Resident microbial communities inhibit growth and antibiotic resistance evolution of *Escherichia coli* in human gut microbiome samples

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Abstract

Countering the rise of antibiotic resistant pathogens requires improved understanding of how resistance emerges and spreads in individual species, which are often embedded in complex microbial communities such as the human gut microbiome. Interactions with other microorganisms in such communities might suppress growth and resistance evolution of individual species (e.g. via resource competition), but could also potentially accelerate resistance evolution via horizontal transfer of resistance genes. It remains unclear how these different effects balance out, partly because it is difficult to observe them directly. Here, we used a gut microcosm approach to quantify the effect of three different human gut microbiome communities on growth and resistance evolution of a focal strain of *Escherichia coli*. We found the resident microbial communities not only suppressed growth and colonization by focal *E. coli*, they also prevented it from evolving antibiotic resistance upon exposure to a beta-lactam antibiotic. With samples from all three human donors, our focal *E. coli* strain only evolved antibiotic resistance in the absence of the resident microbial community, even though we found highly effective resistance plasmids in resident microbial communities. We identified genetic constraints (lack of transfer genes) that can explain why our focal strain failed to acquire some of these plasmids, and for other plasmids further experiments revealed physical constraints on conjugative transfer. This suggests interactions with resident microbiota can inhibit antibiotic resistance evolution of individual species, and the potential for this to be counterbalanced by horizontal gene transfer depends on *in situ* transfer dynamics.

Introduction

The over- and inappropriate use of antibiotics has promoted the evolution of resistance in pathogens, resulting in a crisis for human health care [1]. To combat this problem, it is important to understand the underlying mechanisms of how resistance is acquired by bacteria and spreads within bacterial populations and communities [2,3]. A large body of research has used direct observations of resistance evolution in simplified laboratory conditions to understand how antibiotics drive the spread of resistance [4,5]. A key limitation of this approach is that it excludes interactions with other microorganisms, which we can expect to be important for bacteria evolving in natural or clinical settings because they spend most of their time in dense and diverse microbial communities. Interactions in species-rich microbial communities might negatively affect growth of individual species via, for example, competition for resources or niche space [6]. This may in turn inhibit antibiotic resistance evolution of individual species, because reduced population growth should reduce the supply of new genetic variation. On the other hand, interspecific interactions also potentially have positive effects on growth and evolution of individual species via, for example, exchange of genetic material [7] or cross-feeding [9,10] . In support, observations of bacteria isolated from natural and clinical settings indicate genes involved in antibiotic resistance evolution are often horizontally transferable [11,12]. Despite this, direct observations of how these different types of effects balance out are lacking. Consequently, it remains unclear how interactions with species-rich microbial communities affect growth and antibiotic resistance evolution of individual species or strains of bacteria.

The impact of interactions with other microorganisms for antibiotic resistance evolution is likely to be particularly important in the human gastrointestinal tract. This is one of the most

61 densely inhabited environments in the world, colonized by a rich diversity of bacteria, viruses
62 and eukarya, which are embedded in a network of biotic interactions [13,14]. Interactions
63 among microorganisms in the gut microbiome (which we take here to mean the resident
64 microorganisms, their genes and the local abiotic environment, following Marchesi & Ravel
65 [15] and Foster et al. [14]) play an important role for human health [16]. For example, the
66 microbiome protects the host from pathogens by minimizing the potential niche space for an
67 invading species [17,18]. This suggests competitive interactions with other microorganisms are
68 common, which we would expect to inhibit population growth of individual taxa and in turn
69 constrain their ability to evolve antibiotic resistance. On the other hand, some interactions in
70 the gut may be mutualistic [10] or modify the effects of antibiotics on individual species [19],
71 potentially resulting in a net positive effect on growth. Moreover, recent metagenomic studies
72 [20–22] showed the gut microbiome harbours a variety of mobile genetic elements, often
73 carrying resistance and virulence genes, that are shared by community members. Consistent
74 with this, horizontal transfer of resistance genes within individual hosts is central to resistance
75 evolution in several key pathogens found in the gastrointestinal tract [23–25]. This suggests
76 interactions with other microorganisms in the gut microbiome can also promote growth and
77 resistance of individual taxa. We aimed to quantify the net effect of interactions with species-
78 rich communities of other microorganisms, in particular those found in the human
79 gastrointestinal tract, for growth and resistance evolution of a given strain that newly arrives
80 in the community.
81
82 We approached this question using a human gut microcosm system consisting of anaerobic
83 fermentors filled with human faecal slurry, including the resident microbial community and
84 the beta-lactam antibiotic ampicillin, to which bacteria can evolve resistance either
85 chromosomally or mediated horizontally by picking up a beta-lactamase gene. Because the

86 microbiota in faecal samples reflects the diversity of the distant human gastrointestinal tract
87 [26], this approach allowed us to produce microcosms containing species-rich communities
88 sampled from human gut microbiomes. We aimed to determine how interactions with this
89 resident microbial community affected growth and resistance evolution of *Escherichia coli*.
90 We focused on *E. coli* because it is a ubiquitous gut commensal [27] and key opportunistic
91 pathogen [28] for which antibiotic resistance is an increasing problem [29]. We inoculated each
92 microcosm with a tagged, focal *E. coli* strain, before tracking its growth and resistance
93 evolution in the presence and absence of antibiotics. By also including microcosms containing
94 sterilized versions of the same faecal slurry (where the resident microbial community had been
95 deactivated), we quantified the net effect of interactions with the resident microbial
96 community. This approach allowed us to (i) track growth and resistance evolution of the focal
97 strain in the presence and absence of resident microbial communities sampled from multiple
98 human donors; (ii) isolate plasmid-carrying *E. coli* strains from the resident microbial
99 community and identify constraints on horizontal transfer of resistance genes; (iii) characterize
100 the resident microbial communities and how they changed over time. Our results show the
101 resident microbial community inhibits both growth and resistance evolution of *E. coli*, despite
102 the presence of resistance plasmids that can be conjugatively transferred to our focal strain in
103 certain physical conditions.
104

105 # Results

106

107 *Resident microbial communities suppressed growth of a focal* E. coli *strain*

108 We cultivated our focal *E. coli* strain in anaerobic microcosms in the presence and absence of
109 an antibiotic and three different samples of gastrointestinal microbiomes, each from a different
110 human donor, for seven days (Sup. Fig 1). Antibiotic treatment (ampicillin) reduced focal strain

111 abundance (effect of antibiotic in a generalized linear mixed model with zero inflation,
112 glmmadmb, χ^2 = 14.14, d.f. = 1, P < 0.001; Fig 1). Inclusion of the resident microbial
113 community from human faecal samples also reduced focal strain abundance over time, which
114 we inferred by comparing the community treatments (faecal slurries including the resident
115 microbial community) to the community-free treatments (sterilized versions of the same faecal
116 slurries; effect of community in glmmadmb, χ^2 = 6.65, d.f. = 1, P = 0.01; Fig 1). The
117 suppressive effect was strongest for populations exposed to both antibiotics and the resident
118 microbial community (antibiotic×community interaction in glmmadmb, χ^2 = 5.2, d.f. = 1, P =
119 0.02; Fig 1), resulting in extinction of the focal strain (below our detection limit) in populations
120 exposed to the resident microbial communities from human donors 1 and 3, and to very low
121 abundance with the community from human donor 2 (Fig 1). Average abundance of the focal
122 strain depended on which human donor sample was used to prepare the microcosms (effect of
123 human donor in glmmadmb, χ^2 = 12.08, d.f. = 2, P = 0.002). For example, in the absence of
124 ampicillin only the resident microbial community from human donor 1 was associated with a
125 rapid decrease of our focal strain under the detection limit. Despite the suppressive effect of all
126 three resident microbial communities, we found no evidence that abiotic factors in the faecal
127 slurry were suppressive for the focal strain: there was no significant variation in average focal
128 strain abundance among the community-free treatments and the control treatment containing
129 only the basal growth medium that was used to prepare faecal slurries (linear mixed effects
130 model, glmer: χ^2 = 0.41, d.f. = 3, P = 0.94). In summary, the resident microbial communities
131 sampled from three human donors each suppressed growth of a focal *E. coli* strain growing in
132 anaerobic fermentors filled with faecal slurry, and this effect was amplified by adding
133 antibiotics.
134
135 *Stable total bacterial abundance but variable community composition over time*

136 We used flow cytometry to estimate total bacterial abundance in microcosms containing
137 resident microbial communities, where we observed suppression of the focal strain above. In
138 antibiotic-free microcosms, total bacterial abundance was approximately stable over time (>10^9
139 cells/ml; Fig 2), and was higher on average than in microcosms exposed to antibiotics (effect
140 of antibiotic in a linear mixed effects model, lmm: $\chi^2 = 10.37$, d.f. = 1, $P = 0.001$). However,
141 the suppressive effect of the antibiotic varied over time (antibiotic×time interaction in lmm: χ^2
142 = 101.81, d.f. = 7, $P < 0.001$), being strongest at the beginning of the experiment. The effect
143 of the antibiotic also varied across communities from different human donors (antibiotic×donor
144 interaction in lmm: $\chi^2 = 79.30$, d.f. = 2, $P < 0.001$), with those from human donors 1 and 2
145 showing a stronger recovery after the first application of ampicillin (which resulted in a drop
146 in abundance after 24h) than the community from human donor 3. These results show our
147 experimental set-up sustained high numbers of microorganisms in the community treatments
148 over time in both the presence and absence of antibiotics.
149
150 To investigate the community composition in these microcosms, we used amplicon sequencing
151 of the variable regions 3 and 4 of the 16s rRNA gene. This revealed similar levels of within-
152 sample diversity (Shannon's alpha diversity; Sup. Fig 2) in microbiome samples from the three
153 human donors at the start of the experiment. Within-sample diversity then decreased slightly
154 over the first 24h of the experiment, and significantly between 24h and 168h (effect of time in
155 a linear mixed effects model including data from 24h and 168h, lme: $F_{1, 14} = 481.43$, $P < 0.001$).
156 This applied across the three human donors (effect of human donor, lme: $F_{2, 15} = 2.07$, $P =$
157 0.16), which also showed similar shifts in taxonomic composition over time (Fig 3).
158 Communities at 0h were dominated by the families *Lachnospiraceae* and *Ruminococcaceae*,
159 plus *Prevotellaceae* for human donor 3. Over time these groups became less abundant relative
160 to *Enterobacteriaceae* and *Bacteroidaceae* (note the focal strain also contributes to the total

161 abundance of *Enterobacteriaceae* in some microcosms, although our analysis of total bacterial
162 abundance above indicates the focal strain was always in the minority compared to other
163 *Enterobacteriaceae*, ranging between 0% and ~30% depending on the treatment group).
164 Compared to changes over time, ampicillin had a weak effect on within-sample diversity (effect
165 of antibiotic: $F_{1, 14} = 11.37$, $P = 0.006$). This interpretation was supported by an alternative
166 analysis (Principal Coordinate Analysis; Sup. Fig 3) based on Bray-Curtis dissimilarities. Thus,
167 despite approximately stable total abundance, we saw changes in community composition over
168 time that were more pronounced than differences among communities from different human
169 donors or antibiotic treatments.
170

171 *Antibiotic resistance evolved only in the absence of resident microbial*
172 *communities*

173 We screened for the emergence of antibiotic-resistant variants of the focal strain (that had
174 acquired resistance to ampicillin) after every growth cycle by plating each population onto
175 antibiotic-selective plates (8 μg/ml ampicillin; approximating the minimal inhibitory
176 concentration (MIC) of the focal strain). We never observed resistant variants of the focal strain
177 in any of the community treatments (populations exposed to the resident microbial
178 communities from human microbiome samples). By contrast, in community-free treatments
179 (basal growth medium and sterilized human faecal slurries) resistant variants appeared toward
180 the end of the experiment at 120h (slurry from human donor 1) and 144h (basal growth medium
181 and slurry from human donor 3), although not in sterilized samples from human donor 2 (Fig
182 1). Thus, the resident microbial community from human microbiome samples suppressed
183 antibiotic resistance evolution in our focal strain.
184

185 To investigate genetic mechanisms associated with resistance evolution and general adaptation
186 to our experimental conditions, we performed whole-genome sequencing for two sets of focal
187 strain isolates from the final time point: 8 ampicillin-resistant colony isolates from ampicillin
188 plates (one from each of the eight populations where we observed the emergence of antibiotic
189 resistance during the experiment) and 33 randomly selected colony isolates from ampicillin-
190 free plates (each from a different population and across all treatments). In the antibiotic-
191 resistant isolates, all SNPs and the deletion we found (Table 1) were in genes related to
192 membranes (*ompR, ftsI, opgB*), general stress response (*rpoC, relA*) or transcription regulation
193 (*rpoD*). Two genes were mutated independently in multiple colony isolates: *rpoC* and *rpoD*.
194 We also detected an insertion sequence (IS) movement between *perR* and *insN* in two colony
195 isolates. Of genes where we detected mutations in a single colony isolate, *ftsI* [30], *relA* [31]
196 and *ompR* [32] have each been previously annotated as being involved in resistance to beta-
197 lactam antibiotics. Consistent with a role in ampicillin resistance, *ompR* was also mutated in
198 three randomly selected colony isolates, all from populations that had been exposed to
199 ampicillin during the experiment (Sup. Table 1). We found six other genes mutated in parallel
200 in between 2 and 5 randomly selected colony isolates (Sup. Table 1). Five of these were
201 mutated in isolates from both antibiotic and antibiotic-free treatments. This included *insN*
202 (involved in transposition) and *gtrS* (involved in aerobic respiration), both mutated in five
203 isolates. Across the two sets of colony isolates (randomly selected and antibiotic-resistant),
204 three other loci were mutated in both sets. These were *rpoD* (only in isolates that had been
205 exposed to antibiotics), *opgB* and *yaiO* (both in isolates from antibiotic and antibiotic-free
206 treatments). In summary, we found some parallel genetic changes specific to antibiotic
207 treatments and consistent with known resistance mechanisms, plus other genetic changes that
208 occurred across antibiotic and antibiotic-free treatments and are therefore more likely involved
209 in general adaptation.

210

211 *Resistance plasmids in resident* E. coli *and constraints on their transfer*

212 We next sought to explain why we never observed antibiotic resistance evolution of the focal
213 strain in the presence of resident microbial communities, which we had expected to harbour
214 beneficial resistance genes [21,22,33–35]. We hypothesized this could have been due to a lack
215 of horizontally transferable resistance genes in the resident microbial communities. However,
216 we detected ampicillin resistant *E. coli* in the resident microbial communities from human
217 donors 1 and 3 (by selective plating), and after sequencing their genomes we identified several
218 antibiotic resistance genes that were associated with plasmid genes in these isolates (Fig 4;
219 Sup. Table 2). In the isolates from human donor 1, the plasmid formed one large contig with
220 three known resistance genes (Fig 4; Sup. Table 2), including the one conferring resistance to
221 beta-lactam antibiotics. We also identified two IncF replicons on this plasmid and a complete
222 set of *tra* genes, which are involved in conjugative plasmid transfer. For genome assemblies
223 from isolates of human donor 3, we also found the plasmid replicon and resistance genes on
224 the same contig in one out of six sequenced isolates. The other five genome assemblies
225 contained the same resistance genes and replicon across multiple smaller contigs (Sup. Table
226 2). Mapping the corresponding sequencing reads to the single contig found in one isolate
227 revealed complete coverage in all five cases, consistent with the putative plasmid having the
228 same genomic structure across each of the isolates. This putative plasmid from human donor 3
229 also carried multiple resistance genes, including beta-lactamases, and an IncQ replicon, which
230 is a part of the *repA* gene [36], but we detected no *tra* genes (Fig 4; Sup. Table 2).

231

232 We hypothesized that the lack of plasmid-driven resistance evolution in our focal strain might
233 have been caused by constraints on conjugative transfer that made these plasmids inaccessible.
234 Using a conjugative mating assay on agar, we never found transconjugants of our focal strain

235 when it was mixed with a plasmid-carrying isolate from human donor 3. This is consistent with
236 the lack of *tra* genes on this plasmid and suggests it could not be transferred into our focal
237 strain by conjugation in the absence of other drivers of horizontal gene transfer (e.g. phages or
238 other plasmids). This is also consistent with past work suggesting IncQ plasmids are
239 mobilizable rather than conjugative [37,38], and that we did not detect any other plasmid
240 replicons in the same isolates that might have belonged to helper plasmids that could mobilize
241 the IncQ plasmid. However, for the plasmid from human donor 1 we found transconjugants of
242 our focal strain at the end of the mating assay, which we confirmed by colony PCR (Sup. Fig
243 4). This suggests this plasmid was conjugative and could be transferred to our focal strain,
244 consistent with the presence of *tra* genes on this plasmid (Fig 4; Sup. Table 2).
245
246 Given that the resistance plasmid from human donor 1 was transferable into our focal strain,
247 why did it not spread in the main experiment above? We hypothesized this could result from
248 plasmid-borne resistance being less beneficial than resistance acquired by chromosomal
249 mutation (as we observed in the main experiment). We would expect a net benefit of resistance
250 to result in increased population growth at the antibiotic concentration applied during the
251 experiment (7.2 μg/ml). We found acquisition of the plasmid conferred a much larger increase
252 in population growth across all non-zero concentrations than that observed for evolved colony
253 isolates that had evolved resistance via chromosomal mutation during the main experiment
254 (Fig 5A). The minimal inhibitory concentration for evolved isolates with chromosome-borne
255 resistance was 2-4 times higher than for the ancestral focal strain, whereas the MIC of the
256 transconjugant focal strain (with the plasmid from human donor 1) was higher than the highest
257 concentration we tested (Fig 5A). The transconjugant also did not show reduced growth in the
258 absence of antibiotics (at 0 μg/ml in Fig 5A), indicating the large increase in resistance was not
259 offset by large fitness costs. This suggests resistance conferred by the plasmid from human

260 donor 1 has a strong benefit compared to chromosomally acquired resistance. Therefore the
261 lack of plasmid spread in the main experiment cannot be attributed to a weak benefit compared
262 to chromosomal resistance.
263
264 Another possible explanation for the lack of transfer of the resistance plasmid from human
265 donor 1 in the main experiment is that conjugative transfer of this plasmid might be specific to
266 particular environmental conditions. This has been observed for other plasmids across various
267 experimental abiotic conditions [39,40]. We tested this by carrying out mating assays as above,
268 but in a range of different experimental conditions. We detected transconjugants that had
269 acquired the plasmid at a final frequency of around 0.2% (as a fraction of the total recipient
270 population) after mixing the plasmid-carrying isolate from human donor 1 and the focal strain
271 on an agar surface, but we did not find any transconjugants after doing the same experiment in
272 three different types of liquid growth medium (lysogeny broth (LB), anaerobic LB, and
273 anaerobic community-free faecal slurry as in the main experiment; Fig 5B). This shows transfer
274 of the conjugative plasmid we isolated from human donor 1 requires particular abiotic
275 conditions, which may explain why our focal strain failed to evolve resistance via horizontal
276 gene transfer in the presence of resident microbial communities.
277

278 # Discussion

279
280 We found resistance via chromosomal mutation to an important class of antibiotics (beta-
281 lactams) evolved in a focal *E. coli* strain in our experiment only in the absence of resident
282 microbial communities sampled from healthy human volunteers. The suppressive effect of
283 these resident microbial communities was strong enough that the focal *E. coli* strain was driven
284 towards extinction (below our detection limit) when it was exposed to both antibiotics and the

285 community simultaneously. Consequently, the net effect of the resident microbial communities
286 here was to confer a form of colonization resistance against a non-resident strain, and to prevent
287 that strain from evolving antibiotic resistance. Our analysis of resident *E. coli* isolates (not the
288 focal strain) from the microbial communities showed this occurred despite the presence of
289 beneficial, horizontally transferable plasmids with the potential to confer resistance in our focal
290 strain. Genomic analyses and conjugation experiments with these plasmids showed the *in situ*
291 transfer dynamics depend critically on genetic (the presence of genes encoding the machinery
292 for conjugative transfer) and abiotic (physical structure of the environment) factors. This is
293 important because ultimately it is the *in situ* transfer dynamics that will determine whether or
294 not horizontal transfer of beneficial resistance genes is sufficient to counteract the growth-
295 suppressive effects of interactions with the community and confer a net benefit to invading
296 lineages.
297
298 The first key implication of our work is that as well as suppressing growth and colonization by
299 invading strains [18,41], the gastrointestinal microbiota can inhibit antibiotic resistance
300 evolution. There are several possible ecological mechanisms by which the microbiota may
301 suppress growth of invading lineages [42], such as niche and nutrient competition [43], direct
302 killing via bacteriocins [17,44], phage production [45] or by changing the concentration of
303 compounds such as primary and secondary bile acids [46,47]. It was not our aim to pull apart
304 the mechanisms by which bacteria in the microbiota compete with or suppress *E. coli* (studied
305 in more detail elsewhere; [44,48]), although supernatant experiments revealed no evidence of
306 direct inhibition via phages (see Methods). Crucially, no matter how interactions with the
307 microbiota suppress growth of an invading lineage, we expect the reduced population size,
308 growth and replication to in turn reduce the supply of new genetic variation. Our experiment
309 showed that this can be strong enough to prevent antibiotic resistance evolution. By contrast,

310 in the absence of the resident microbiota, resistance evolved in eight replicate populations. This
311 is consistent with *in vitro* work with malaria parasites showing competition between two
312 species under resource limitation impeded drug resistance evolution [49] and recent studies
313 with *Pseudomonas fluorescens* showing that a eukaryotic predator [50] or a bacteriophage [51]
314 can suppress the emergence of antibiotic resistance. Thus, suppression of an invading lineage
315 via interactions with resident microbiota may frequently have a knock-on effect on resistance
316 evolution.
317
318 A second key implication of our data concerns the genetic and environmental constraints on
319 horizontal gene transfer that determine whether or not the growth-suppressive effects of the
320 microbiota are counter-balanced by horizontal transfer of beneficial genes. We found the
321 unavailability of one putative plasmid to the invading focal strain was associated with a lack
322 of known conjugative transfer machinery. This plasmid belonged to the IncQ group, which is
323 considered mobilizable but not conjugative [37]. The other plasmid we isolated was
324 conjugative, but transfer depended on the abiotic conditions. This suggests the potential for
325 plasmid transfer to allow invading lineages to overcome the suppressive effects of the
326 microbiota depends critically on whether they are conjugative (which can be predicted from
327 sequence data) and on the sensitivity of conjugative transfer to local physical conditions (which
328 is harder to predict from sequence data). Consistent with this, previous research has shown
329 conjugative transfer in *E. coli* and other species is sensitive to the physical experimental
330 conditions [40,52]. Furthermore, mating pair formation machinery, usually encoded by the
331 plasmid, in some cases promotes biofilm formation, which can in turn promote the spread of
332 plasmids [53]. This raises the question of whether some plasmids have evolved to manipulate
333 the physical structure of bacterial populations to promote transfer and overcome the otherwise
334 suppressive effect of interactions with plasmid-free cells. Plasmids are clearly sometimes

335 transferred *in vivo*, as has been observed in animal models [54,55] and human gut microbiomes
336 [33,34,56]. In line with plasmids being key vectors of beta-lactamases [57], the conjugative
337 plasmid we identified was highly effective in terms of resistance. This suggests plasmid-borne
338 resistance will be under strong positive selection once established and can spread rapidly via
339 clonal expansion. However, our experiment showed the initial horizontal transfer required for
340 such spread is sensitive to genetic and abiotic constraints.
341
342 Our approach allowed us to isolate the effect of interactions between diverse microbial
343 communities and a focal *E. coli* strain. Our amplicon sequence data showed the communities
344 had a representative taxonomic composition for healthy human donors. There were still diverse
345 communities present at the end of the seven-day experiment, albeit with a change in the relative
346 abundance of different taxa. The observed rise of *Enterobacteriaceae* and *Bacteroidaceae* has
347 been seen in other experiments with gastrointestinal communities (e.g. [58]) and might be
348 explained by the nutrient content of the medium, micromolar oxygen levels or such *in vitro*
349 systems favouring faster population growth [59]. More importantly, the shift in community
350 composition over the seven-day experiment does not explain the observed suppressive effect
351 of the communities on the focal strain, because this was already visible after one day. Thus,
352 although our experimental system likely differs from the gastrointestinal tracts these bacteria
353 were isolated from in ways that affect community composition, cultivating them *in vitro*
354 allowed us to quantify the effect of species-rich communities sampled from gastrointestinal
355 tracts on resistance evolution, and we observed a consistent effect across the time series for
356 each donor. A key challenge for future work will be to determine how such effects are modified
357 *in vivo* by local spatial structure [60] and immune responses [61]. Indeed, interactions mediated
358 via the host immune system are another possible mechanism of colonization resistance [62–
359 64]. We suspect real progress will require a combination of animal models (direct observations

360 *in vivo*, but non-human microbiomes [24]), comparative analysis of human microbiota and
361 infection outcomes (e.g. [65]), and further work with systems such as ours measuring the
362 effects of human microbiota directly in simplified conditions.
363
364 In conclusion, we showed species-rich microbial communities sampled from human
365 gastrointestinal tracts can suppress growth and resistance evolution of an invading lineage.
366 Given the variety and likely common occurrence of mechanisms that can generate such
367 suppression of invaders (e.g. resource competition), these types of effects are likely common
368 in species-rich communities such as the mammalian gastrointestinal tract. Our other data
369 showed, whether or not these suppressive effects are counter-balanced by beneficial horizontal
370 gene transfer, depends on genetic and environmental constraints that can impede the spread of
371 resistance plasmids. This has important implications for the prediction of resistance evolution
372 from genetic and metagenomics data, such as those widely collected through surveillance
373 efforts [66,67]: identifying mobile resistance genes in a diverse community is not enough to
374 predict resistance evolution, requiring in addition information about genetic and environmental
375 constraints on *in situ* transfer dynamics.
376

377 # Material and Methods

378

379 *Bacterial strains*

380 We used *Escherichia coli* K12 MG1655 carrying a Streptomycin resistance mutation (*rpsL*
381 K43R) as the focal strain. Two days prior to the experiment we streaked the focal strain on LB-
382 agar (Sigma-Aldrich, Buchs, Switzerland) and incubated overnight at 37 °C. To incubate the
383 focal strain cultures anaerobically prior to the microcosm experiment, we prepared 42 Hungate
384 tubes (VWR, Schlieren, Switzerland) with LB (Sigma-Aldrich), which was supplemented with

385 0.5 g/l L-Cysteine and 0.001 g/l Resazurin, flushed the headspace with nitrogen, sealed the
386 tubes with a rubber stopper and autoclaved them. One day before the experiment, we randomly
387 picked 42 colonies and inoculated them in the 42 Hungate tubes containing anaerobic LB, and
388 incubated at 37 °C overnight with 220 rpm shaking. We then used these 42 independent
389 cultures of the focal strain to inoculate the main experiment described below.
390
391 *Human microbiome samples*
392 We used three stool samples from anonymous, consenting human donors. Participants received
393 an information sheet and a consent form with the sampling kit. The consent form stated that by
394 donating a sample they consent to taking part in the study; no other written or oral consent
395 form was collected because of the fully anonymized sampling protocol. Inclusion criteria were:
396 older than 18 years, not obese, not recovering from surgery and no antibiotics in the last six
397 months. The sampling protocol was approved by the ETHZ Ethics Commission (EK 2016-N-
398 55). All samples were collected at the Department of Environmental Systems Science, ETH
399 Zürich on the 15th of May 2018. Each sample was collected in a 500 ml plastic specimen
400 container (Sigma-Aldrich) and kept anaerobic using one AnaeroGen anaerobic sachet (Thermo
401 Scientific, Basel, Switzerland). The three samples used for the experiment were randomly
402 selected from a larger number of donated samples. We collected the samples in the morning
403 before the experiment and kept them for maximum one hour before processing. To prepare
404 faecal slurry from each sample, we re-suspended 20 g of sample in 200 ml anaerobic peptone
405 wash (1 g/l peptone, 0.5 g/l L-Cysteine, 0.5 g/l bile salts and 0.001 g/l Resazurin; Sigma-
406 Aldrich) to prepare a 10% (w/v) faecal slurry. We then stirred the slurry for 15 min on a
407 magnetic stirrer to homogenize, followed by 10 min of resting to sediment. At this point we
408 removed 100 ml of each faecal slurry ('fresh slurry'), which we used later to re-introduce the
409 resident microbial community to sterilized slurry (for the community treatments). To sterilize

410 the faecal slurries, we transferred 100 ml to a 250 ml Schott bottle, flushed the headspace with
411 Nitrogen gas, sealed them with rubber stoppers and autoclaved for 20 min at 121 °C.
412
413 *Inoculating anaerobic gut microcosms, sampling and bacterial enumeration*
414 For the start of the experiment (Sup. Fig 1), we filled 42 Hungate tubes with 7 ml of basal
415 medium (2 g/l Peptone, 2 g/l Tryptone, 2 g/l Yeast extract, 0.1 g/l NaCl, 0.04g K_2HPO_4, 0.04
416 g/l KH_2PO_4, 0.01 g/l $MgSO_4x7H_2O$, 0.01 g/l $CaCl_2x6H_2O$, 2g/l $NaHCO_3$, 2 ml Tween 80, 0.005
417 g/l Hemin, 0.5 g/l L-Cysteine, 0.5 g/l bile salts, 2g/l Starch, 1.5 g/l casein, 0.001g/l Resazurin,
418 pH adjusted to 7, addition of 0.001g/l Menadion after autoclaving; Sigma-Aldrich), and for the
419 subsequent re-inoculation cycles with 6.5 ml of basal medium. We flushed the head space of
420 each tube with nitrogen gas, sealed it with a rubber septum and autoclaved to produce anaerobic
421 microcosms containing only basal medium.
422
423 On day one of the experiment, we introduced faecal slurry and antibiotics to each tube
424 according to a fully factorial design (Sup. Fig 1), with three replicate microcosms in each
425 combination of Human Donor (1, 2 or 3), Community (present or absent) and Antibiotic (with
426 or without). In the community-free treatments, we added 850 µl of sterile slurry. In the
427 community treatments, we added 350 µl of fresh slurry and 500 µl of sterilized slurry. In the
428 antibiotic treatment, we added ampicillin to a final concentration of 7.2 µg/ml, approximating
429 the IC90 (concentration required to reduce growth by 90%) for the focal strain; this was
430 introduced 2h after the focal strain had been inoculated (8 µl of focal *E. coli* from one of the
431 42 overnight cultures introduced at 0h; approximately 1:1000 dilution). As a control treatment
432 testing for the effect of sterilized slurry, we also inoculated the focal strain into three replicate
433 microcosms containing only the basal medium (supplemented with 850 µl of peptone wash to
434 equalize the volume with the slurry treatments), and we did this with and without antibiotic

435 treatment. We incubated all microcosms at 37 °C in a static incubator. After 24h we transferred
436 a sample of 800 µl from each microcosm to a new microcosm (containing basal medium plus
437 500 µl of sterile slurry from the corresponding human donor in the community and community-
438 free treatments, or basal medium plus peptone wash for the basal medium treatment,
439 supplemented with ampicillin at each transfer in the antibiotic treatments), and we repeated
440 this for seven days.
441
442 To estimate the abundance of the focal strain during the experiment, we used a combination of
443 selective plating and colony PCR. For selective plating, we serially diluted the samples and
444 plated them on Chromatic MH agar (Liofilchem, Roseto degli Abruzzi, Italy), which allowed
445 us in a first step to discriminate *E. coli* from other species based on colony colour. By
446 supplementing these agar plates with Streptomycin (200 µg/ml), to which our focal strain is
447 resistant, we selected against other *E. coli* that were not resistant to Streptomycin. To screen
448 for variants of our focal strain that acquired resistance to Ampicillin during the experiment, we
449 additionally plated each sample onto the same agar supplemented with both Streptomycin (200
450 µg/ml, Sigma-Aldrich) and Ampicillin (8 µg/ml, Sigma-Aldrich). We did this after every
451 growth cycle. Despite initial screening of microbiome samples revealing no resident *E. coli*
452 that could grow on our selective plates, later in the experiment we found such bacteria to be
453 present in some samples (that is, non-focal strain *E. coli* that could grow on our plates and were
454 presumably very rare at the beginning of the experiment). To discriminate between these *E.*
455 *coli* and our focal strain, we used colony PCR with specific primers (forward (5'- AGA CGA
456 CCA ATA GCC GCT TT-3'); reverse (5'- TTG ATG TTC CGC TGA CGT CT-3')). For
457 colony PCR, we picked 10 colonies randomly for each time point and treatment. The PCR
458 reaction mix consisted of 2x GoTaq green master mix, 2.5 µM of each primer and nuclease
459 free water. The thermal cycle program ran on a labcycler (Sensoquest, Göttingen, Germany)

460 with 6 min 95°C initial denaturation and the 30 cycles of 95°C for 1 min, 58°C for 30s, 72°C
461 for 35s and a final elongation step of 72°C for 5 min. For gel electrophoresis we transferred 5
462 µl of the PCR reaction to a 1.5% agarose gel stained with sybr safe (Sigma-Aldrich) and
463 visualized by UV illumination. Focal strain abundance was then estimated by multiplying the
464 frequency of the focal strain determined by colony PCR with the total colony count for each
465 plate. To account for the possibility that the focal strain was still abundant in populations where
466 we found 0/10 colonies (that is, where it could have been rare relative to resident *E. coli* but
467 still present), we additionally screened the DNA extracted from the community (described
468 below) of the final time point by PCR (as described above for the colony PCR, but using 30 ng
469 of DNA as template). We did this for all microcosms from the community treatment and
470 detected PCR products in all cases where we detected focal strain by plating and colony PCR,
471 and none of the cases where we did not, consistent with our analysis of individual colonies and
472 suggesting that in those microcosms the focal strain had been completely excluded during the
473 experiment.
474
475 To estimate total microbial abundance in each microcosm supplemented with the microbiome
476 we used flow cytometry. We diluted samples by 1:10000 with phosphate buffered saline (PBS)
477 and stained them with Sybr green (Sigma-Aldrich). We used a Novocyte 2000R (ACEA
478 Biosciences Inc. , San Diego, CA, USA), equipped with a 488 nm laser and the standard filter
479 setup for the flow cytometric measurements.
480
481 We froze samples after every transfer from each microcosm at -80 °C, and at the end of the
482 experiment we isolated two sets of focal strain colony isolates for sequencing. First, we
483 randomly picked a single focal strain colony isolate from each microcosm where the focal
484 strain was detected at the end of the experiment (from Streptomycin plates; *n*=33). Second, we

485 randomly picked a single ampicillin-resistant colony isolate of the focal strain from each of the
486 8 populations at the end of the experiment where they were detected (from Streptomycin +
487 Ampicillin plates; *n*=8). We grew each colony isolate overnight in LB (with Ampicillin for the
488 ampicillin-resistant isolates), mixed 1:1 with 50% glycerol and stored at -80 °C.
489
490 *Supernatant assay for potential phage killing*
491 We also screened the initial faecal slurries from each human donor for evidence of lysis of our
492 focal strain, which might be caused by lytic phages. We centrifuged each faecal slurry (10 min,
493 2000 g), filtered the supernatant with cellulose (Melitta, Egerkingen, Switzerland), then 0.45
494 and 0.22 µm syringe filters, then supplemented with $MgSO_4$ and $CaCl_2$ (final concentration 8
495 µM), and stored at 4 °C. We then made lawns of the focal strain on agar and added 10 µl of
496 supernatant extract from each human donor and incubated 24h at 37 °C. We never observed
497 any plaques or evidence of lysis.
498
499 *Whole genome sequencing and Bioinformatics*
500 We sequenced all of the randomly selected (*n*=33) and ampicillin-resistant (*n*=8) focal strain
501 colony isolates. Prior to DNA extraction, we grew each isolate overnight in LB, then
502 concentrated the liquid cultures by centrifugation and extracted DNA with the Magattract kit
503 (Qiagen, Hilden;Germany) according to the manufacturer's protocol. Quality and quantity of
504 DNA was assessed with Nanodrop (Thermo Fisher) and Qubit (Thermo Fisher). At the
505 Functional Genomics Center (ETH Zürich / University of Zürich) DNA was processed with
506 Nextera library prep kit and sequenced on the Illumina Hiseq 4000 platform. We filtered raw
507 sequencing reads with trimmomatic [68] and mapped the reads to the reference with snippy
508 (https://github.com/tseemann/snippy) to detect variants. Deletions were identified by using the
509 coverage analysis tool of CLC Genomics Workbench 11 (Qiagen) with a read threshold value

510 of 0 and a window size of 1. We identified IS elements with ISfinder (Siguier 2006) in the
511 ancestor genome and used these sequences to detect IS movements in the evolved strains with
512 ismapper[70].
513
514 We additionally sequenced 12 ampicillin-resistant resident *E. coli* colony isolates (not the focal
515 strain). We isolated these colony isolates from microcosms filled with faecal slurry from human
516 donors 1 and 3 at the final time point of the experiment (6 for each donor, each from a different
517 microcosm). We picked, grew and sequenced these colony isolates as described above for focal
518 strain isolates. We then made de Novo assemblies of the resulting sequences with spades [71]
519 and annotated them with prokka [72]. We screened for known antibiotic resistance genes and
520 the presence of plasmid replicons by a local blast query against the resfinder [73] and
521 plasmidfinder [36] databases.
522 To identify genes that are involved in mating pair formation or mobilization of the plasmid we
523 screened the genome annotation files and blasted potential candidate genes against the NCBI
524 nucleotide database to verify them.
525
526 *Mating experiments with plasmids from resident* E. coli
527 We aimed to determine whether the resident *E. coli* colony isolates could act as plasmid donors
528 for our focal strain (transferring antibiotic resistance plasmids via conjugation). Because the
529 replicate *E. coli* colony isolates that we sequenced from each human donor (1 and 3) were
530 almost identical on the DNA sequence level (Sup. Table 2), we randomly chose one colony
531 isolate from each human donor as a potential plasmid donor strain. We used a focal strain as
532 the potential plasmid recipient in these experiments, which was only different from the focal
533 strain used in the main experiment by addition of a dTomato marker and a Chloramphenicol
534 resistance cassette (enabling us to detect transconjugants by selective plating). In the first set

535 of mating experiments, we grew overnight cultures of the potential plasmid donor strains and
536 the potential plasmid recipient strain in LB at 37 °C with shaking. We then pelleted the
537 overnight cultures by centrifugation (3000 rpm, 5 min), washed them twice with PBS and
538 resuspended them in 300 µl PBS. We then mixed the donor and recipient strains 1:1 (v:v), and
539 transferred 100 µl of this mixture to each of three replicate LB plates, and incubated the plates
540 for 6h at 37 °C. After that, we washed the cells off the plate with 500 µl PBS and streaked out
541 50 µl of this plate wash on LB plates supplemented with Ampicillin and Chloramphenicol. To
542 verify plasmid uptake by the transconjugants, we used a colony-PCR screen with primers
543 targeting the recipient strain (same primer set we used above to identify the focal strain) and
544 three additional primer sets targeting the beta-lactamase gene *blaTem-1b* (blaFW: 5'-
545 TGCAACTTTATCCGCCTCCA-3'; blaRV 5'-TTGAGAGTTTTCGCCCCGAA-3'), the
546 *traC* gene (traFW 5'- TCGATAAACGCCAGCTGGTT-3'; traRV 5'-
547 AGGTGAAAACCCACAGCGAA-3') and the replicon *IncFIC (FII)* (IncFW 5'-
548 CACCATCCTGCACTTACAATGC-3'; IncRV 5'- TCAGGCCCGGTTAAAAGACA-3')
549 with the same PCR reaction mix and settings as described above.
550
551 To test whether environmental conditions affected conjugation efficiency, we performed a
552 second set of mating experiments in four different conditions: solid LB agar, liquid LB, liquid
553 anaerobic LB and anaerobic sterile faecal slurry. We prepared the liquid LB (Sigma-Aldrich)
554 and LB agar (Sigma-Aldrich) for all treatments in the same way (independent of whether they
555 were aerobic or anaerobic), supplementing them with 0.5 g/l L-Cysteine and 0.001 g/l
556 Resazurin (reducing agent and anaerobic indicator respectively). For the anaerobic LB
557 treatment, we transferred 0.9 ml LB to each Hungate tube, flushed the headspace with nitrogen
558 and sealed it with a rubber stopper before autoclaving. For the anaerobic sterile faecal slurry
559 treatment, we added 0.45 ml LB to each Hungate tube and 0.45ml thawed slurry under

560 anaerobic conditions, before flushing the headspace with Nitrogen, sealing and autoclaving.
561 Prior to each mating assay, recipient and donor strains were inoculated, washed, concentrated
562 and mixed exactly the same way as described above for the first set of mating experiments. For
563 each solid and liquid treatment four replicates were inoculated with 100 µl of the 1:1 donor
564 recipient mix, either under aerobic or anaerobic conditions according to the respective
565 treatment and all tubes and plates were incubated for 6h under static conditions at 37 °C. We
566 stopped the mating assay by either vortexing the liquid cultures or washing off the cells from
567 the plates with 1 ml of PBS. 100 µl of each bacterial suspension was then plated on selective
568 agar plates containing either Chloramphenicol to count total number of recipient cells or a mix
569 of Chloramphenicol and Ampicillin to count the number of transconjugants. After 24h we
570 calculated transconjugant frequencies by dividing cfu counts of plasmid-positive colonies by
571 the total cfu count of recipient cells [55].
572
573 We measured susceptibility to ampicillin for the ancestral focal strain from the main
574 experiment, the focal strain used in the conjugation experiment (with the dTomato tag), one
575 focal strain transconjugant (with the plasmid from the resident *E. coli* isolate of human donor
576 1), the resident *E. coli* isolate of human donor 1 (carrying the same plasmid), and all eight
577 ampicillin resistant focal strain isolates from the main experiment. We did this by measuring
578 OD600 after 24h incubation at various concentrations of ampicillin. We prepared overnight
579 cultures of each isolate in a randomly organized master plate, and then inoculated the
580 susceptibility assay using a pin replicator to transfer approximately 1 µl of the overnight
581 cultures to assay plates filled with 100 µl of 0-60 µg/ml ampicillin per well. We measured OD
582 at 0h and after 24h with a NanoQuant infinite M200Pro platereader (Tecan).
583
584 *Amplicon sequencing*

585 We thawed samples of fresh faecal slurry from 0h and samples from each microcosm in the
586 community treatments after 24h and 168h on ice and homogenised them by vortexing. We
587 concentrated 3 ml of each slurry sample by centrifuge subsequently twice 1.5 ml of the same
588 slurry sample directly in the bead beating extraction tubes provided by the extraction kit
589 (Qiagen). We extracted the DNA following the protocol of the powerlyzer power soil kit
590 (Qiagen). DNA yield and quality were checked by Qubit and Nanodrop.
591
592 We amplified the V3 and V4 region of the 16s rRNA gene with three slightly modified
593 universal primers [74] with an increment of a 1-nt frame shift in each primer [75] to increase
594 Miseq sequencing output performance between target region and Illumina adapter. The target
595 region was amplified by limited 17 cycle PCR with all three primer sets in one reaction for
596 each sample. We cleaned up PCR products and in a second PCR adapters with the Illumina
597 barcodes of the Nextera XT index Kit v2 were attached. We checked 10 randomly selected
598 samples on the Tapestation (Agilent, Basel, Switzerland) for the proper fragment size. We
599 quantified library by qPCR with the KAPA library quantification Kit (KAPA Biosystems,
600 Wilmington, MA, USA) on the LightCycler 480 (Roche, Basel, Switzerland). We normalized
601 quantified samples, pooled them in equimolar amounts and loaded the library with 5% PhiX
602 on the illumnina MiSeq platform with the Reagent Kit V3 at the Genetic Diversity Center
603 (ETHZ).
604
605 Sequencing reads were trimmed on both ends with seqtk (https://github.com/lh3/seqtk) and
606 amplicons were merged into pairs with flash [76]. USEARCH [77] was then used to trim primer
607 sites and amplicons were quality filtered using prinseq [78]. We clustered the quality filtered
608 sequences into zero-radius operational taxonomic units (ZOTU) using USEARCH. We used
609 Sintax implemented in the USEARCH pipeline with the SILVA database [79] for the

610 taxonomic assignment of the ZOTU sequences. For the analysis of taxonomic data including
611 plotting Shannnon diversity, relative proportions of taxa and to generate the PCoA plots, we
612 used the Phyloseq package in R [80].
613
614 *Statistical analyses*
615 We used R 3.5.1 [81] for all analyses. To test whether focal strain abundance differed between
616 treatments, we used a generalized linear mixed effects model with the glmmadmb function of
617 the glmmADMB package, with zero inflation and a poisson error distribution [82]. For this
618 analysis we excluded the basal medium treatment and used time, antibiotic (with / without),
619 resident microbial community (with / without) and human donor (1 / 2 / 3) as fixed effects and
620 replicate population as a random effect. The model was reduced by removing non-significant
621 ($P>0.05$) interactions using F-tests. P values for interaction terms in the reduced model were
622 obtained with type II Wald chisquare tests. To test whether there was an inhibitory effect of
623 sterilized slurry on the focal strain, we used the glmer function of the lme4 [83] R package. For
624 this analysis we included only community-free and antibiotic-free treatments, with focal strain
625 abundance as the response variable, donor as a fixed effect, replicate population as a random
626 effect, and a poisson error distribution.
627
628 To analyse differences in total bacterial abundance in the community treatments, we used a
629 linear mixed effects model with the lmer function of lme4 [83], with a poisson error
630 distribution. Time, donor and antibiotic were fixed effects and replicate population a random
631 affect. To analyse variation of Shannon diversity, we used a linear mixed effects model with
632 the lme function of nlme [84]. We excluded time point 0h from the analysis, and included
633 Time, Donor and Antibiotic as fixed effects and replicate population as a random effect.
634 Finally, to analyze similarities of microbiome samples based on the 16s rRNA data, we applied

635 the Bray-Curtis distance metric with the ordinate function of the Phyloseq R package to get
636 coordinates for the principal coordinate analysis (PCoA). On this dataset we ran a
637 permutational multivariate analysis of variance (PERMANOVA) with the adonis function of
638 vegan [85], using the distance matrix obtained from the PCoA analysis but omitting time point
639 0h. We did this separately for time points 24h and 168h .
640
641
642 *Data availability*
643 Sequences have been deposited in the European Nucleotide Archive under the study accession
644 number PRJEB32627 for the whole genome sequences of single colonies and PRJEB33429 for
645 the 16s rRNA gene amplicon data. Other data will be available through the Dryad repository
646 upon publication.
647
648
649
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655
656 *Competing interests*
657 The authors declare that no competing interests exist.
658

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660

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913 **Figure legends**

914

915 **Fig. 1. Resident microbial communities suppressed growth and resistance evolution of**
916 **focal *E. coli*.** Each panel shows abundance of the focal *E. coli* strain (in colony-forming units
917 per ml) over seven days for either basal medium (top row) or with faecal slurry from one of
918 three human donors, in the absence (left panels) or presence (right panels) of ampicillin applied
919 after 2h and thereafter at each transfer. Empty symbols show community-free treatments; filled
920 symbols show treatments with the resident microbial community; red symbols show
921 microcosms where we detected ampicillin-resistant colony isolates of the focal strain. The three
922 lines in each treatment show three replicate microcosms. Microcosms where we detected no
923 focal strain colonies are plotted at 10^0.

924

925 **Fig. 2. Total bacterial abundance over time in community treatments with and without**
926 **antibiotics.** Each panel shows data from a single human donor (rows) with or without
927 ampicillin (columns), with the three replicate communities in each panel shown by three
928 different lines. Total bacterial abundance was measured by flow cytometry (see Methods).

929

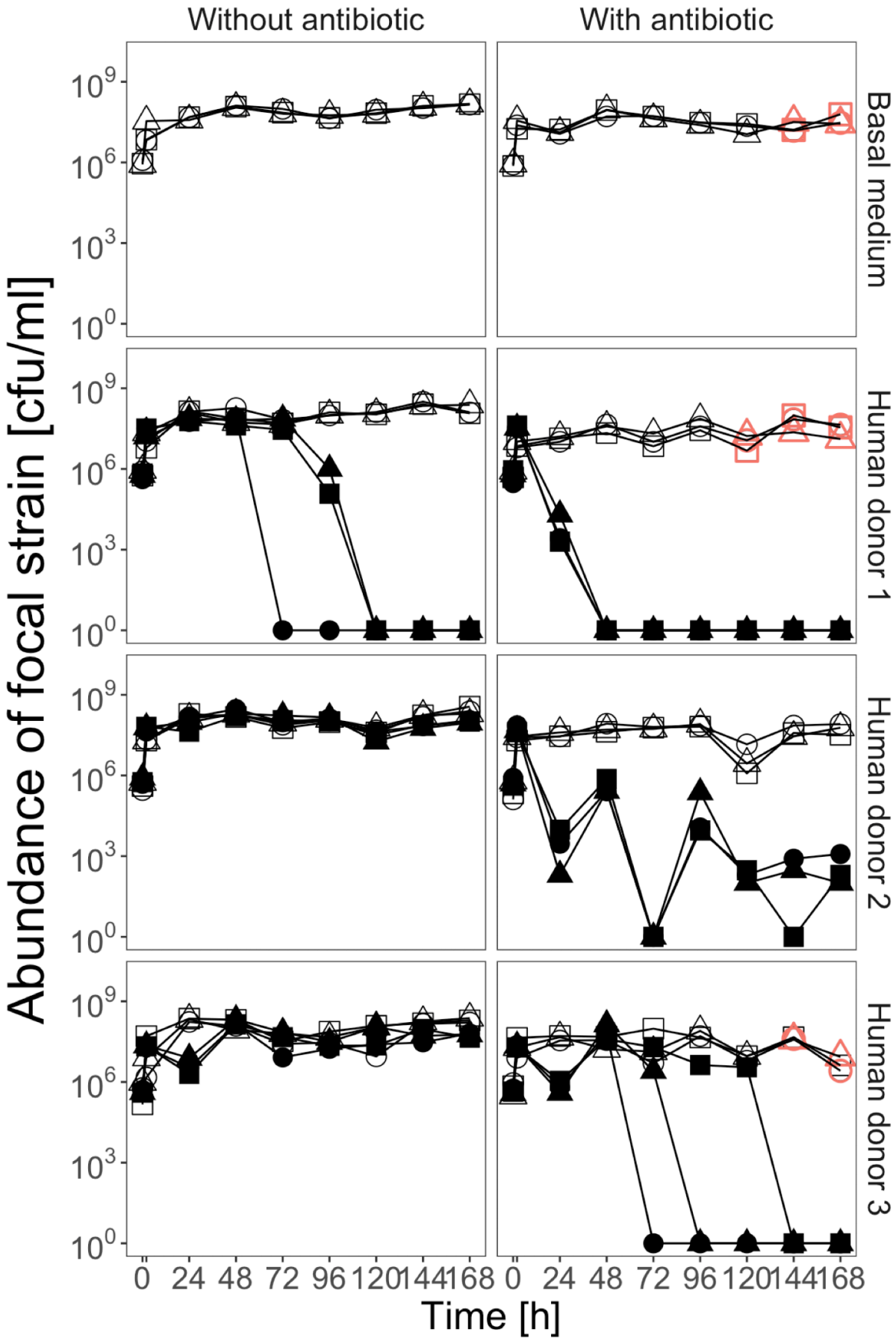
930 **Fig. 3. Changes in community composition over time.** Relative abundance of the 15 most
931 prevalent bacterial families in each microcosm at 0h, 24h and 168h.

932

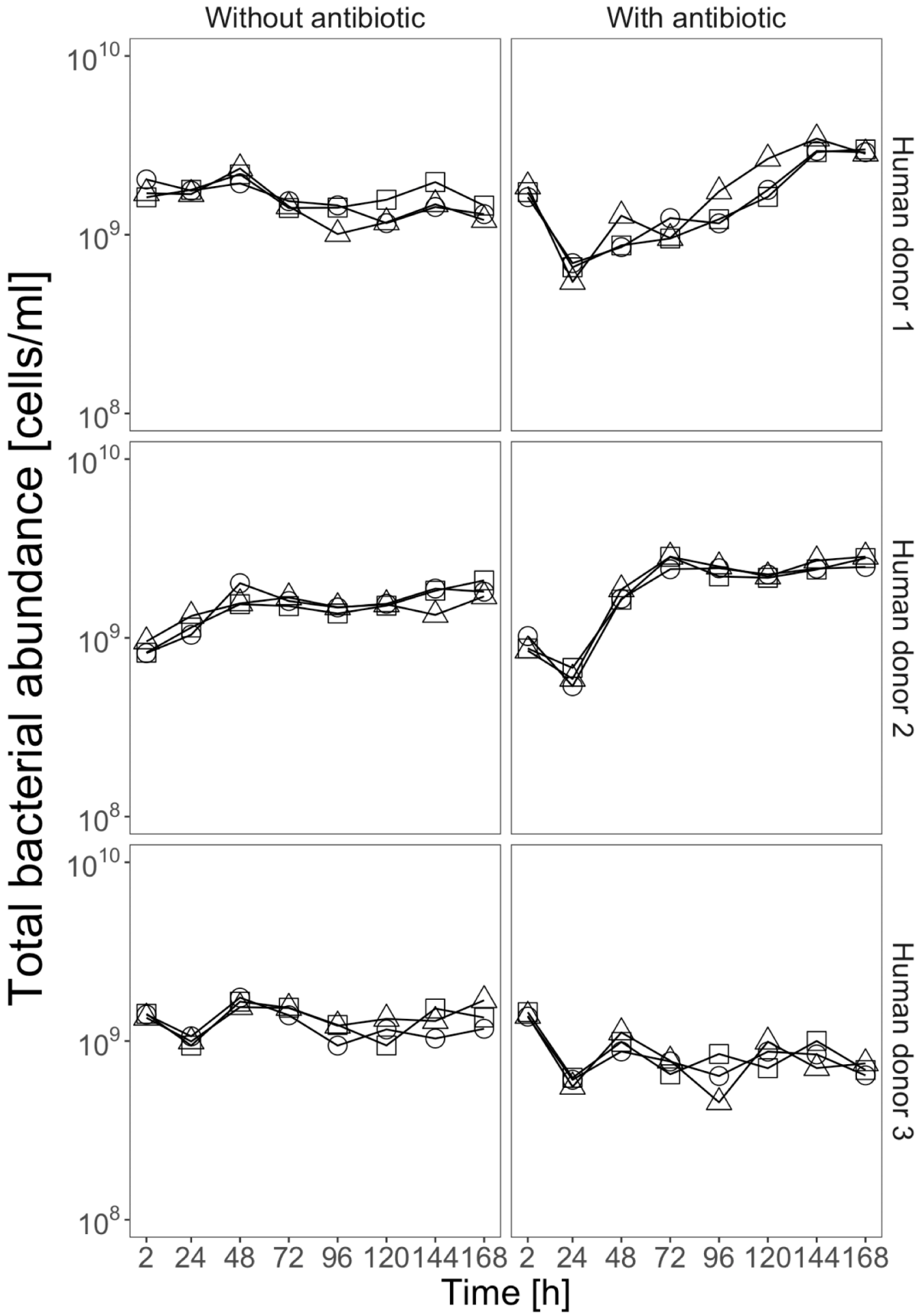
933 **Fig. 4. Schematic maps of resistance genes, replicons and transfer-related genes for the**
934 **putative plasmid contigs found in the sequenced resident *E. coli* isolates.** (A) Putative
935 plasmid contig of human donor 1 isolate with indicated replicon sequences (IncFIC, IncFIA),
936 genes involved in horizontal gene transfer (*tra* and *trb*) and resistance genes (*blaTem*, *sul2*,
937 *aph(3)*). (B) Map of putative plasmid contig of human donor 3 isolate with indicated replication

938 genes (*repA* and *repC*) and resistance genes (*blaTem*, *sul2*, *aph(3)*, *aph(6)*, *tet(A)*). Colours on
939 the ring stand for coding (black) and non-coding regions (green).
940
941 **Fig. 5. Transfer of a resistance plasmid from the resident microbial community is**
942 **sensitive to abiotic conditions, and it confers a large increase in resistance.** (A) Antibiotic
943 susceptibility of the ancestral focal strain, the version of the focal strain used to isolate
944 transconjugants (see Methods), the focal strain with the plasmid (transconjugant), the resident
945 *E. coli* isolate used as the plasmid donor, and eight evolved focal strain colony isolates that we
946 isolated on ampicillin plates and that had chromosomal mutations (Sup. Table 2). Each point
947 shows mean ±s.e. (B) Transconjugant frequency (as a percentage of the total recipient
948 population) after mating experiments in various conditions. The recipient strain was a tagged
949 version of the ancestral focal strain and the plasmid donor was a resident *E. coli* isolate from
950 human donor 1. For the faecal slurry treatment we used sterilized faecal slurry from human
951 donor 1.
952
953
954

955 Fig.1


Without antibiotic
With antibiotic
Basal medium
Human donor 1
Human donor 2
Human donor 3
Abundance of focal strain [cfu/ml]
10^9
10^6
10^3
10^0
0 24 48 72 96 120 144 168
Time [h]

956 Fig.2


Without antibiotic
With antibiotic
Human donor 1
Human donor 2
Human donor 3
Total bacterial abundance [cells/ml]
10^{10}
10^{9}
10^{8}
2 24 48 72 96 120 144 168
Time [h]

957

958

959 Fig. 3

960

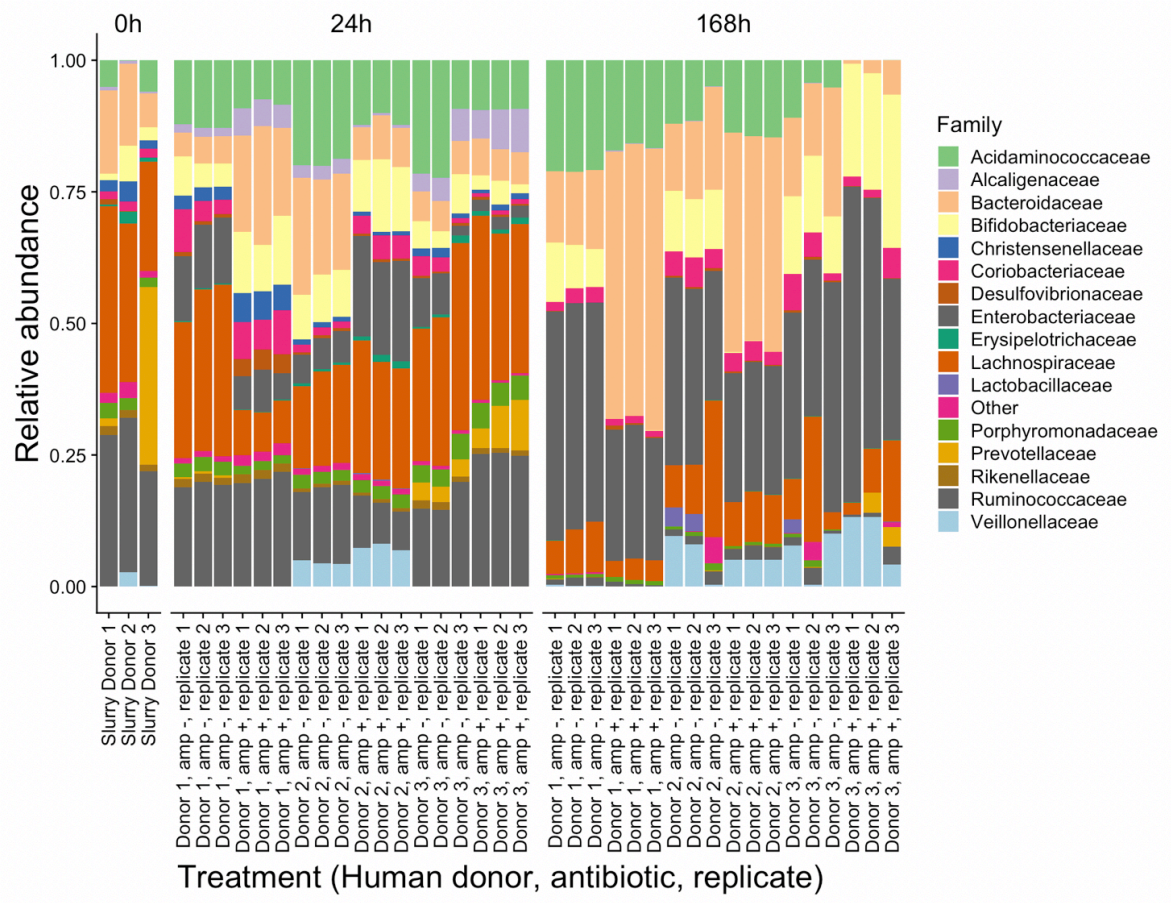


Table 1: Genes mutated in ampicillin resistant colony isolates of the focal strain from the end of the experiment. Asterisks indicate genes also mutated in randomly selected clones from ampicillin free plates (see Sup. Table 1).

Treatment group	Human donor	Replicate	*afuB*	*afuC*	*cspB <> cspF*	*cyoA*	*fecI*	*ftsI*	*insA <> uspC*	*insB1*	*insD1*	*ompR*	*opgB**	*perR*	*perR <> insN*	*relA*	*rpoC*	*rpoD*	*yaiO**	*yehE*	*yjhD <> insO*	*yjhD <> yjhE*
Basal medium with antibiotic	None	1			x			●							x							x
		2		x																x		
		3													x	●	●					
Community-free with antibiotic	1	1							x		x			x				●				
		2										●										
		3	x			x							●						x			
Community-free with antibiotic	3	1								x								●				
		2					x										▲				x	

▲ Deletion x Insertion ● SNP

964 Fig. 4

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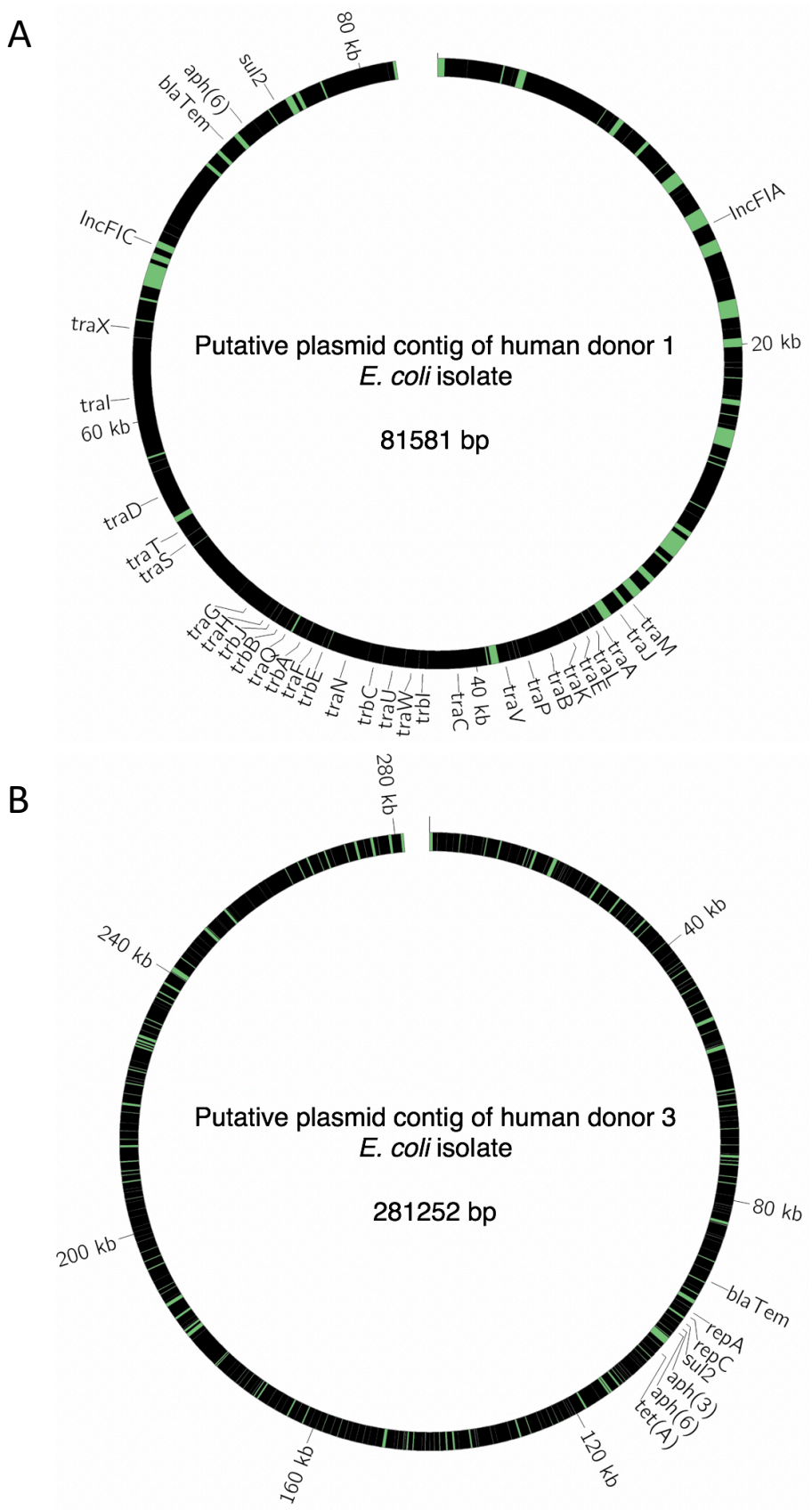

968 Fig. 5

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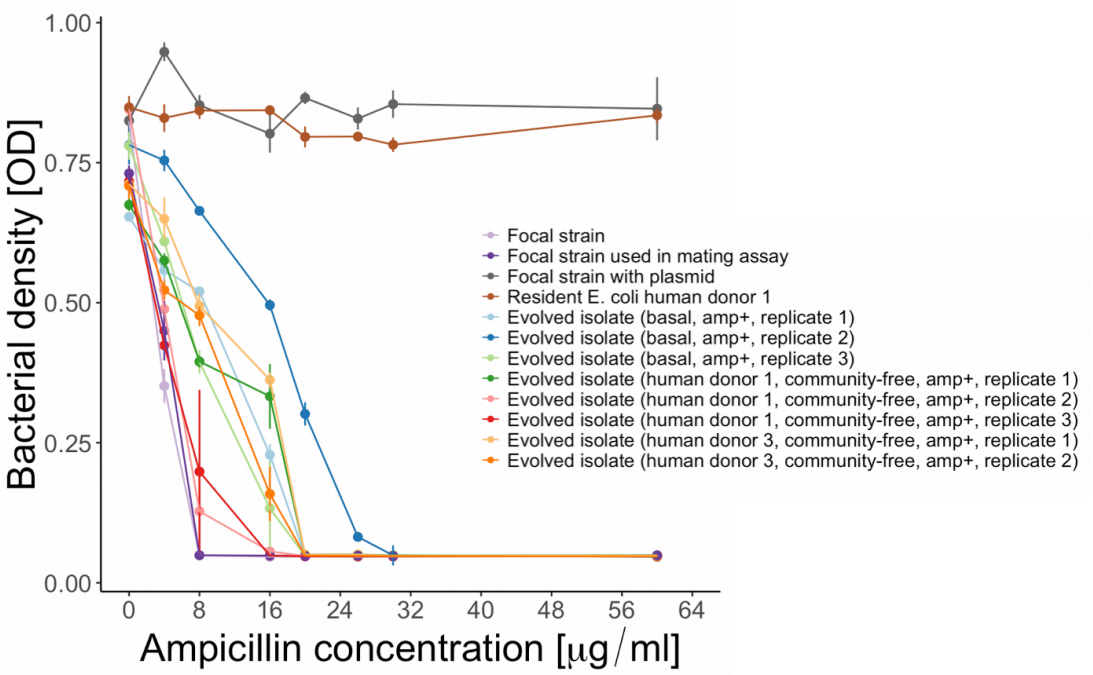

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B

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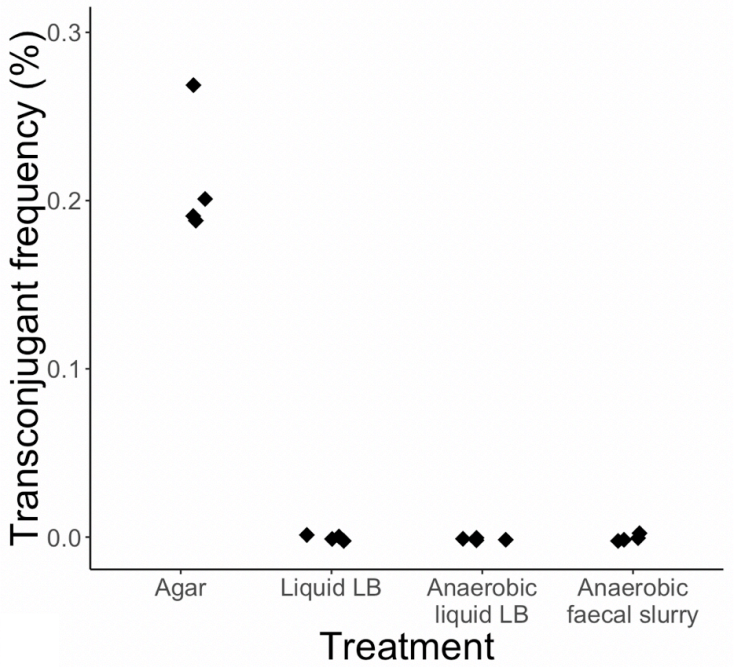

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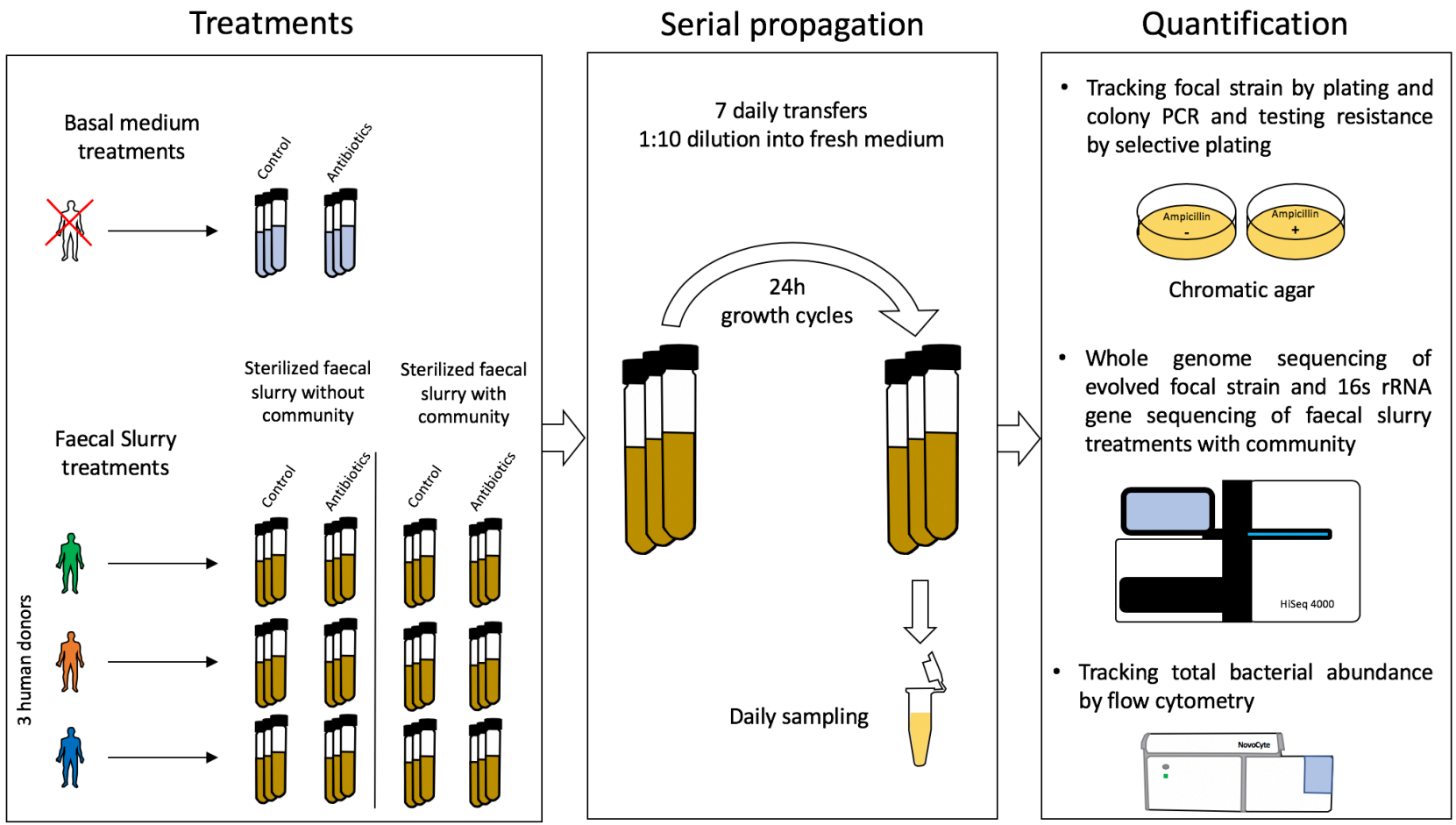
Supplementary material

Sup. Fig. 1



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985 **Sup Fig 1: Summary of experimental evolution in faecal slurry.** Treatments consisted of basal medium only, basal medium supplemented with
986 sterilized faecal slurry (without community) from one of three human donors, or basal medium supplemented with sterilized faecal slurry to which
987 the resident microbial community had been reintroduced (with community). After inoculation, all treatments were incubated for 2h at 37 °C, before
988 7 µg/ml ampicillin was added in the antibiotic treatment. Every 24h, we sampled each microcosm and transferred an aliquot to fresh medium
989 (either basal medium or sterilized faecal slurry) with or without antibiotics. We serially diluted each sample and spread it on chromatic agar plates
990 with or without antibiotics to quantify focal strain abundance (verified by colony PCR) and to screen for resistance. We sequenced focal strain
991 isolates from the final time point and investigated community composition by 16s rRNA gene amplicon sequencing. We monitored total bacterial
992 abundance in the community treatments by flow cytometry.

993

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Supplementary Table 1 (continued on next page): Genomic variants found in randomly selected colony isolates of the focal strain picked from ampicillin-free agar plates at the end of the experiment.

Treatment group	Human donor	Replicate	*arpA <> iclR*	*casA <> ygcB*	*clsA*	*entD*	*frsA <> phoE*	*gatY <> fbaB*	*gatZ*	*gfvA+B+C, insA+B*	*gtrS*	*hsdS*	*insI1, insX*	*insN*	*intD*	*intD, xisD,exoD, peaD*	*iraM <> ycgX*	*mmuM+P, afuB+C, insB1*	*ompF <> asnS*	*ompR*	*ompT*	*opgB*	*rpoD*	*serS*	*tfaD*	*tfaD <> ybcY*	*tfaX <> appY*	*waaS*	*waaU*	*wbbK*	*wecB*	*yaiO*	*yaiP*	*yaiS <> tauA*	*ybbD*	*ybcy*	*ybfK <> kdpE*	*yddK*	*ydjO*	*yedR <> yedS*	*yfcV <> sixA*	*yfjI*	*ygeF*	*yhhI*	*yibV*	*yjbS*	*yjgL*	*yjhB*	*yjiS <> yjiT*	*Δ 566060-584707*	*Δ 568024-586058*
Basal medium without antibiotic	None	1												X																																					
		2											▲					▲														X																			
		3	No mutations detected																																																
Community-free without antibiotic	1	1	No mutations detected																																																
		2																						●																											
		3	No mutations detected																																																
Community without antibiotic	1	1	Focal strain below detection limit																																																
		2																																																	
		3																																																	
Community-free without antibiotic	2	1	No mutations detected																																																
		2																																																	
		3									▲											●								●																					
Community without antibiotic	2	1	No mutations detected																																																
		2																																																	
		3																																																	
Community-free without antibiotic	3	1																																							X										
		2									▲																	▲					▲										x							
		3										●																																							
Community without antibiotic	3	1								x					●						●				●	●									●															▲	
		2												X																																					
		3				X	●									▲											▲					X				●															▲

▲ Deletion X Insertion ● SNP

Continuation supplementary Table 1: Genomic variants found in randomly selected colony isolates of the focal strain picked from ampicillin-free agar plates at the end of the experiment.

Treatment group	Human donor	Replicate	*arpA <> iclR*	*casA <> ygcB*	*clsA*	*entD*	*frsA <> phoE*	*gatY <> fbaB*	*gatZ*	*gfvA+B+C, insA+B*	*gtrS*	*hsdS*	*insI1, insX*	*insN*	*intD*	*intD, xisD,exoD, peaD*	*iraM <> ycgX*	*mmuM+P, afuB+C,*	*ompF <> asnS*	*ompR*	*ompT*	*opgB*	*rpoD*	*serS*	*tfaD*	*tfaD <> ybcY*	*tfaX <> appY*	*waaS*	*waaU*	*wbbK*	*wecB*	*yaiO*	*yaiP*	*yaiS <> tauA*	*ybbD*	*ybcy*	*ybfK <> kdpE*	*yddK*	*ydjO*	*yedR <> yedS*	*yfcV <> sixA*	*yfjI*	*ygeF*	*yhhI*	*yibV*	*yjbS*	*yjgL*	*yjhB*	*yjiS <> yjiT*	Δ 566060-584707	Δ 568024-586058
Basal medium with antibiotic	None	1												x																																						
		2	No mutations detected																																																	
		3																																																		
Community-free with antibiotic	1	1																					●											▲				▲		x											
		2									▲																																									
		3												x						●																																
Community with antibiotic	1	1	Focal strain below detection limit																																																	
		2																																																		
		3																																																		
Community-free with antibiotic	2	1									▲								x																																	
		2	▲		x															●																									▲			▲				
		3						●												●							▲																									
Community with antibiotic	2	1																																																		
		2		▲													▲											▲	▲	▲							▲		▲		▲		▲			▲	▲				
		3									▲																																									
Community-free with antibiotic	3	1												x																																			▲			
		2	No mutations detected																																																	
		3							x																																											
Community with antibiotic	3	1	Focal strain below detection limit																																																	
		2																																																		
		3																																																		

▲ Deletion X Insertion ● SNP

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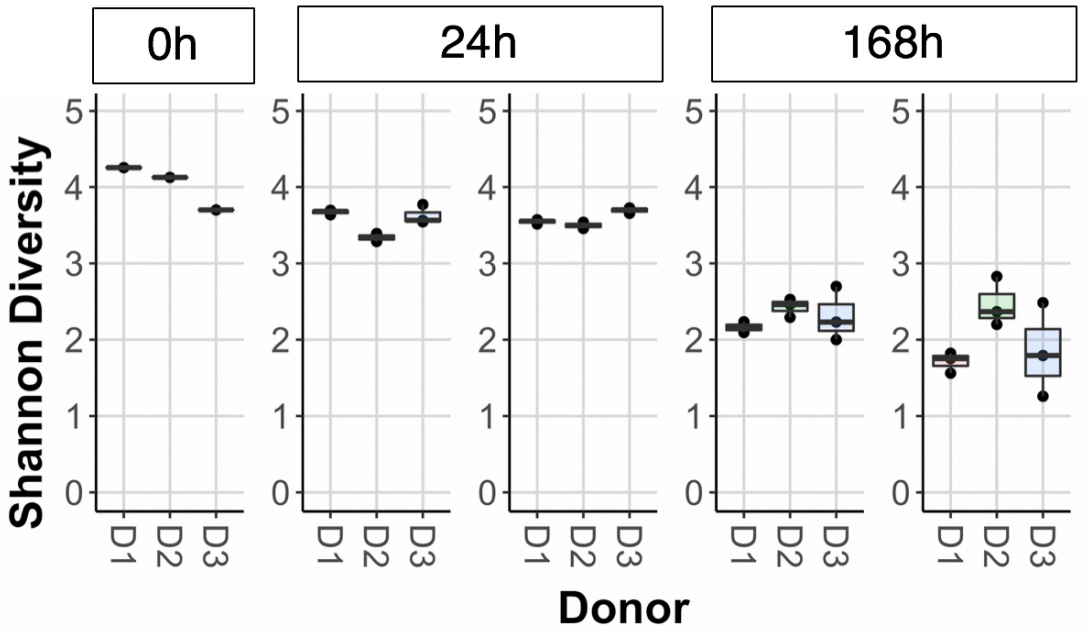
Supplementary Table 2: Antibiotic resistance genes, plasmid replicons and genes involved in conjugative transfer found on the putative plasmids of isolates from the resident *E. coli* community of each replicate population

		Putative plasmid from human donor 1 without antibiotics of replicate community:			Putative plasmid from human donor 1 with antibiotics of replicate community:			Putative plasmid from human donor 3 without antibiotics of replicate community:			Putative plasmid from human donor 3 with antibiotics of replicate community:		
Genomic feature	**Function**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
blaTem-1b	Beta lactam resistance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
aph(3")	Aminoglycoside resistance	×	×	×	×	×	×	✓	✓	✓	✓	✓	✓
aph(6)	Aminoglycoside resistance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
sul2	Sulphonamide resistance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
tet(A)	Tetracycline resistance	×	×	×	×	×	×	✓	✓	✓	✓	✓	✓
IncQ1	Replicon	×	×	×	×	×	×	✓	✓	✓	✓	✓	✓
IncFIC(FII)	Replicon	✓	✓	✓	✓	✓	✓	×	×	×	×	×	×
IncFIA	Replicon	✓	✓	✓	✓	✓	✓	×	×	×	×	×	×
tra genes [a]	Involved in conjugative transfer	✓	✓	✓	✓	✓	✓	×	×	×	×	×	×

[a] traA, traB, traC, traD, traE, traF, traG, traH, traI, traK, traL, traM, traN, traP, traQ, traS, traT, traU, traV, traW, traX, traY

Sup. Fig. 2



Sup Fig 2: Within-sample diversity over time. Diversity is estimated here using Shannon's diversity index in each initial sample, after 24h and 168h in each treatment (three replicates per treatment group and time point, except for at 0h).

1017 Sup. Fig. 3

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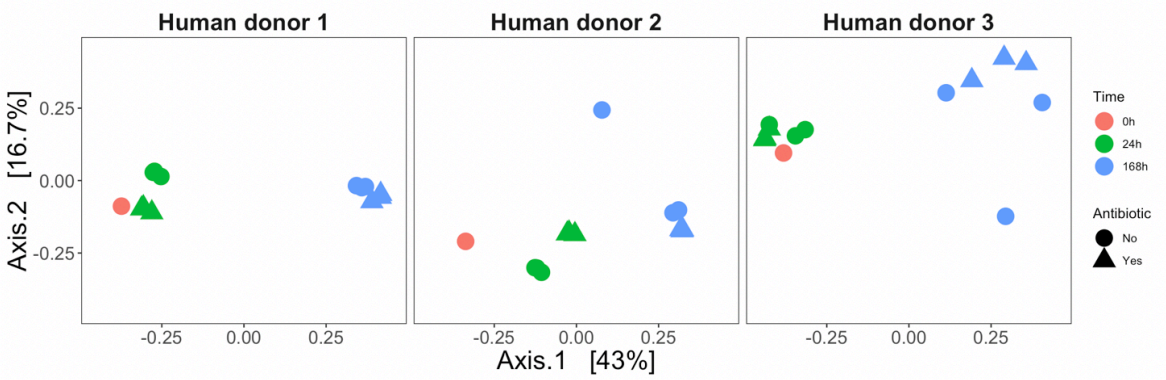

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1025 **Sup Fig 3: Variable similarity of microbial communities across time and treatment**

1026 **groups.** Each panel shows samples from a single human donor, with the same axes used in

1027 each panel. Points show the initial sample (0h) and microcosms from 24h and 168h with and

1028 without antibiotics (legend at right). Similarities between communities were calculated by

1029 Bray-Curtis distance and plotted using Principal Coordinate Analysis (see Methods).

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1031 Sup. Fig. 4

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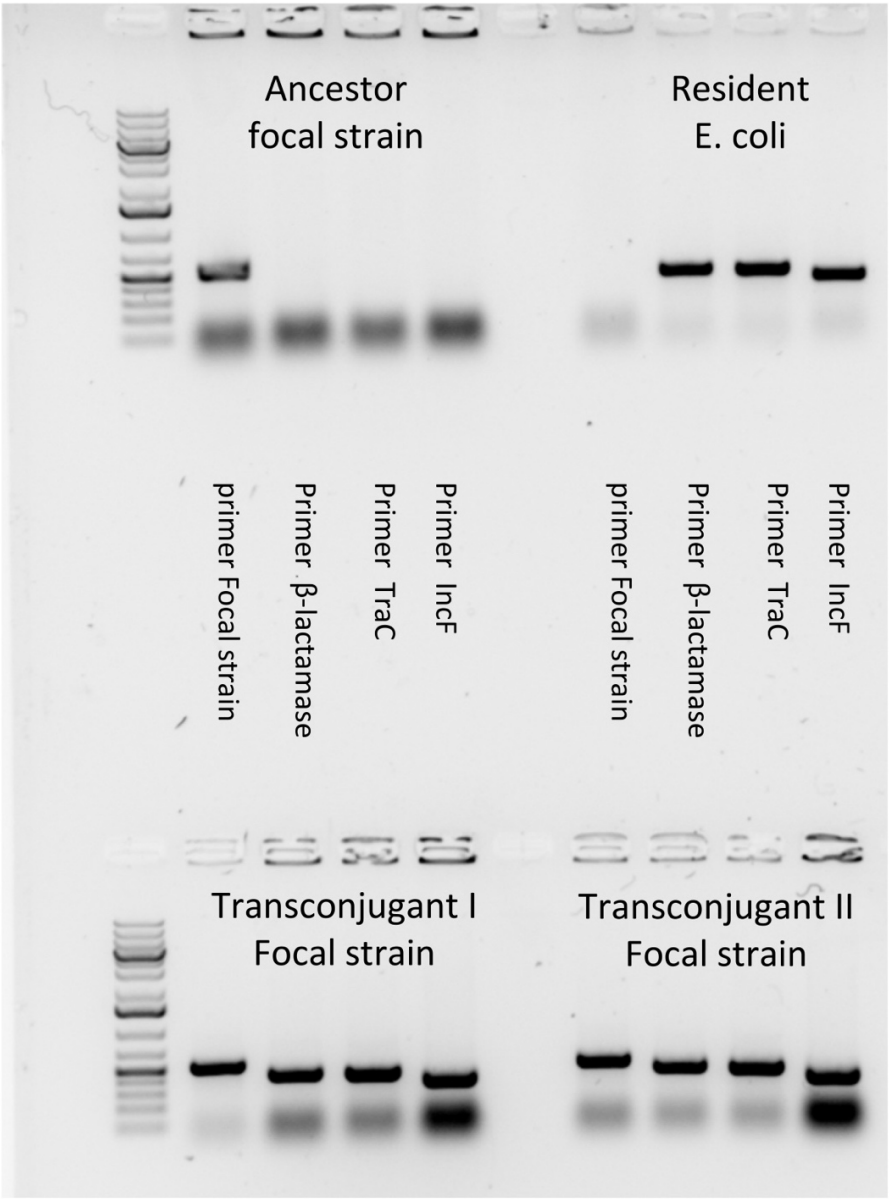

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1046 **Sup Fig 4: Agarose gel electrophoresis picture of the PCR products specific for plasmid**

1047 **genes and a chromosomal marker of the focal strain.** We used these primer sets to verify

1048 plasmid uptake of the transconjugants. Primers are given in the main text in the methods

1049 section.

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