT dynamics by directly affecting transfer rates as well as the survival of bacteria not carrying the AMR gene [16–18]. This has been shown to occur for transduction in *S. aureus*, where the addition of antibiotics induced a higher proportion of transducing phage compared to lytic phage [56]. On the other hand, some studies correctly argue that it is equally important to understand the dynamics of HGT in the absence of antibiotics. Effectively, it is common for bacterial populations to rapidly transition between being exposed to antibiotics or not, with the most obvious example being individuals transiently consuming antibiotics. Consequently, understanding the dynamics of HGT of AMR both in the presence and absence of antibiotics is essential.

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HGT of AMR has been studied in laboratory setting, consequently data around which models can be built has been generated and is available. Naturally, using these external data sources for purposes they were not originally designed for can require assumptions to be made in the model structure and parameters. In addition, it is essential to bear in mind how these data were originally collected, since for example combining sources which look at bacteria in multiple environments to derive parameters in a single environment-specific model is far from ideal. On the other hand, the fact that almost a quarter of the studies we reviewed (6/26) assumed all of their parameter values is worrying. While the purpose of some of these studies was to exclusively test a range of parameter values to identify conditions for a specific event to occur (e.g. AMR prevalence increases), the absence of any clear sources for the limits of these ranges is questionable.

Regarding model structures, the majority of studies relied on deterministic models. To allow variability in the dynamics and therefore increased realism, studies more often choose to sample their parameter values, run their deterministic model, and repeat this process a number of times (as can be seen in [30,36,41,44,46,47,53]), a simpler alternative to developing new stochastic models. Sensitivity analysis is extremely important in any case since the size of the modelled bacteria population is often above 10^6; a small change in parameter value can lead to a greater change in the results. Despite this, seven studies exclusively relied on a deterministic model without sampling parameters or performing sensitivity analyses [28,31,34,38–40,50]. Interestingly, except for one [28], all of these are studies which also generated their own parameter values experimentally. Although they capture variation when measuring the parameters experimentally, often providing distributions for their values, they then only retain fixed point estimates for their corresponding model parameter values instead of sampling them from these distributions, and only use these fixed estimates to derive their conclusions. Acknowledging variability in microbiological observations by specifying distributions rather than point estimates is essential, and this must be represented in the corresponding mathematical models.

This also raises the question of how to best represent these microbiological events in mathematical models. Effectively, almost all of the models here describe transfer as a mass-action process (25/26). However, as stated above this approach is acceptable for conjugation, but might not fully apply to transformation, where transfer depends on the density of DNA in the surrounding environment rather than the number, and transduction, which follows vector-like dynamics with the phage acting as carriers of resistance genes between bacteria. Unfortunately, the latter has only been studied once in *E. coli* in cattle, without any experimental support for many parameter values [53]. The degree of modelling complexity required to accurately represent HGT is therefore unclear. This is also true for models designed to understand the public health implications of HGT of AMR genes, for which the level of detail required to represent within-host dynamics be clarified. In addition, since transfer dynamics have thus far been mostly studied in bacterial culture, mostly “short” time-frames have been explored (hours or days), with long term dynamics remaining unclear despite our knowledge that even resistant bacteria can colonise us for weeks or months [57–59]. To best guide our public health policies with mathematical modelling, we must first clarify the complexity of the process we are actually attempting to model, and the time required to fully capture its *in-vivo* dynamics.

This is the first attempt at providing an overview of existing mathematical modelling work on HGT of AMR genes. Our systematic review methods, with two individuals separately screening titles and abstracts of candidate studies, allowed us to identify and bring together key studies on this topic.

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Using our list of comparison elements, we extracted and contrasted essential information between studies, overall allowing us to obtain a broad overview of the field and identify research gaps. However, our approach also has some limitations. Firstly, it was necessary for us to specify "math* model*" rather than just "model*" in the search, since otherwise it would have returned results on experimental models (e.g. mice) as opposed to mathematical models. Effectively, repeating our search with "model*" instead of "math* model*" yields 1,860 and 578 results on PubMed and Web of Science respectively, as opposed to our 54 and 30 results. The consequence of our choice however was that ten relevant studies were missed in the search, and were only identified by screening the references of already included studies. These ten studies were missed in the original literature search due to the absence of at least one of the search terms, with some studies for example referring to their models as "mass action models" instead of "mathematical models". In terms of study comparisons, although we originally considered this, we were unable to extract any meaningful quantitative data (e.g. estimated gene transfer rates) common to all studies due to the high variability of study designs. This variability also prevented us from identifying common measures of study quality we could report aside from the presence or absence of sensitivity analysis.

This systematic review allowed us to identify key research gaps on the dynamics of HGT of AMR. Firstly, we recommend that future studies should focus on developing models of transformation and transduction to determine the required complexity to represent these dynamics. In parallel, since the basic dynamics of conjugation are already reasonably well understood, future studies on this mechanism should focus on other bacterial species than *E. coli*, preferably in a setting where inter-specific HGT and the movement of multiple, separate AMR genes can both be observed. The optimal solution to address these research questions would be to design frameworks to study HGT of AMR that encompass both laboratory and modelling work; this would ensure that the data collected are appropriate for the modelling needs, and that the actual model is a good representation of the situation measured in the laboratory. We therefore believe that, to fully understand the complexity of both the biology and the dynamics of HGT, collaboration of both microbiologists and mathematical modellers would be the best strategy for future research on this topic, and that studies should attempt to generate both their own data and models to reduce the assumptions they require.

Studying the effect of HGT of AMR on bacterial evolutionary dynamics is a necessary first step to understand the overall importance of this process. This has been the focus of the vast majority of the studies identified in this review, however the public health implications remain vastly unknown. This is related to the observation that the majority of studies model bacteria in an *in-vitro* setting; to understand the public health impact of HGT of AMR, it is essential to expand this to include other bacterial environments such as within humans and animals. In addition, important differences have been identified between transfer rates estimated *in-vitro* and *in-vivo*, with *in-vivo* transduction rates in *S. aureus* for example being much higher than expected [14]. This difference in dynamics is attributable to the fact that *in-vitro* conditions fail to capture essential biological mechanisms influencing bacteria and therefore HGT [6,10]. We therefore recommend that future studies should evaluate HGT of AMR in more complex scenarios than *in-vitro* cultures, perhaps using *in-vivo* models such as mice [50], to truly assess the potential consequences of this phenomenon on human well-being.

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CONCLUSIONS

In this systematic review, we aimed to assess the current state of mathematical modelling as a tool to improve our understanding of horizontal gene transfer of antimicrobial resistance. From the 26 studies identified, we found that the majority focused on conjugation in *E. coli*, exploring evolutionary dynamics of HGT in culture. Whilst this provides a solid base for a key method of HGT, future work must also consider HGT by transformation and transduction which drive most of the HGT in other bacteria. Importantly for public health implications, only one bacterial species was considered in most models when we know that inter species transfer is responsible for many of our epidemic AMR clones and much of the work was fitted to data in the absence of antibiotic exposure. Crucially, to answer these questions we must first clarify the level of modelling complexity required to accurately represent HGT dynamics. This complex topic requires close collaboration between mathematical modellers and microbiologists in order to determine the full impact of these processes on our ability to control the public health threat posed by antimicrobial resistance.

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