

1 **The perfect condition for the rising of superbugs: person-to-person contagion and**  
2 **antibiotic use are the key factors responsible for the positive correlation between**  
3 **antibiotic resistance gene diversity and virulence gene diversity in human**  
4 **metagenomes**

5

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24 **1. Abstract**

25 This study aims to understand the cause of the recent observation that humans with a  
26 higher diversity of virulence genes in their metagenomes tend to be precisely those with  
27 higher diversity of antibiotic-resistance genes. We simulated the transferring of  
28 virulence and antibiotic-resistance genes in a community of interacting people where  
29 some take antibiotics. The diversities of the two genes types became positively  
30 correlated whenever the contagion probability between two people was higher than the  
31 probability of losing resistant genes. However, no such positive correlations arise if no  
32 one takes antibiotics. This finding holds even under changes of several simulations'  
33 parameters, such as the relative or total diversity of virulence and resistance genes, the  
34 contagion probability between individuals, the loss rate of resistance genes, or the social  
35 network type. Because the loss rate of resistance genes may be shallow, we conclude  
36 that the contagion between people and antibiotic usage is the leading cause of  
37 establishing the positive correlation mentioned above. Therefore, antibiotic use and  
38 something as prosaic as the contagion between people may facilitate the emergence of  
39 virulent and multi-resistant bacteria in people's metagenomes with a high diversity of  
40 both gene types. These superbugs may then circulate in the community.

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## 45 **2. Introduction**

46 Since the 1940s, antibiotics have been used in health contexts in medicine and  
47 veterinary and as growth promoters in livestock and agriculture (Castanon, 2007). As an  
48 incredible example of Darwinian selection, bacteria worldwide have gradually become  
49 resistant to several antibiotics. Such spread of resistance has had terrible consequences.  
50 For example, there were about 875 500 disability-adjusted life-years and more than 33  
51 000 deaths in European Economic Area due to antibiotic resistance in 2015 (Cassini et  
52 al., 2019).

53 Bacterial communities are often very complex, eventually comprising both pathogenic  
54 and non-pathogenic bacteria. The human microbiome, defined as the set of  
55 microorganisms that colonize humans (body's surfaces and biofluids, including tissues  
56 such as skin, mucosa, and, most importantly, the gastrointestinal tract) comprises about  
57  $3.8 \times 10^{13}$  bacterial cells (Sender et al., 2016), spanning thousands of taxa.

58 Virulence factors are proteins that help bacteria in colonizing a host or biome. These  
59 traits are easily spread in bacterial populations or microbiomes by horizontal gene  
60 transfer, which can potentially convert mutualistic or commensal bacteria into  
61 pathogens able to progress into new tissues, triggering an infectious disease. We  
62 recently found a positive correlation between antibiotic resistance genes' diversity and  
63 virulence genes' diversity across human gut microbiomes (Escudeiro et al., 2019).  
64 Could this positive correlation result from administering antibiotics in sick people due  
65 to bacterial infections, eventually selecting bacteria encoding virulence and resistance  
66 determinants simultaneously? This hypothesis is unlikely to be adequate because, even  
67 when the objective of taking antibiotics is to kill or inhibit the growth of pathogenic  
68 bacteria, many non-pathogenic (mutualistic or commensal) strains and species are  
69 undoubtedly affected. Therefore, an explanation for the positive correlation mentioned  
70 above is still missing.

71 Both virulence and resistance genes present in commensal bacteria and pathogenic  
72 bacteria spread between people's metagenomes. This dissemination may contribute to  
73 the accumulation of virulence and resistance genes in some people when themselves or  
74 their contacts take antibiotics. Meanwhile, pathogenic bacteria's presence triggers the

75 administration of antibiotics. Therefore, contagion (the dissemination of bacteria and  
76 their genes) between people should play a role in keeping the correlation between  
77 resistance and virulence genes' diversity. Microbes' transmission from mother to child  
78 is already well documented (Blaser and Falkow, 2009; Nayfach et al., 2016; Ferretti et  
79 al., 2018; Yassour et al., 2018; Nogueira et al., 2019). A recent study highlighted that  
80 the oral and gut microbiomes of people belonging to the same household share  
81 similarities in bacterial strains, regardless of these people's genetic relationship (Brito et  
82 al., 2019). These studies suggest that bacteria in human microbiomes can have a shared  
83 exposure or result from person to person transfer on the social network. This suggestion  
84 is supported by a study that showed that social interactions shape the chimpanzee's  
85 microbiomes (Moeller et al., 2016).

86 This work aims to find the key factors leading to the positive correlation between the  
87 diversity of virulence and antibiotic resistance genes observed across human  
88 metagenomes (Escudeiro et al., 2019). To this end, we simulated the transfer of  
89 bacterial pathogens and antibiotic resistance and virulence genes in a human-to-human  
90 transmission network. We show that a positive correlation between the diversity of  
91 antibiotic resistance coding genes and those coding for virulence emerges whenever the  
92 contagion rate between individuals is higher than the probability that metagenomes lose  
93 resistant genes, independently of all the other parameters of the simulations. This simple  
94 rule explains the positive correlation between virulence genes' diversity and antibiotic  
95 resistance genes' diversity.

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### 99 **3. Methods**

#### 100 **2.1 Building the human network**

101 We simulated a network where each node represents a person or, more precisely, a  
102 person's metagenome. To simplify language, sometimes we use the words person or  
103 people, meaning a person's metagenome or people's metagenomes, respectively. The  
104 edges represent possible transmission avenues of microorganisms.

105 We built the social contact network following the Watts and Strogatz method (Watts  
106 and Strogatz, 1998). In a regular network, each node links to the  $n$  nearest nodes. In  
107 non-regular networks, each node's link has a certain probability  $p$  of being reconnected  
108 to another randomly chosen node. The parameter  $p$  represents the probability of each  
109 connection to be modified. We defined the network type by the value assigned to the  
110 parameter  $p$  (for example, a regular network when  $p = 0$ , whereas  $p = 1$  results in a  
111 random network). Unless noted, we performed simulations with  $p = 0.5$ .

112

#### 113 **2.2 The metagenome, pathogenic bacteria, and antibiotic administration**

114 The model considers the transmission of bacterial pathogens (capable of causing  
115 infections), as well as virulence and antibiotic resistance genes, between people. These  
116 non-housekeeping genes are present in the metagenome. We focused on the presence or  
117 absence of genes encoding different functions, irrespectively of its copy-number in the  
118 metagenome. In the simulations, each gene represents a gene family (with similar  
119 functions). We divided resistance genes into groups, each group having the same  
120 number of families. Each group represents genes associated with resistance to an  
121 antibiotic. Of note, we did not consider resistance to multiple drugs in our simulations.  
122 Therefore, there will be as many groups as there are antibiotics accounted for in the  
123 simulations. We define the diversity of a specific gene kind as the number of genes of  
124 that type present in a human metagenome.

125 To simulate the migration of bacteria from individuals outside the network or the  
126 contagion from sources such as food or contaminated water, we inserted five different

127 bacterial pathogenic species into random individuals per cycle. To simplify language,  
128 we assume that pathogenic bacteria belong to different species, but, in reality, some of  
129 them may constitute different strains of the same species. In this model, the only  
130 difference between species is the antibiotic to which they are susceptible, as explained  
131 below.

132 Individuals infected by pathogenic bacteria feel sick and take an antibiotic. The  
133 antibiotic administered is specific for the bacteria that caused the illness. The antibiotic  
134 selects cells carrying resistance genes by eliminating the remaining susceptible bacteria.  
135 In this work, we assume that all families of resistance genes are present in all  
136 metagenomes, but in two different possible states: in some metagenomes, they are  
137 present in low copy number, so they are not transmissible to other individuals in the  
138 network; in other metagenomes, the copy number of resistance genes is high due to the  
139 selective pressure of antibiotics to which they were previously submitted. In the latter  
140 case, resistance genes are transmissible from person to person.

141 Moreover, upon antibiotic consumption, the following events can occur: (i) elimination  
142 of a pathogenic bacterial species; (ii) selection in the metagenome of resistance genes  
143 belonging to the same group of resistance to an antibiotic, which means their copy  
144 number gets so high that they become transferable; (iii) loss of resistance genes  
145 associated with other antibiotics with a given probability (becoming non-transferable  
146 but still present in minute copy number); (iv) virulence genes disappear from the  
147 metagenome with a given probability.

148 Several processes lead to gene loss. Genes are lost because of the selective pressure by  
149 antibiotics and because we assume that resistance determinants impose a fitness cost (in  
150 the absence of antibiotics). To include this cost in the simulations, we consider that each  
151 metagenome may lose specific resistance genes according to a “loss rate” (with this  
152 process, these genes become non-transferable).

153

## 154 **2.3 Algorithm of the program**

155 Each simulation is composed of several cycles. In each cycle, we considered all  
156 procedures described in the pseudocode (Table 1; see also the flowchart in Fig. 1). We  
157 performed exploratory simulations to parameterize our model. We fixed a set of  
158 parameters as default (Table 2). The main steps of the program in each cycle are:

159 i) Transfer of pathogenic bacteria, virulence and resistance genes between people (i.e.,  
160 between linked nodes), according to specific contagion probabilities of pathogens and  
161 virulence and resistance genes of the metagenome. With this process, the diversity of  
162 genes present in the recipient metagenome increases.

163 ii) To look for people infected by at least one pathogenic bacterial species. These people  
164 take antibiotics (chosen according to the pathogen). The antibiotic eliminates the  
165 pathogenic species and selects the resistance genes associated with the antibiotic used.  
166 According to a certain probability, the antibiotic also eliminates virulence genes and  
167 resistance genes unrelated to the administered antibiotic. Finally, the metagenome loses  
168 a few more resistance genes not associated with the antibiotic, according to the loss rate  
169 probability. The cause of this loss is the fitness cost of resistance genes.

170 iii) The metagenomes of people that did not take an antibiotic in this cycle lose  
171 resistance genes according to the loss rate probability. This loss is a consequence of the  
172 fitness cost imposed by resistance genes on their hosts, which is not happening with  
173 virulence genes.

174 iv) Insert the five bacterial pathogenic species in five individuals randomly chosen from  
175 the population.

176

## 177 **2.4 Statistical analysis**

178 We considered that  $Y$  (diversity of resistance genes) correlates with  $X$  (diversity of  
179 virulence genes), according to:

180  $Y = a.X + b$

181 In this equation, the parameter  $a$  represents the linear regression slope, while  $b$   
182 represents the point at which the line crosses the y-axis.

183 Given the complexity of human interactions, it is paramount to simplify the computer  
184 simulations. A simplified model allows us to comprehend the effect of specific factors  
185 in our simulations, which would otherwise be extremely difficult to detect. As these  
186 simplifications do not allow us to make quantitative inferences, we make qualitative  
187 analyses. The focus is always on the correlation or linear regression slope signal  
188 between the diversity of virulence and antibiotic resistance genes and whether the  
189 correlation is significantly different from zero. Accordingly, the null hypothesis is that  
190 there is no correlation between antibiotic resistance genes' diversity and virulence  
191 genes' diversity. The alternative hypothesis is that there is a correlation between  
192 antibiotic resistance genes' diversity and virulence genes. We define  $\alpha = 1 \times 10^{-6}$ ,  
193 rejecting the null hypothesis if P-value  $< \alpha$ .

194 We performed the data analyses described above, and the Student's t-tests (see  
195 Supplementary Information) with R – version 3.5.1 (R Core Team, 2015).

196



## 197 **4. Results**

### 198 **4.1 The number of diseases and the probability of contagion**

199 This work aims to understand the positive correlation between antibiotic resistance  
200 genes' diversity and virulence genes in metagenomes across human populations  
201 observed by Escudeiro et al. (2019). As explained in the Methods section, we assumed  
202 that people establish a fixed network of contacts between them and that there is the  
203 transmission of pathogenic bacteria along with antibiotic-resistance and virulence genes  
204 between connected people. In the simulations, five different pathogenic bacteria,  
205 belonging to distinct species, circulate between linked people. When pathogenic  
206 bacteria infect an individual, that person takes an antibiotic. The antibiotic eliminates  
207 only the pathogenic species associated with the administered antibiotic, even if more  
208 than one species infects that individual. The antibiotic also removes a certain percentage  
209 of virulence and resistance genes.

210 In principle, the bacterial pathogen contagion probability parameter could have any  
211 value in the simulations. Given the importance of this parameter, we must calibrate its  
212 value according to the model's other conditions. We assumed that individuals are not  
213 affected by more than two infectious diseases at the same time. Therefore, we started  
214 this study searching for the parameters that led individuals to have a maximum of two  
215 pathogenic bacterial species or strain simultaneously at a given cycle.

216 We performed simulations with different bacterial pathogen contagion probabilities, and  
217 counted the number of pathogenic bacterial species that each individual has per cycle.  
218 As we can see in Table 3, when the bacteria pathogen contagion probability is 0.2, some  
219 individuals in a specific cycle (out of two million possibilities) became infected by three  
220 pathogenic bacteria. For this reason, we settled the bacterial pathogen contagion  
221 probability to be less than 0.2 in our simulations.

222 In each cycle of the simulation, we introduced five pathogenic bacterial species into the  
223 population. Then, we counted the total number of pathogenic species present in the  
224 population. If this number is equal to five, then the only pathogenic species in the  
225 population are those that were inserted (simulating immigration into the network),  
226 which means that, before the insertion, all pathogenic bacterial species had disappeared

227 in that cycle. As it is unrealistic that all bacterial species disappear simultaneously, we  
228 looked for a contagion value below 0.2 that minimizes the number of times that all  
229 bacteria disappear at the same time. As shown in Table 4, the number of times that  
230 pathogenic species disappear increases with a bacterial pathogen contagion probability  
231 of 0.1 or less. Therefore, we defined that this probability is 0.15 in the simulations.

232

#### 233 **4.2 Calibration of the contagion probability**

234 As previously explained, individuals take antibiotics whenever pathogenic bacteria  
235 infect them. However, antibiotics remove other bacteria present in the microbiome  
236 carrying antibiotic-resistance and virulence genes, beyond pathogenic bacteria.  
237 Therefore, it is essential to calibrate the probability of passing these genes by avoiding  
238 their population's loss. These genes disappeared from the community when the number  
239 of eliminated genes was higher than the number of genes passed between individuals.

240 To better understand the impact of the gene contagion probability parameter, we then  
241 studied the simpler case: only antibiotics can eliminate genes, and there is no fitness  
242 cost for harboring resistance genes (hence, loss rate = 0).

243 As we can see in Fig. 2, when the gene contagion probability was less than 0.005 (Figs  
244 2A and 2B), virulence genes disappeared from the population. On the other hand, when  
245 the contagion probability of genes was higher than 0.01 (Figs 2E and 2F), several  
246 individuals had the maximum diversity of genes in their metagenome, which does not  
247 correspond to the observation in (Escudeiro et al., 2019). Following our results, we  
248 assumed that the gene contagion probability must be 0.005 or 0.01 (Figs 2C and 2D).

249

#### 250 **4.3 Correlation between diversities is positive if gene contagion probability is** 251 **higher than the resistance gene loss rate**

252

253 We studied the correlation between virulence genes' diversity and the diversity of  
254 resistance genes effect for different combinations of gene contagion probability and  
255 resistance gene loss rate. For that, we fixed all the other parameters (see Table 2). Fig. 3  
256 shows that if the gene contagion probability is higher, the same or only slightly lower  
257 than the loss rate, the correlation between the diversity of virulence genes and the  
258 diversity of resistance genes is positive (Supp. Table 1, Fig. 3).

259

260

#### 261 **4.4 Correlations maintain signal even when people take antibiotics randomly**

262 Until now, we have studied the correlations when people take antibiotics because  
263 bacterial pathogens infected them through their contacts in the network. Here we  
264 examine what happens if individuals take antibiotics at random, not because pathogens  
265 infected them. We chose these individuals randomly from the population in each cycle.  
266 In the previous simulations, there were 13/1000 individuals, on average, taking  
267 antibiotics in each cycle. Thus, in this section, we considered that the probability that a  
268 random individual takes antibiotics is 0.013. At the end of simulations, we obtained the  
269 same correlations' signals when assuming that people take antibiotics randomly or  
270 because pathogens infected them through their contacts in the network (compare Supp.  
271 Table 1 and Fig. 3C with Supp. Table 2.1 and Supp. Fig. 2.1 respectively). In other  
272 words, whatever are the reasons for taking antibiotics, the correlation between  
273 diversities is positive if gene contagion probability is higher than the resistance gene  
274 loss rate.

275

#### 276 **4.5 Taking antibiotics is crucial for a positive correlation between virulence and 277 resistance genes' diversity**

278 In the previous sections, we showed that if the gene contagion probability is higher than  
279 the loss rate, the outcome is a positive correlation between virulence and resistance

280 genes' diversity. Here we show that taking antibiotics is crucial for this outcome (Supp.  
281 Fig. 3.1).

282 If no one takes antibiotics, there is no counter-selective pressure on commensal bacteria  
283 encoding virulence genes. The result is that virulence genes' diversity gets the  
284 maximum possible value in everyone's metagenome in the community (in Supp. Fig.  
285 3.1 A, B and C, all the dots converge to the right). If the loss rate is null (if there is no  
286 fitness cost of resistance), all metagenomes accumulate every possible virulence and  
287 resistance gene families, so their diversity attains the maximum achievable value (in  
288 Supp. Fig. 3.1 A, all the dots congregate to a single point at the top right corner). If the  
289 loss rate is low, there is some diversity of resistance genes in the population (in Supp.  
290 Fig. 3.1 B, all the dots distribute in a vertical line on the right side). Finally, if the loss  
291 rate is high, more resistance genes are lost than those that accumulate through  
292 contagion, so all metagenomes lose all virulence genes (see Suppl. Fig 3.1 C, where all  
293 the dots congregate to a single point at the bottom right corner).

294

#### 295 **4.6 Positive correlations are robust under changes in the main simulated system's** 296 **properties.**

297 We have seen that the positive correlation between virulence and resistance genes'  
298 diversity is positive if the gene contagion probability is higher than the loss rate (Fig.  
299 3C). We then analyzed the robustness of this result. The next five subsections show the  
300 impact of changing the simulations' parameters or changing the network itself. We  
301 studied the following parameters: population size, the ratio between virulence genes and  
302 antibiotic resistance genes, the elimination probability under antibiotic intake, the  
303 proportion of the population harboring antibiotic-resistance genes in their metagenome,  
304 and the network type.

305

#### 306 **4.6.1 Population size has no impact on the correlations' signal**

307 Due to computer power constraints, we had to assume that the population has just a  
308 thousand people. Therefore, it is essential to understand whether population size  
309 impacts the correlations' signals. We performed simulations with a population size of  
310 3000 individuals, instead of 1000 individuals, for the 14 conditions shown in Fig. 3C.  
311 Although there were significant differences between the slopes in three cases, we didn't  
312 observe a change of the correlation's signal from the cases where the population size  
313 was 1000 individuals (Supp. Table 4.1 and Supp. Fig. 4.1). An increase in the  
314 population size leads to a rise in the number of intermediaries between two distant  
315 individuals. Therefore, for virulence genes and antibiotic resistance genes to be  
316 transferred between these two faraway individuals, more contacts are needed and,  
317 consequently, more time is required to achieve a stable correlation.

318

#### 319 **4.6.2 The ratios between virulence and antibiotic resistance genes diversities have** 320 **no impact on correlations' signal**

321 In all the other sections, we considered that virulence and resistance genes have the  
322 same total diversity. Here, we studied the effect of assuming that the diversity of  
323 virulence genes is different from that of resistance genes for the same 14 conditions of  
324 gene contagion probability and loss rate studied in the previous section. For that, we  
325 performed simulations similar to the previous ones, but with the following ratios  
326 between virulence and antibiotic resistance genes: 1:2, 1:4, 2:1, 4:1. Although there  
327 were significant differences between the slopes in 48 out of 56 cases, we didn't observe  
328 a change of the correlation's signal (Supp. Tables 5.1 to 5.4 and Supp. Figs 5.1 to 5.4).

329

330 **4.6.3 The correlation's signal is robust under changes in the gene elimination**  
331 **probability when people take antibiotics**

332 When an individual takes an antibiotic, virulence genes and resistance genes are  
333 eliminated from the metagenome with a probability of 0.7 (except for resistance genes  
334 corresponding to the antibiotic used, which are selected, not eliminated). In this section,  
335 we analyzed the impact of using other elimination probabilities when an individual  
336 takes an antibiotic. For that, we performed simulations similar to the previous ones, for  
337 the same 14 conditions of gene contagion probability and loss rate, but where the  
338 probability of eliminating genes under antibiotic intake is 0.3 and 0.5 for all gene types  
339 (instead of 0.7). In 19 out of 28 cases, the slopes were not significantly different from  
340 those obtained with a probability of 0.7 (Supp. Tables 6.1 to 6.2). The slopes were  
341 different in the other nine cases, but the signal remained the same (Supp. Tables 6.1 to  
342 6.2 and Supp. Figs 6.1 and 6.2).

343 We also checked the impact of setting the probability of eliminating antibiotic resistance  
344 genes different from that of eliminating virulence genes. Although the slopes were  
345 significantly different in 51 out of 84 tested cases, the slopes' signal remained the same  
346 (Supp. Tables 7.1 to 7.6 and Supp. Figs 7.1 to 7.6). Overall, these results show that the  
347 slope's signal is robust under changes in the elimination probability.

348

349 **4.6.4 The initial proportion of metagenomes containing antibiotic-resistance genes**  
350 **has no impact on correlations' signal**

351 In the previous sections, we considered that every individual carries all the antibiotic  
352 resistance genes in two alternative states at the beginning of the simulation. Either  
353 resistance genes were present at low copy numbers (hence being unable to be  
354 transmitted to other people) or at high copy numbers because they previously selected  
355 by antibiotic exposure (thus transmitting to other people). In this section, we study the  
356 effect of considering that, initially, only 10% of the metagenomes contain antibiotic-  
357 resistance genes. With this parameter changed, the simulations take more time to  
358 stabilize because 90% of the population receives resistance genes only through  
359 contagion. We performed simulations similar to the ones shown in Figure 3, but with

360 5000 cycles. The final slopes are not significantly different from the case where all  
361 metagenomes initially contain antibiotic-resistance genes (Supp. Table 8.1 and Supp.  
362 Fig 8.1).

363

#### 364 **4.6.5 The network type has no impact on correlations' signal**

365 The simulations leading to Fig. 3 were performed in a network with a rewiring  
366 probability of  $p = 0.5$  (see Methods). We then performed similar simulations but in a  
367 regular ( $p = 0$ ) and in a random ( $p = 1$ ) networks. This parameter did not change the  
368 correlation signals (see Suppl. Tables 9.1 and 9.2). However, the time needed (number  
369 of cycles) to reach a stable distribution was lower for higher values of  $p$  (Supp. Fig. 9.3)

370

371 This section 3.5 shows that the simulated system's main parameters have no impact on  
372 the correlation's signal between the virulence and resistance genes diversities.

373

## 374 **5. Discussion**

375 Antibiotics affect hundreds of commensal and mutualist bacterial strains and species,  
376 even if their target is bacterial pathogens. Moreover, healthy animals often take  
377 antibiotics, given the properties of these drugs as growth-promoters. With these two  
378 processes, antibiotic-sensitive bacteria are counter-selected, raising the frequency of  
379 antibiotic resistance cells in metagenomes. Meanwhile, metagenomes, both from sick  
380 and healthy people, harbor virulence genes. This paper aimed to understand why there is  
381 a positive correlation between the diversity of virulence and antibiotic-resistance genes  
382 among human populations' microbiomes (Escudeiro et al., 2019).

383 Our simulations' main result is that a positive correlation emerges if the contagion  
384 probability is higher than the loss rate of antibiotic-resistance genes. We can understand  
385 this result in the following way.

386 In the absence of infection by bacterial pathogens, people do not take antibiotics (in that  
387 particular cycle), and thus, the diversity of virulence genes increase through contagion  
388 with other people. However, two opposing forces play a role in resistance genes of the  
389 microbiomes of people not taking antibiotics. Contagion from other people in the  
390 network makes the diversity of resistance genes to increase, whereas gene loss  
391 decreases it. At the end of a cycle, the diversity of resistance genes increases exclusively  
392 if the effect of contagion is higher than that of gene loss. The gene loss is just the  
393 consequence of the fitness cost imposed by resistance determinants (chromosomal  
394 mutations or genes) in competition with susceptible cells. However, the contagion effect  
395 has two main contributors: the contagion probability and the number of connections  
396 (which depends on the network type and varies from person to person in non-regular  
397 networks). Figs. 3C and the corresponding figures in Supplementary File (Suppl. Figs.  
398 4.1, 5.1 – 5.4, 6.1, 6.2, 7.1 – 7.6, 8.1, 9.1 and 9.2) show that if the contagion rate is  
399 higher than the loss rate, a positive correlation emerges between the diversity of  
400 antibiotic resistance genes and virulence genes.

401 At first, one might expect to see a negative correlation whenever the contagion  
402 probability is lower than the loss rate, but that is not always the case. Indeed, when the  
403 contagion probability is only slightly lower than the loss rate, the correlation is positive.



404 For example, if the contagion probability is 0.005 and the loss rate is 0.01, the  
405 correlation is still positive (Fig. 3A and 3C, Supp. Table 1). The reason for these  
406 counter-intuitive cases is that, in each cycle, one individual contacts with four other  
407 individuals, and during each of these contacts they share bacteria from its microbiomes.  
408 In turn, each individual can only be medicated with antibiotics once (at the end of a  
409 cycle). That implies that the rate of loss of resistance genes applies only once in a cycle.  
410 Therefore, the impact of the contagion rate is higher than the loss rate of resistance  
411 genes.

412 Importantly, our conclusion that a positive correlation emerges if the contagion  
413 probability is higher than the loss rate of antibiotic-resistance genes is robust under  
414 changes of the population size (Supp. Tables 4.1), the ratio between virulence and  
415 antibiotic resistance genes (Supp. Tables 5.1 to 5.4), the elimination probability under  
416 antibiotic intake (Supp. Tables 6.1 to 7.6), or the network type (Supp. Tables 9.1 and  
417 9.2).

418 We assumed that, by default, resistance determinants are already present in little  
419 amounts in all metagenomes because they are a part of the natural bacterial lifestyle,  
420 and human beings have used massive quantities of antibiotics since the 1940s. What is  
421 the impact of this assumption? As shown in Supp. Tables 8.1, if we assumed that,  
422 initially, only 10% of the metagenomes contain antibiotic-resistance genes, the final  
423 correlations between the diversity of resistance genes and the diversity of virulence  
424 genes are the same as in the default case. The only difference is that more cycles are  
425 needed to stabilize the correlation.

426 The contagion probability between people and the loss rate of antibiotic-resistance  
427 genes are the two critical parameters of our main result, so it is relevant to know their  
428 actual values. Human microbiomes' interest strongly increased in recent years, yet we  
429 still do not know how much is the contagion probability of non-housekeeping genes.  
430 For example, we know that human microbiomes are more similar among humans living  
431 together, irrespective of the genetic relatedness, suggesting that transmission is a critical  
432 factor of the microbiome constitution (Rothschild et al., 2018).

433 Sarowska and colleagues recently reviewed the fate of the so-called extraintestinal  
434 pathogenic *Escherichia coli* (ExPEC), which are facultative pathogens of the normal  
435 human intestinal microbiome. ExPEC pathogenicity relies on many virulence genes,  
436 and pathogenicity islands, or mobile genetic elements (such as plasmids) encoding some  
437 of them. One of the authors' conclusions is precisely the difficulty in assigning ExPEC  
438 transmission to people due to the delay between ExPEC colonization and infection:  
439 ExPEC cell can live in human intestines for months or even years before starting an  
440 infection (Sarowska et al., 2019). The same problem applies to the transmission rate of  
441 antibiotic-resistance genes: there is very little data on transmission rates between people  
442 (Andersson and Hughes, 2017).

443 We have seen that the relationship between the contagion rate and loss rate is paramount  
444 to understand the positive correlation between resistance and virulence genes diversity.  
445 So, we now discuss how much is the loss rate of resistance determinants in human  
446 metagenomes. Several longitudinal studies have shown that antibiotic-resistance genes  
447 often remain tens of days, sometimes months, in human gut microbiomes (Horcajada et  
448 al., 2002; Lautenbach et al., 2006; O'Fallon et al., 2009; Rogers et al., 2012). While still  
449 harboring resistance genes, people most probably contact with several other people. Yet,  
450 it is still unclear what is the relationship between contagion and loss rates.

451 As explained in the methods section, the loss of antibiotic resistance results from the  
452 fitness cost of resistance determinants on bacterial cells (compared to otherwise  
453 isogenic susceptible cells). Several studies have shown that resistance determinants,  
454 here broadly comprising resistance mutations and resistance genes encoded in the  
455 chromosome or plasmids, impose a fitness cost on their hosts (giving the sensitive  
456 strains a growth advantage) (Andersson and Levin, 1999). However, several  
457 mechanisms decrease or even eliminate it. First, compensatory mutations, which mask  
458 the deleterious effects of resistance mutations, have been observed in several studies  
459 (Levin et al., 1997; Schrag et al., 1997; Bjorkman et al., 2000; Maisnier-Patin and  
460 Andersson, 2004; Nilsson et al., 2006). Second, resistance mutations can even be  
461 beneficial in specific resistance genetic backgrounds (Trindade et al., 2009). Third,  
462 while resistance plasmids often impose a fitness cost to their hosts, it has also been  
463 observed that plasmid and/or cells need just a few hundreds of bacterial generations to

464 adapt to each other (Bouma and Lenski, 1988; Modi and Adams, 1991; Dahlberg and  
465 Chao, 2003; Dionisio et al., 2005; Harrison et al., 2015). Fourth, plasmids sometimes  
466 increase the fitness of bacteria that already harbor a resistance mutation (Silva et al.,  
467 2011); likewise, some resistance mutations increase the fitness of plasmid bearing cells  
468 (Silva et al., 2011). The same may happen with two plasmids: one of them  
469 compensating for the fitness-cost of the other (Silva et al., 2011; San Millan et al.,  
470 2014). Fifth, plasmids may interact with other plasmids to facilitate their transfer (Gama  
471 et al., 2017c, 2017a, 2017b, 2018). Sixth, a few works suggested that plasmids appear  
472 costly because their fitness effect is often measured a long time after its isolation from  
473 nature (Lau et al., 2013; Gama et al., 2018).

474 Together, these six factors suggest that the fitness cost of resistance determinants is  
475 often very low or null, allowing the permanence of resistance determinants in  
476 microbiomes for long periods. This stability of resistance determinants implies that their  
477 loss rate, the probability that a metagenome loses a particular resistance gene or  
478 mutation, is undoubtedly lower than the contagion probability. Therefore, antibiotic  
479 consumption and contagion between people lead to a positive correlation between the  
480 diversity of resistance genes and virulence genes.

481

## 482 **6. Concluding remarks**

483 The simple fact that people contaminate between themselves, and antibiotic use, is chief  
484 to explain the positive correlation between antibiotic resistance gene diversity and  
485 virulence gene diversity across human metagenomes. This result is robust and general  
486 because we made very few assumptions. This result also has worrying health  
487 implications: people with a higher diversity of resistance genes in their metagenomes  
488 have a higher diversity of virulence genes. Such co-presence may potentiate the  
489 appearance of plasmids or bacteria encoding virulence and resistance genes  
490 simultaneously. Meanwhile, the current restrictive measures due to the COVID-19  
491 pandemic may weaken this correlation between the diversity of resistance genes and  
492 antibiotics and virulence factors due to a decrease in the contagion rate (Domingues et  
493 al., 2020).

494 **7. Conflict of Interest**

495 The authors declare that the research was conducted in the absence of any commercial  
496 or financial relationships that could be construed as a potential conflict of interest.

497 **8. Author Contributions**

498 CD, JR, TN, and FD conceived the study and designed the simulations. CD and JR  
499 wrote the computer program; CD, JR, TN, JP, and FD analyzed the data. CD, JR, TN,  
500 and FD wrote the first draft of the manuscript, with contributions of JP and FM. All  
501 authors contributed to manuscript revision, read, and approved the submitted version.

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508 This manuscript has been released as a pre-print at BioRxiv, (Domingues et al.).

509

510

511 **11. References**

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642 **12. Figure legends**

643 **Figure 1. Flowchart of the program.** After the network's construction, the program  
644 performs several cycles where, eventually, there is gene transfer between nodes  
645 (people). Some individuals get sick and take antibiotics. Some genes are lost due to  
646 antibiotic pressure or the fitness cost imposed by resistance genes.

647 **Figure 2. Effect of the gene transmission probability.** A to F: the relationship  
648 between the diversity of resistance genes (vertical axes) and the diversity of virulence  
649 genes (horizontal axes). Each dot represents the case of an individual metagenome. A  
650 and B: disappearance of the diversity of virulence genes; C and D: positive correlation  
651 between the diversity of resistance genes and the diversity of virulence genes; E and F:  
652 positive correlation between the diversity of resistance genes and the diversity of  
653 virulence, with many individuals having a high diversity of the two gene types.  
654 Parameters as follows. In all cases, we set resistance genes loss rate = 0. In A, when the  
655 gene contagion probability is low (0.0005), virulence genes disappeared from the  
656 network. In B, gene contagion probability = 0.0025 (R = 0.309, slope = 11.00, p-value =  
657  $1.47 \times 10^{-23}$ ); In C, gene contagion probability = 0.005 (R = 0.934, slope = 0.798, p-value  
658 =  $\sim 0$ ); In D, gene contagion probability = 0.01 (R = 0.973, slope = 0.757, p-value =  $\sim 0$ );  
659 In E, gene contagion probability = 0.015 (R = 0.972, slope = 0.754, p-value =  $\sim 0$ ); In F,  
660 gene contagion probability = 0.02 (R = 0.976, slope = 0.751, p-value =  $\sim 0$ ).

661 **Figure 3. Effect of the relative values of the gene contagion probability and the**  
662 **resistance genes loss rate.** A and B: the relationship between the diversity of virulence  
663 genes (horizontal axes) and the diversity of resistance genes (vertical axes). Each dot  
664 represents the case of an individual metagenome. In both A and B, the gene contagion  
665 probability = 0.005. A: resistance genes loss rate = 0, which is lower than the gene  
666 contagion probability, resulting in a positive slope; (R = 0.929, slope = 0.775, p-value  $\sim$   
667 0). B: resistance genes loss rate = 0.03, which higher than the gene contagion  
668 probability, resulting in a negative slope; (R = -0.682, slope = -0.174, p-value =  
669  $1.19 \times 10^{-137}$ ). C: Slope of the regression between the diversity of virulence and  
670 resistance genes according to the loss rate (horizontal axes) and the gene contagion  
671 probability (vertical axes). Green: positive slopes; Red: negative slopes; Blue: the slope  
672 is not significantly different from zero (p-value  $\geq 1 \times 10^{-6}$ ).

Table 1- Pseudocode of the program\*.

Process	Pseudo Code
Gene transfer	For each connection between two individuals do (for each individual of the connection do (get the genes present in each individual metagenome; transmit genes to the other individual of the connection according to the gene contagion probability))
Transfer of bacterial pathogens	For each connection between two individuals do (for each individual of the connection do (get the pathogenic species present in each individual; transmit pathogen to the other individual of the connection according to the bacterial pathogen contagion probability))
Screening individuals	of For each individual do (check if the individual has a pathogenic bacteria)
Antibiotic effect	Choose an antibiotic randomly. Select all resistance genes associated with the chosen antibiotic. Eliminate resistance genes not associated with the chosen antibiotic according to the probability of eliminating genes under antibiotic intake. Eliminate virulence genes according to the probability of eliminating genes under antibiotic intake.
Loss rate of resistance genes under antibiotic consumption	Eliminate resistance genes not associated with the chosen antibiotic according to the loss rate probability.
Loss rate of resistance genes without antibiotic consumption	Eliminate resistance genes according to the loss rate probability.
Immigration of bacterial pathogen into the network	of For each bacterial species do (select a random individual; insert the bacterial pathogen in the individual)

\*The program code was implemented in the Python programming language.

Table 2 - Parameters and default values used in simulations.

Parameters	Default values	Changing values
Rewiring connectivity probability $p$	0.5	0 or 1
Number of individuals	1000	3000
Number of virulence genes	100	200, 400
Number of resistance genes	100	200, 400
Number of pathogenic bacterial species	5	NA
Number of antibiotics	5	NA
Gene contagion probability	0.005, 0.01	0.0005, 0.0025, 0.015, 0.02
Bacterial pathogen contagion probability	0.15	0.05, 0.1, 0.2, 0.25
Probability of eliminating genes under antibiotic intake	0.7	0.3, 0.5
The loss rate of resistance genes	0, 0.005, 0.01, 0.015, 0.02, 0.025, 0.03	NA

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Table 3 – Number of pathogenic species according to the bacterial pathogen contagion probability.

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Number of pathogenic species (in 2 000 000 possibilities)						
Bacterial pathogen contagion probability	0	1	2	3	4	5
0.05	1987473	12496	31	0	0	0
0.1	1982852	17094	54	0	0	0
0.15	1973053	26763	184	0	0	0
0.2	1940458	58759	779	4	0	0
0.25	104967	262575	527204	705479	399253	522

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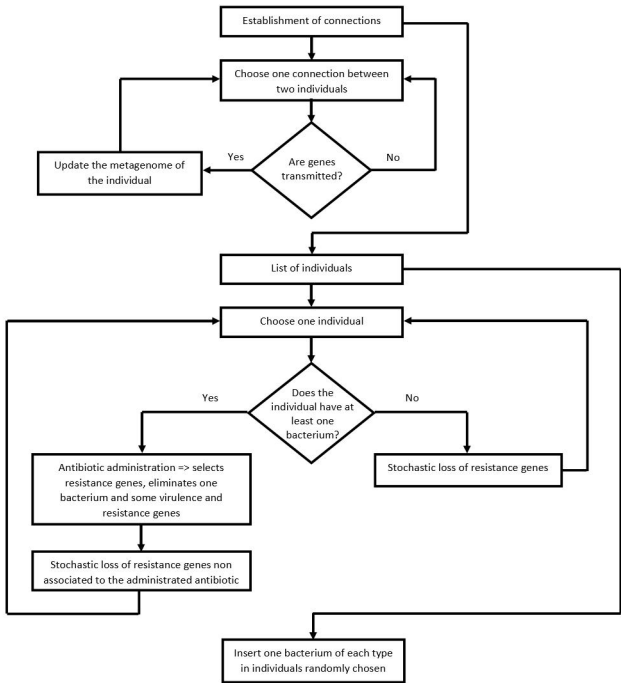
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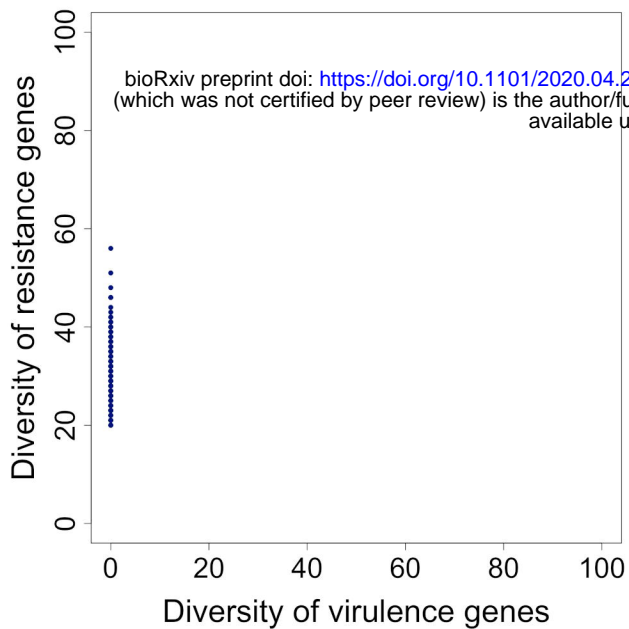
Table 4 - Simultaneous extinction of all pathogenic bacterial species according to the bacterial pathogen contagion probability.

Bacterial pathogen contagion probability	Number of times that all pathogenic bacterial species disappeared (in 2 000 possibilities)
0.05	570
0.1	70
0.15	2

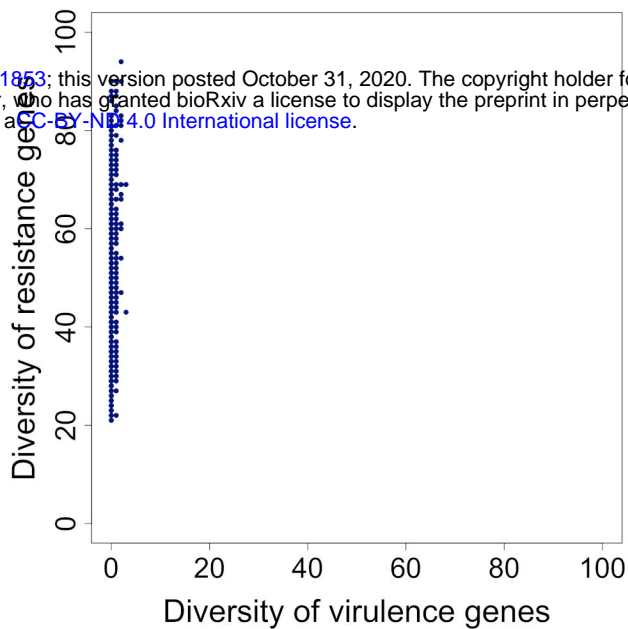
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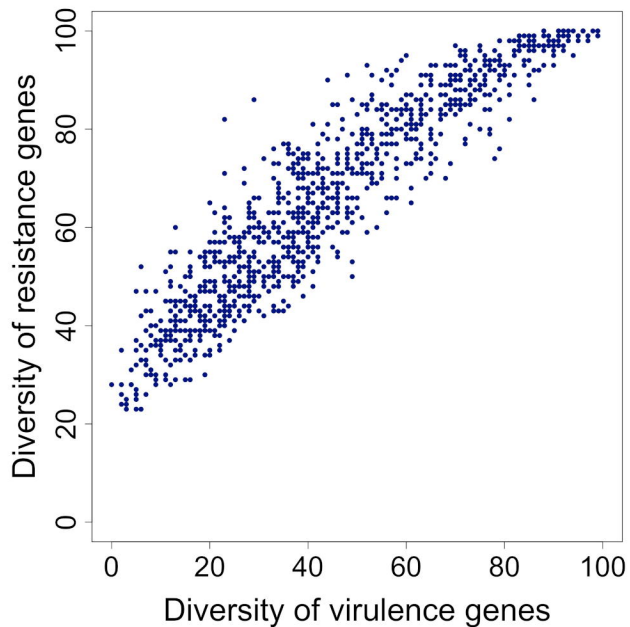
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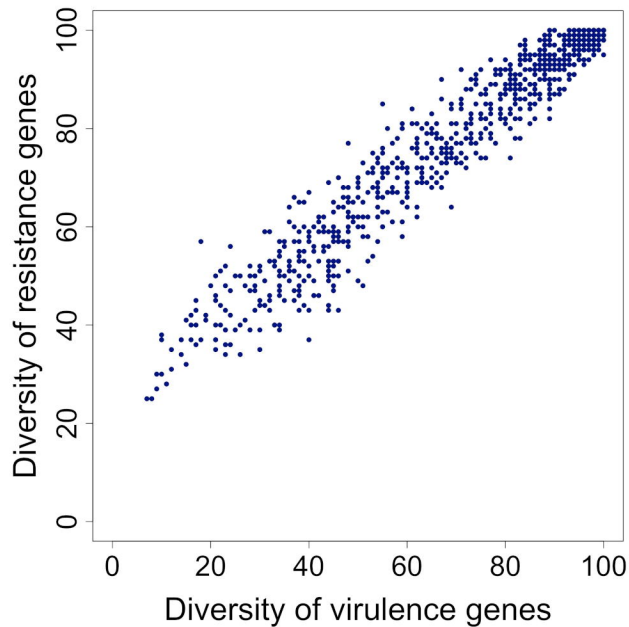
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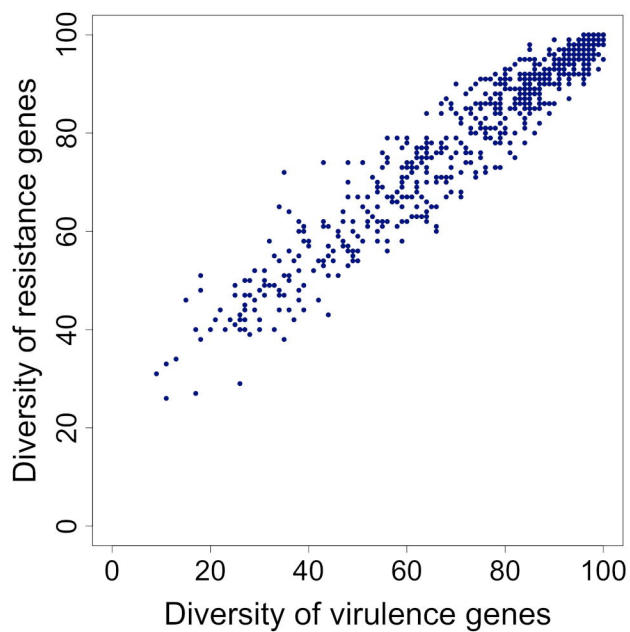
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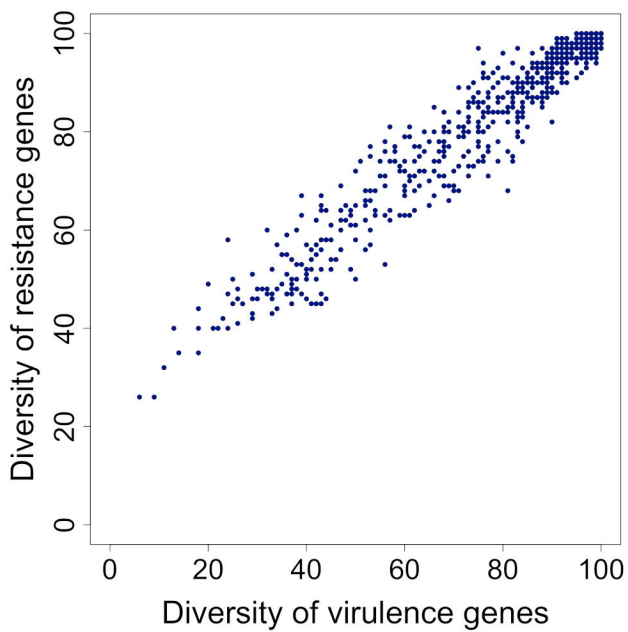
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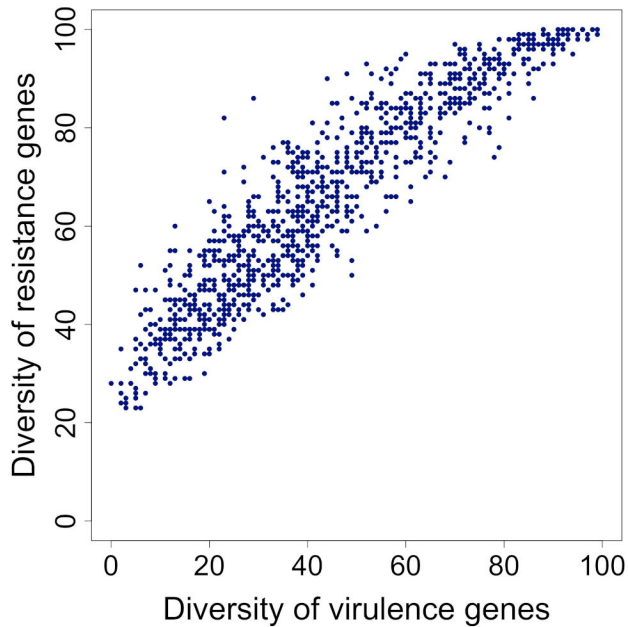
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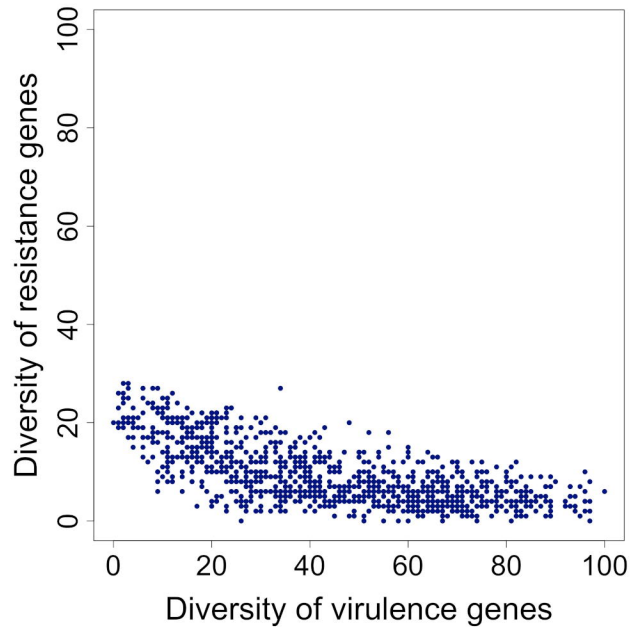
F



A



B



C

