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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 the extent of its use 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 utilised our results to assess the current state of this research field. Of the 43 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

## 34 INTRODUCTION

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

48 The consequences of HGT of AMR in a bacterial population are varied and can change depending on  
49 the setting that this process occurs in. Firstly, HGT can often be at the origin of new combinations of  
50 resistances to multiple antimicrobials in single bacteria strains [8]. This is amplified by the fact that  
51 HGT can occur both intra- and inter-species [9], therefore allowing for mixing between many different  
52 gene pools. Fortunately, these resistance mechanisms often impose a fitness cost which reduces the  
53 competitiveness of bacteria with AMR genes in settings where antibiotics are absent [10], thereby  
54 limiting the increase in the prevalence of these bacteria in the environment. Studying HGT of AMR can  
55 be further complicated by differences in transfer rates and importance of transfer mechanisms  
56 between bacterial species [11], with transformation for example being rare for *Staphylococcus aureus*  
57 [12] but common for *Neisseria gonorrhoea* [13], and by differences between rates estimated *in-vitro*  
58 and *in-vivo*, as was seen with transduction in *Staphylococcus aureus* [14] and conjugation in *Klebsiella*  
59 *pneumoniae* and *Escherichia coli* [15]. Lastly, HGT dynamics appear to vary depending on the presence  
60 or absence of antibiotics in the surrounding environment [16–20], therefore requiring studies to be  
61 conducted in multiple settings to fully capture this process.

62 It is essential to fully understand HGT of AMR since it can impact the overall transmission of AMR, and  
63 therefore the predicted effect of interventions against bacterial infections, to varying degrees  
64 depending on the setting. A most striking example of this is phage therapy, where bacteriophages are  
65 proposed as antimicrobials. A risk is that therapeutic phages could perform transduction and increase  
66 the proportion of bacteria in the patient that carry a resistance gene. In that case, if the phage therapy  
67 treatment fails to clear all the bacteria this could leave the patient at a higher risk of antimicrobial-  
68 resistant bacteria infection [21,22]. In addition to the aforementioned differences between bacterial  
69 species, HGT mechanisms themselves are biologically complex. For example, the capacity to form a  
70 conjugative bridge generally requires the presence of a specific set of “*tra*” genes [23]. These can  
71 themselves be transferred, leading to an increase through time in the prevalence of bacteria that can  
72 perform conjugation. Transformation gene expression is extremely variable depending on the  
73 environmental conditions that bacteria are exposed to [6], therefore we cannot realistically assume  
74 that bacteria are able to perform transformation at all times. Finally, some phages can either undergo  
75 a “lytic cycle”, where they immediately replicate upon infecting a bacterium, or a “lysogenic cycle”,

76 where they first integrate into the bacterial genome for a variable duration [12]. Consequently,  
77 transduction dynamics can be further complicated by the characteristics of the phage life cycle.

78 HGT is therefore complex in its dynamics, and studying these requires appropriate tools. Mathematical  
79 modelling is often used to study infectious disease processes [24]. It provides a simulation  
80 environment that can be informed by real-life data, in which dynamics can be disentangled and easily  
81 studied. Mathematical models can be split into “deterministic models”, which always generate the  
82 same results for a given set of parameter values [24], and “stochastic models”, which generate  
83 variability in their results using random events [24]. Mathematical modelling is already being used to  
84 study AMR dynamics and their public health implications [25,26]. For example, it has been employed  
85 to study within-host bacterial dynamics (i.e. the bacterial processes that occur during colonisation or  
86 infection of a host) and derive conclusions on patterns of AMR seen in the host population [27].  
87 Consequently, it can provide novel insight into optimal strategies to combat AMR spread by analysing  
88 the effect that these have on the transmission dynamics [28]. However, existing models may not  
89 always capture the relevant and complex microbiological dynamics of HGT. In this systematic review,  
90 we aimed to find modelling studies which focus on HGT of AMR, to record their methods and research  
91 questions, and hence, to identify potential research gaps and areas for improvement in this field.

92 **METHODS**

93 The methodology of our systematic review follows the recommended PRISMA guidelines [29].

94 Inclusion criteria:

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

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

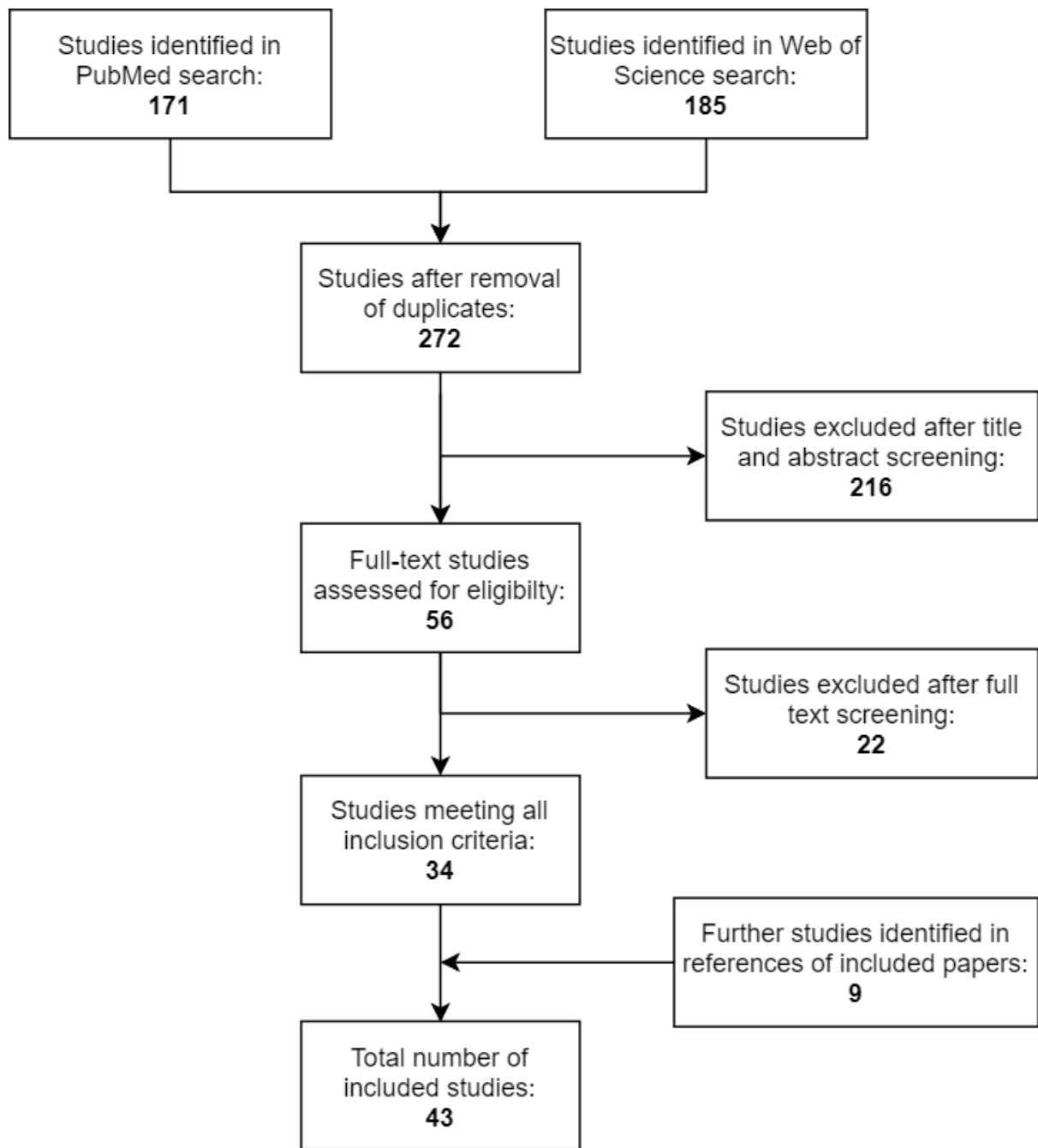
101 Screening process:

102 The entire screening process is summarised in Figure 1. We searched two databases on the 26<sup>th</sup> of  
103 April 2019 using the following terms:

- 104 - PubMed search: “(antimicrobial OR antibacterial OR antibiotic) resist\* AND (horizontal  
105 transfer OR mobile genetic element OR plasmid OR transformation OR conjugation OR  
106 transduction OR phage) AND (math\* OR dynamic\*) model\*”, 171 results
- 107 - Web of Science search: “TS = ((antimicrobial OR antibacterial OR antibiotic) resist\* AND  
108 (horizontal transfer OR mobile genetic element OR plasmid OR transformation OR  
109 conjugation OR transduction OR phage) AND (math\* OR dynamic\*) model\*)”, 185 results

110 After removal of duplicates, these combined searches yielded a list of 272 studies. Both QL and GK  
111 independently screened the titles and abstracts of all 272 studies. 54 studies were retained by both  
112 authors, and two more were discussed and retained after an additional screen of the methods due to  
113 uncertainty, leading to a total of 56 studies retained after the first screening step.

114 The full texts of these 56 studies were then screened by QL, leading to 34 studies being retained as  
115 relevant for this review. Finally, by screening the reference lists in these 34 studies, nine more were  
116 included, leading to a total of 43 studies to discuss in this review.



117

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

119

120 Information extracted from the included studies:

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

123

124

125

126

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

| <b>RECORDED ELEMENT</b>                      | <b>SIGNIFICATION</b>   | <b>POSSIBLE VALUES</b>                              |
|--|--|---|
| <b>Transfer mechanism</b>                    | Biological mechanism of horizontal gene transfer modelled  | “Conjugation” or “Transformation” or “Transduction” |
| <b>Bacteria</b>                              | Any species of bacteria explicitly modelled  | -   |
| <b>Aim of the study</b>                      | 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”         |
| <b>Bacterial environment</b>                 | Any environment which contained bacteria in the model  | -   |
| <b>Antibiotic effect considered</b>          | Whether one or more antibiotic(s) were present in the model(s)   | “Yes” or “No”                                       |
| <b>Multiple resistances considered</b>       | Whether the model(s) tracked multiple resistance genes that could be transferred separately  | “Yes” or “No”                                       |
| <b>Fitness cost of resistance considered</b> | Whether the model(s) included a fitness cost for bacteria carrying a resistance gene   | “Yes” or “No”                                       |
| <b>Source of model parameters</b>            | 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”   |
| <b>Type of model</b>                         | 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”           |
| <b>Type of parameter values</b>              | If the model(s) structure was “Deterministic”, whether the parameter values were constant or were sampled from distributions before each model run   | “Constant” or “Sampled”                             |
| <b>Sensitivity analysis performed</b>        | Whether the study performed any type of sensitivity analysis of the effect of model parameter values on the results  | “Yes” or “No”                                       |

129

130

131 Note that in our analysis, “Type of parameter values” and “Sensitivity analysis performed” are two  
132 independent criteria. We can therefore report that a study only uses “Constant” parameter values,  
133 yet still performs a sensitivity analysis. If a study is reported to have “Sampled” parameters, this  
134 means that the values of the parameters vary for each model run, and that this is represented in  
135 the main results, with figures showing model output with ranges instead of single lines for  
136 example. If a sensitivity analysis was performed, this means that the authors report conducting  
137 such a procedure to support their findings (e.g. to argue that their choice of “Constant” parameter  
138 values is a reasonable assumption, and does not significantly affect their results).

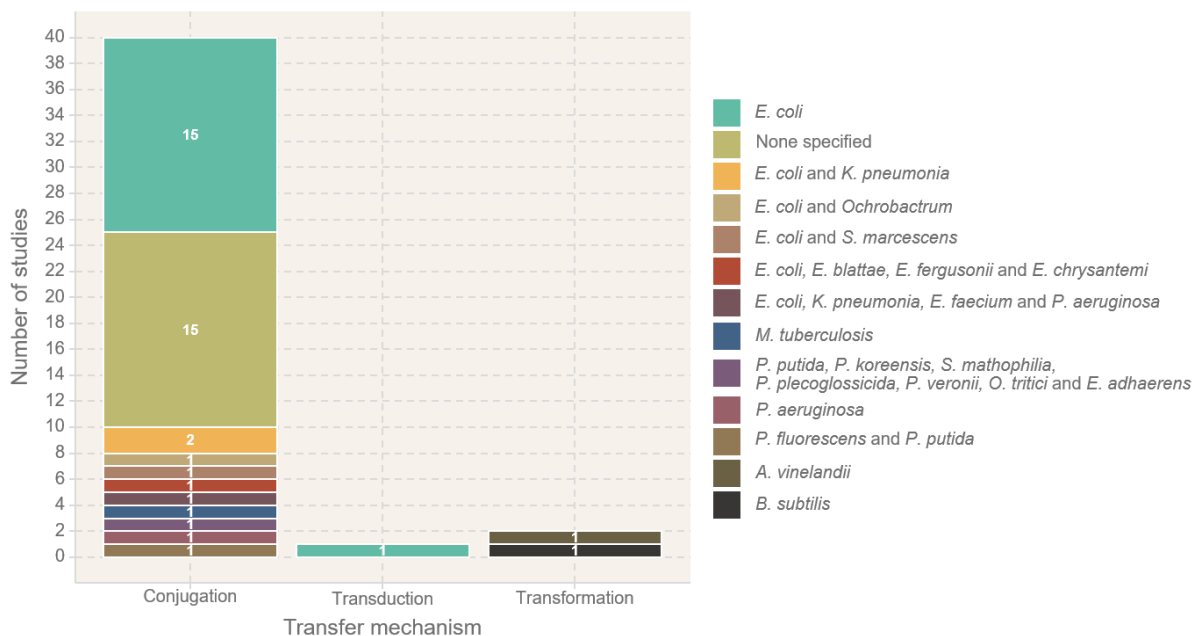


139 **RESULTS**

140 The table showing all of the recorded elements from the 43 included studies can be found in the  
 141 Supplementary Material of this paper.

142 Firstly, when looking at the transfer mechanism modelled by these studies, we observe that almost all  
 143 exclusively focus on conjugation (40 out of 43) [30–69] (Figure 2). Of the remaining three, two focused  
 144 on transformation [70,71], and one on transduction [72]. Additionally, more than a third of the studies  
 145 (16/43) chose exclusively *Escherichia coli* (*E. coli*) as the bacteria in which to model the transfer  
 146 processes [30,34,36,41–46,52,53,59,64,66,68,72] (Figure 2). It is also worth noting that another third  
 147 of the studies (15/43) do not model a specific organism, and instead indicate that they are looking at  
 148 bacteria in general [31,32,37,38,48,51,54,56–58,61,62,65,67,69]. Finally, while eight studies applied  
 149 their model to more than one bacterial species [33,35,39,40,47,49,60,63], only four of these modelled  
 150 two strains of bacteria simultaneously and captured inter-species transfer of resistance genes  
 151 [39,49,60,63].

152



153

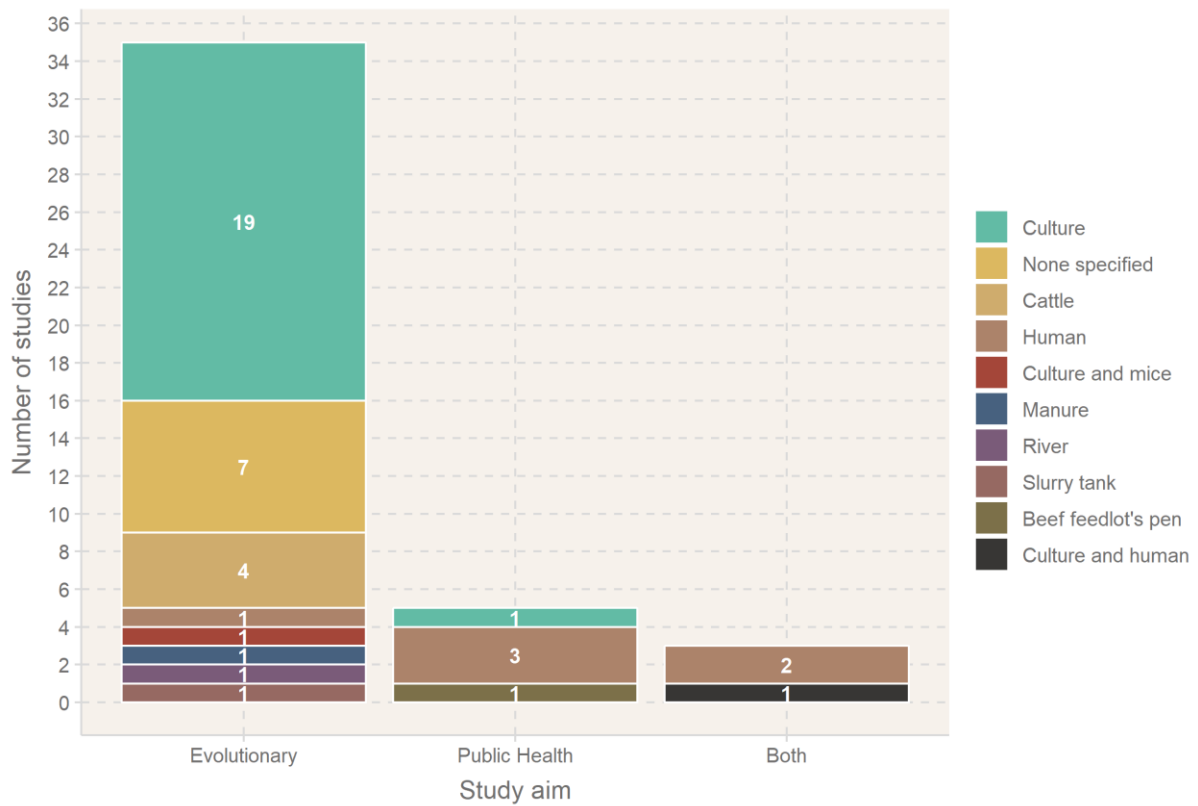
154 **Figure 2.** Transfer mechanisms and bacterial species modelled in the 43 studies included in our review.

155

156 In terms of the aims of these studies, all except eight [32,55,58,60,63–65,69] used modelling  
 157 approaches exclusively to improve the understanding of bacterial evolutionary dynamics (Figure 3).  
 158 This covered questions such as how the prevalence of resistance genes in the bacterial population  
 159 changes over time (as in [34] for example), or how the rise of multi-drug resistant bacteria varied  
 160 under different environmental conditions (as in [30] for example). Inversely, the remaining eight  
 161 studies [32,55,58,60,63–65,69] attempted to place at least some of their results in a public health  
 162 setting by, for example, quantifying the impact of transfer on the incidence of multi-drug resistant  
 163 bacteria infection in humans [32,69]. In accordance with this previous point, almost half of the studies  
 164 (20/43) modelled bacteria exclusively in culture [33–42,47,49,50,52,53,58,59,66,70,71], and only

165 seven modelled bacteria in humans [30,32,55,60,63,65,69] (Figure 3). In the remaining studies, seven  
 166 did not specify an environment for their bacteria [31,48,56,57,61,62,67].

167



168

169 **Figure 3.** Aims and environments modelled in the 43 studies included in our review.

170

171 Almost all of the studies included a bacterial fitness cost for the carriage of a resistance gene in their  
 172 models (Table 2), except for six [32,42,48,63,66,71]. On the other hand, despite the fact that in reality  
 173 bacteria can acquire multiple AMR genes independently (i.e. the acquisition of each gene is a separate  
 174 HGT event), only four studies included this feature [30,32,60,69] (Table 2). Lastly, it is important to  
 175 note that almost half of the studies did not model the presence of antibiotics, and therefore did not  
 176 consider the effect of antibiotics on transfer rates [33–36,39–42,47,52,53,59,63,66,68,71,72] (Table  
 177 2).

178

179 **Table 2.** Summary of the presence or absence of model characteristics in the 43 studies we reviewed.

|            | Include antibiotic effect | Include multiple AMR genes | Include fitness cost | Include sensitivity analysis |
|------------|---------------------------|----------------------------|----------------------|------------------------------|
| <b>Yes</b> | 26                        | 4                          | 37                   | 29                           |
| <b>No</b>  | 17                        | 39                         | 6                    | 14                           |

180

181 Almost half of these modelling studies (19/43) included their own experimental work to generate data  
182 and estimate at least some parameter values for their models [33–36,39–42,47,49,51–  
183 54,59,66,68,70,71] (Figure 4). On the other hand, more than half (23/43) chose to assume the values  
184 of at least some of their parameters, without explicitly citing any sources to support their choices, and  
185 a quarter (12/43) assumed the values of all of their parameters [31,32,37,38,65,67]. Finally, a third  
186 (15/43) used previous studies to obtain at least some of their parameter values. For these, except for  
187 three studies (two of which were each the direct follow-up of another one on the same topic [44,50],  
188 and one an analysis of data collected during an outbreak [63]), more than one previous study was  
189 taken to estimate the value of parameters, with a median number of studies of 8 and a maximum of  
190 42.

191



192

193 **Figure 4.** Sources of parameter values in the 43 studies included in our review. “Assume” (top,  
194 green): no clear reference is given to support the choice of parameter value; “Experimental” (right,  
195 orange): the study generated its own experimental data to support the choice of parameter value;  
196 “External” (left, brown): the study references a previous study to support the choice of parameter  
197 value. Studies in an overlap region used each of the corresponding methods at least once to  
198 estimate the value of their parameters.

199

200 Finally, more than three quarters of the studies (33/43) exclusively relied on deterministic models to  
201 obtain their results [30,32,34,36–40,42,43,45,47–51,53–56,58,59,61,63–69,71–73]. All of these  
202 deterministic models were composed of a set of ordinary differential equations to track the different  
203 sub-populations (susceptible bacteria, resistant bacteria etc...) through time. As for the ten studies  
204 which relied on stochastic models [31,33,35,41,44,52,57,60,62,70], most of these were agent-based  
205 models, where the bacteria were tracked individually [31,33,41,52,57,60], while the remaining ones  
206 either used stochastic differential equations [44,62,70] or difference equations [35]. Out of the studies  
207 which exclusively used deterministic models, only eight acknowledge variability in the parameter  
208 values by running their model multiple times and sampling parameters from distributions instead of  
209 assuming them to be constant [32,38,43,46,56,64,65,72]. Nevertheless, most studies performed  
210 sensitivity analyses of the effect of their parameter values on their model results (Table 2). Overall,  
211 nine studies still relied solely on a deterministic model without either sampling their parameter values  
212 or performing sensitivity analyses [30,36,40,42,48,54,55,58,68]. We also noted that except for the one  
213 study on transduction [72], all the studies modelled transfer as a mass-action process. This assumes  
214 that the number of transfer events is determined by multiplying the number of bacteria that can  
215 receive the gene, the number of bacteria that can transfer the gene, and the rate at which transfer  
216 occurs. This is therefore generally written as some form of  $\beta \cdot S \cdot R / N$ , where  $\beta$  is a rate of transfer, S is  
217 the number of bacteria that can receive the resistance gene, R is the number of bacteria that can  
218 provide the resistance gene, and N is the total bacterial population in the system.

219 **DISCUSSION**

220 We used a systematic literature review of mathematical models of horizontal gene transfer (HGT) to  
221 determine our current understanding of the dynamics of HGT of AMR. The first main observation from  
222 our results is that the majority of studies assessed only focus on HGT by conjugation (40 out of 43).  
223 The likely reason for this is the simplicity of conjugation dynamics. Effectively, these are comparable  
224 to infections transmitted upon contact, such as influenza, where established modelling exists using  
225 mass-action dynamics [24]. Consequently, modelling conjugation does not require much complexity  
226 to be added to these models. However, we know that transformation and transduction also contribute  
227 to HGT [7,14] and the lack of studies on these mechanisms is worrying.

228 Conjugation, transformation and transduction fundamentally differ in their biology, making it essential  
229 to study each of them in their own modelling framework; it is unknown whether models of conjugation  
230 could be directly applied to transformation and transduction. When looking at the studies which  
231 attempted to model these two processes, we first see that the one which focused on transduction  
232 [72] attempted to place it in a complex setting, with the phage able to undergo both lytic and lysogenic  
233 cycle, and the possibility for some bacteria to be resistant to phage infection. Transduction is  
234 represented as a multi-step process in this model, as opposed to relying on a single rate. The phage  
235 must first successfully infect a bacterium, then pick up a resistance gene, before successfully  
236 transferring this gene to a different bacterium. This model aims to accurately represent most of the  
237 biological complexity of transduction, which necessarily requires many assumptions regarding  
238 parameter values. Further study of this trade-off would be greatly beneficial; it is currently unclear  
239 whether this complexity is required, at the cost of more assumptions, or if the process of transduction  
240 could be simplified and modelled using fewer parameters, which could be estimated from  
241 experimental data. The two studies which focused on transformation [70,71] applied similar mass-  
242 action dynamics to this process as what can be seen in models of conjugation. However, this approach  
243 assumes that the number of resistance genes available in the environment is equivalent to the number  
244 of bacteria carrying these genes. This is questionable, as we would only expect these genes to be  
245 available in the environment after the bacteria die and release their genetic material; while it is  
246 possible for bacteria to actively release their genetic material while still alive, the extent of this  
247 phenomenon is unclear [6]. Further exploration of this assumption, and perhaps redesigns of model  
248 structures for transformation would be of value.

249 *E. coli* is the most commonly studied model organism for bacteria in general [74]. Its rapid growth and  
250 consistent behaviour in *in-vitro* settings make it amenable to experimental work, including transfer  
251 studies, therefore its overwhelming presence as the organism of choice for studies modelling HGT of  
252 AMR genes is not a surprise. However, HGT is known to occur with varying rates in multiple bacterial  
253 species, consequently it is unlikely that the rates of transfer estimated by looking at *E. coli* are equally  
254 applicable to other bacterial species [7]. In addition, HGT of AMR is a process that can also occur  
255 between bacterial species [9,11], while most models here exclusively focused on *E. coli* alone. Some  
256 resistances in bacterial species are in fact thought to have been originally acquired following a gene  
257 transfer event with another species, such as the *mecA* resistance gene in *Staphylococcus aureus*  
258 acquired from *S. fleurettii* [75].

259 Despite the fact that the carriage of an AMR gene often imposes a reduction in the growth rate of the  
260 bacteria [10], a few studies did not model this (6/43), but only one argued that this element could be  
261 ignored after fitting their model to experimental data [66]. However, this was once more only based

262 on observations *in-vitro*, which are likely to differ from the *in-vivo* reality. Including a fitness cost, while  
263 requiring the estimation of an additional parameter, does not add any particular complexity to the  
264 model structure itself, effectively only requiring a reduced growth rate value for the bacteria carrying  
265 AMR genes as opposed to bacteria susceptible to the modelled antibiotic (as can be seen in [68] for  
266 example), and should therefore be included at least for sensitivity analyses. In addition, although it is  
267 understandable that the first models of HGT of AMR should focus on tracking single genes to  
268 understand the basic dynamics of this process, in reality many bacteria carry multiple AMR genes that  
269 can be transferred independently [8]. However, we only identified four studies in our review which  
270 included more than one independent AMR gene in their model [30,32,60,69]. 13 studies did model  
271 the transfer of multiple linked genes [34,35,66,68,70,40–42,47,49,53,55,59]; however in these cases  
272 a single HGT event causes the transfer of all of these genes, therefore there is little difference between  
273 the model structures of these 13 studies and those of other studies which modelled the transfer of  
274 single genes.

275 Many studies did not allow for the presence of an antibiotic in their model. However, antibiotics are  
276 likely to modify HGT dynamics by directly affecting transfer rates, as well as the survival of bacteria  
277 not carrying the AMR gene [16–20]. The former has been shown to occur for transduction in *S. aureus*,  
278 where the addition of antibiotics induced a higher proportion of transducing phage compared to lytic  
279 phage [76]. On the other hand, some studies correctly argue that it is equally important to understand  
280 the dynamics of HGT in the absence of antibiotics. Effectively, it is common for bacterial populations  
281 to rapidly transition between being exposed to antibiotics or not, with the most obvious example  
282 being individuals transiently consuming antibiotics. Consequently, understanding the dynamics of HGT  
283 of AMR both in the presence and absence of antibiotics is essential.

284 HGT of AMR has been studied in laboratory settings, consequently data around which models can be  
285 built have been generated and are available [7,77]. However, we note that, to the best of our  
286 knowledge, most data appear to focus on conjugation in *in-vitro* settings. The availability of  
287 experimental data on HGT of AMR by transformation or transduction, and on any of the three HGT  
288 mechanisms in more complex settings (such as *in-vivo*), is unclear. This should be investigated in future  
289 work to further refine the recommendations we make here, and identify where more data are needed  
290 to support the development of mathematical models. This is essential to understand which of the gaps  
291 we identify are due to theory outpacing data collection, and which are due to under-utilisation of  
292 available data. In any case, using these external data sources for purposes they were not originally  
293 designed for can require assumptions to be made in the model structure and parameters. In addition,  
294 it is essential to bear in mind how these data were originally collected, since for example combining  
295 sources which look at bacteria in multiple environments to derive parameters in a single environment-  
296 specific model is far from ideal. On the other hand, the fact that a quarter of the studies we reviewed  
297 (12/43) assumed all of their parameter values is worrying. While the purpose of some of these studies  
298 was to exclusively test a range of parameter values to identify conditions for a specific event to occur  
299 (e.g. AMR prevalence increases), the absence of any clear sources for the limits of these ranges is  
300 questionable. Looking at studies which determined their parameter values experimentally, we see  
301 that some of these also assume values such as the initial proportion of bacteria capable of performing  
302 transformation and the rate at which they can gain this ability [70], the bacterial growth rate and the  
303 conjugation rate [40], or the fitness cost of carrying an AMR gene and the rate at which such genes  
304 are lost by the bacteria [34]. Informing models with data is essential to ensure that they are accurate  
305 representations of reality, therefore, as stated above, we believe that further work is required to

306 review the availability of data on HGT of AMR, and the methods that could be used to generate them  
307 when they are currently missing.

308 Regarding model structures, the majority of studies relied on deterministic models. To allow variability  
309 in the dynamics and therefore increased realism, studies more often chose to sample their parameter  
310 values, run their deterministic model, and repeat this process a number of times (as can be seen in  
311 [32,38,43,46,56,64,65,72]), a simpler alternative to developing new stochastic models. Acknowledging  
312 stochasticity when looking at HGT is essential; HGT rates are typically low (estimates from studies in  
313 our review include for example  $5.1 \times 10^{-15}(\text{cells/mL})^{-1}\text{hour}^{-1}$  for conjugation [49] and  $10^{-16}(\text{cells/mL})^{-1}\text{hour}^{-1}$   
314 for transformation [70]). These are therefore models of rare events which, by chance, might  
315 not always occur as expected, a feature which deterministic models fail to capture [24]. Sensitivity  
316 analysis is extremely important in any case since a small change in parameter value can lead to a  
317 greater change in the results. Despite this, nine studies exclusively relied on a deterministic model  
318 without sampling parameters or performing sensitivity analyses [30,36,40,42,48,54,55,58,68].  
319 Interestingly, five of these nine studies also generated their own parameter values experimentally  
320 [36,40,42,54,68]. Although they capture variation when measuring the parameters experimentally,  
321 often providing distributions for their values, they then only retain fixed point estimates for their  
322 corresponding model parameter values instead of sampling them from these distributions, and only  
323 use these fixed estimates to derive their conclusions. Acknowledging variability in microbiological  
324 observations by specifying distributions rather than point estimates is essential, and this must be  
325 represented in the corresponding mathematical models.

326 This also raises the question of how to best represent these microbiological events in mathematical  
327 models. Effectively, almost all of the models here describe transfer as a mass-action process (42/43).  
328 However, as stated above this approach is acceptable for conjugation, but might not fully apply to  
329 transformation, where transfer depends on the density of DNA in the surrounding environment rather  
330 than the number of bacteria, and transduction, which follows vector-like dynamics with the phage  
331 acting as carriers of resistance genes between bacteria. Transformation dynamics might therefore be  
332 better represented by models of environmental transmission of infections (such as [78]), and  
333 transduction by models of vector-borne diseases (such as [79]), as opposed to mass-action models.  
334 The degree of modelling complexity required to accurately represent HGT is therefore unclear. This is  
335 also true for models designed to understand the public health implications of HGT of AMR, for which  
336 the level of detail required to represent within-host dynamics must be clarified. In addition, since  
337 transfer dynamics have thus far been mostly studied in bacterial culture, mostly “short” time-frames  
338 have been explored (hours or days), with long term dynamics remaining unclear despite our  
339 knowledge that even resistant bacteria can colonise us for weeks or months [80–82]. To best guide  
340 our public health policies with mathematical modelling, we must first clarify the complexity of the  
341 process we are actually attempting to model, and the time required to fully capture its *in-vivo*  
342 dynamics.

343 This is the first attempt at providing an overview of existing mathematical modelling work on HGT of  
344 AMR. Our systematic review methods, with two individuals separately screening the titles and  
345 abstracts of candidate studies, allowed us to identify and bring together key studies on this topic.  
346 Using our list of comparison elements, we extracted and contrasted essential information between  
347 studies, overall allowing us to obtain a broad overview of the field and identify research gaps.  
348 However, our approach also has some limitations. Firstly, it was necessary for us to specify “(math\*

349 OR dynamic\*) model\*" rather than just "model\*" in the search, since otherwise it would have  
350 returned results on experimental models (e.g. mice) as opposed to mathematical models. Effectively,  
351 repeating our search with "model\*" instead of "(math\* OR dynamic\*) model\*" yields 2,360 and 1,560  
352 results on PubMed and Web of Science respectively, as opposed to our 171 and 185 results. The  
353 consequence of our choice however was that nine relevant studies were missed in the search, and  
354 were only identified by screening the references of already included studies. These nine studies were  
355 missed in the original literature search due to the absence of at least one of the search terms, with  
356 some studies for example referring to their models as "mass action models" instead of "mathematical  
357 models". In addition, we only searched for studies which modelled transfer of AMR genes, as opposed  
358 to HGT of any gene. This is firstly due to our specific research interest; horizontal transfer of AMR  
359 genes is an especially strong evolutionary driver for bacteria populations, compared to transfer of  
360 other genes. This is because AMR genes can be strongly selected for by environmental factors, such  
361 as the presence of antibiotics, while many other genes are often not subject to such selection  
362 pressures. In addition, AMR genes can be selected in more settings compared to other genes; for  
363 example, genes involved in immune evasion will only be selected for during infection of the host, while  
364 AMR genes can also be selected for during asymptomatic colonisation. The consequences of HGT of  
365 AMR in the bacterial population can therefore be greater than for other genes, which is why we  
366 believe it is important to study this process. Secondly, repeating the search without "(antimicrobial  
367 OR antibacterial OR antibiotic) resist\*" yields 12,236 and 38,148 results on PubMed and Web of  
368 Science respectively, which would be too many to cover in a single systematic review. Nevertheless,  
369 this suggests that there are other studies which model HGT more broadly. These could be a source of  
370 methodologies that could be applied to further develop the specific field of HGT of AMR modelling. In  
371 terms of the elements gathered from the studies to compare them, we were unable to extract any  
372 meaningful quantitative data (e.g. estimated gene transfer rates) common to all studies due to the  
373 high variability of study designs. This variability also prevented us from identifying common measures  
374 of study quality we could report aside from the presence or absence of sensitivity analysis.

375 Studying the effect of HGT of AMR on bacterial evolutionary dynamics is a necessary first step to  
376 understand the overall importance of this process. This has been the focus of the vast majority of the  
377 studies identified in this review, however the public health implications remain vastly unknown. This  
378 is related to the observation that the majority of studies model bacteria in an *in-vitro* setting; to  
379 understand the public health impact of HGT of AMR, it is essential to expand this to include other  
380 bacterial environments such as within humans and animals. In addition, important differences have  
381 been identified between transfer rates estimated *in-vitro* and *in-vivo*, with *in-vivo* transduction rates  
382 in *S. aureus* and conjugation rates in *K. pneumoniae* and *E. coli* for example being much higher than  
383 expected [14,15]. This difference in dynamics is attributable to the fact that *in-vitro* conditions fail to  
384 capture essential biological mechanisms influencing bacteria and therefore HGT [6,10]. Studying HGT  
385 *in-vitro* allows for a controlled environment to understand the basic dynamics of this process and the  
386 factors that might influence them (e.g. antibiotic exposure), and consequently offers a starting point  
387 to inform *in-vivo* models. We therefore recommend that future modelling studies should build upon  
388 the work of existing *in-vitro* studies to evaluate HGT of AMR in more complex scenarios, utilising  
389 parameter estimates from *in-vitro* studies as a baseline and refining them using data generated with  
390 *in-vivo* model organisms such as mice [68]. Due to the added complexity (e.g. immune system,  
391 simultaneous within-host and between-hosts dynamics, rapidly varying host exposure to antibiotics  
392 and therefore selection pressure on the bacteria...), this will require major extensions to existing



393 models. However, we believe that this is necessary to truly assess the potential consequences of HGT  
394 of AMR on human well-being.

395 This systematic review allowed us to identify key research gaps on the dynamics of HGT of AMR. Firstly,  
396 we recommend that future studies should focus on developing models of transformation and  
397 transduction to determine the required complexity to represent these dynamics. Since these  
398 mechanisms fundamentally differ in their biological characteristics, this will likely require substantial,  
399 novel modelling work as opposed to the extension of existing models of conjugation. In parallel, since  
400 the basic dynamics of conjugation are already reasonably well understood, future studies on this  
401 mechanism should focus on other bacterial species than *E. coli*, preferably in a setting where inter-  
402 specific HGT and the movement of multiple, separate AMR genes can both be observed. This should  
403 be achievable simply by re-parameterisation or minor extension of existing models; the greatest  
404 challenge would be to generate new data on HGT in these currently unexplored settings. The optimal  
405 solution to address these research questions would be to design frameworks to study HGT of AMR  
406 that encompass both laboratory and modelling work; this would ensure that the data collected are  
407 appropriate for the modelling needs, and that the actual model is a good representation of the  
408 situation measured in the laboratory. We therefore believe that, to fully understand the complexity  
409 of both the biology and the dynamics of HGT, collaboration of both microbiologists and mathematical  
410 modellers would be the best strategy for future research on this topic, and that studies should attempt  
411 to generate both their own data and models to reduce the assumptions they require.

412 While exclusively microbiological approaches have successfully been used to identify when HGT  
413 occurs, combining these with modelling has allowed us to estimate rates at which these events occur,  
414 and to disentangle the finer temporal dynamics of this process. For example, some studies we  
415 identified in our review which combined microbiology and modelling work answered questions such  
416 as how changing the exposure of bacteria to antibiotics influences HGT rates [49], how a bacterium  
417 interacts in space with its neighbours to perform HGT [31], or how to adjust shaking speed to maximise  
418 contact between bacteria, and thus the rate of HGT, in a liquid culture [66]. Modelling also allows  
419 faster exploration of situations that could be harder to test using only microbiological methods, since  
420 an experiment where the bacteria need to grow for 24 hours in the lab could be completed in a few  
421 seconds using a mathematical model. Crucially, this requires the model to be an accurate  
422 representation of reality, which in turn requires it to be informed by microbiological data to begin  
423 with. Our conclusion here is therefore not that either one of modelling or microbiology is superior to  
424 the other, but that both approaches complement each other. Consequently, we believe that close  
425 cooperation between these two fields would allow us to greatly improve our understanding of  
426 complex microbiological processes, such as HGT of AMR.

427 **CONCLUSIONS**

428 In this systematic review, we aimed to assess the current state of mathematical modelling as a tool to  
429 improve our understanding of horizontal gene transfer of antimicrobial resistance. From the 43  
430 studies identified, we found that the majority focused on conjugation in *E. coli*, exploring evolutionary  
431 dynamics of HGT in culture. Whilst this provides a solid base for a key method of HGT, future work  
432 must also consider HGT by transformation and transduction which are also essential drivers of HGT in  
433 bacteria. Importantly for public health implications, only one bacterial species was considered in most  
434 models when we know that inter species transfer is responsible for many of our epidemic AMR clones  
435 and much of the work was fitted to data in the absence of antibiotic exposure. Crucially, to answer  
436 these questions we must first clarify the level of modelling complexity required to accurately represent  
437 HGT dynamics, as well as the availability and capacity to generate experimental data on these  
438 processes. This complex topic requires close collaboration between mathematical modellers and  
439 microbiologists in order to determine the full impact of these processes on our ability to control the  
440 public health threat posed by antimicrobial resistance.

441

442 **AUTHOR CONTRIBUTIONS**

443 All authors jointly developed the search strategy. QL and GK independently screened the titles and  
444 abstracts of the identified studies. QL then evaluated the full texts of the included studies, and wrote  
445 the first draft of the manuscript. All authors subsequently edited the manuscript.

446

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450

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