

1 Host genetic and environmental factors shape the 2 human gut resistome

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31 **Abstract (max 350 w)**

32 **Background:** Understanding and controlling the spread of antimicrobial resistance is
33 one of the greatest challenges of modern medicine. To this end many efforts focus on
34 characterising the human resistome or the set of antibiotic resistance determinants
35 within the microbiome of an individual. Aside from antibiotic use, other host
36 environmental and genetic factors that may shape the resistome remain relatively
37 underexplored.

38 **Methods:** Using gut metagenome data from 250 TwinsUK female twins, we quantified
39 known antibiotic resistance genes to estimate gut microbiome antibiotic resistance
40 potential for 41 types of antibiotics and resistance mechanisms. Using heritability
41 modelling, we assessed the influence of host genetic and environmental factors on the
42 gut resistome. We then explored links between gut resistome, host health and specific
43 environmental exposures using linear mixed effect models adjusted for age, BMI,
44 alpha diversity and family structure.

45 **Results:** We considered gut microbiome antibiotic resistance to 21 classes of
46 antibiotics, for which resistance genes were detected in over 90% of our population
47 sample. Using twin modelling, we estimated that on average about 25% of resistome
48 variability could be attributed to host genetic influences. Greatest heritability estimates
49 were observed for resistance potential to acriflavine (70%), dalfopristin (51%),
50 clindamycin (48%), aminocoumarin (48%) and the total score summing across all
51 antibiotic resistance genes (38%). As expected, the majority of resistome variability
52 was attributed to host environmental factors specific to an individual. We compared
53 antibiotic resistance profiles to multiple environmental exposures, lifestyle and health
54 factors. The strongest associations were observed with alcohol and vegetable
55 consumption, followed by high cholesterol medication and antibiotic usage. Overall,
56 inter-individual variation in host environment showed modest associations with
57 antibiotic resistance profiles, and host health status had relatively minor signals.

58 **Conclusion:** Our results identify host genetic and environmental influences on the
59 human gut resistome. The findings improve our knowledge of human factors that
60 influence the spread of antibiotic resistance genes and may contribute towards helping
61 to attenuate it.

62

63 **Keywords:** Antibiotic resistance, gut microbiome, heritability, twins

64 **Background**

65 Currently, antibiotics are the most effective treatment for infectious diseases in
66 humans and in animals. However, their intensive use in health care and food
67 production has led to a dramatic increase in antibiotic resistant pathogens [1].
68 Antibiotic resistance is acquired by bacteria through mutation and gene transfer. The
69 human gut is home to trillions of bacteria and can act as a reservoir for antibiotic
70 resistance genes (ARG), where exchange of ARG may take place between bacteria
71 [2, 3]. ARG can be transferred vertically throughout bacterial division and horizontally
72 between bacteria *via* transformation (integration of DNA fragments from the
73 environment), transduction (through a bacteriophage) and conjugation (interaction
74 between two bacteria) [4]. Individuals are constantly exposed to new bacteria that
75 might reach the gastrointestinal track and although the ability of these bacteria to
76 colonise the large intestine is debated [5], their passage through the gut ecosystem
77 may be sufficient to horizontally transfer ARGs to the microbial community. Thus, the
78 host microbiome may have the potential to acquire antibiotic resistance without direct
79 antibiotic exposure. Resistant pathogenic bacteria are a serious health problem, and
80 resistant non-pathogenic bacteria are also of concern due to their potential to transfer
81 ARGs to pathogens. Indeed, the continuous rise of antibiotic resistant bacteria has led
82 to a significant increase in mortality, especially in nosocomial infections [6].

83

84 Advances in technology have allowed for the collective sequencing of whole gut
85 microbiota genomes, or metagenomes [7]. It is therefore possible to identify and
86 potentially quantify ARG carried by bacteria in the gut community through the analysis
87 of gut metagenome data. Several studies have explored the ARG profile of the human
88 gut microbiome [8, 9], or the gut resistome, using different approaches including total
89 number of ARGs in the gut or metrics such as the antibiotic resistance potential (ARP)
90 [10]. ARP estimates the number of ARG copies in a sample, weighted by the relative
91 abundance of taxa carrying the ARG. Although ARP metrics do not measure functional
92 antibiotic resistance, they have been used to explore factors that may shape the gut
93 resistome. For instance, significant ARP differences were observed across countries
94 mirroring differences in country-specific antibiotic consumption [11], where higher
95 antibiotic use in human, and also farm animals, was related to greater ARP levels.
96 Medicinal antibiotic use plays an important role in shaping the gut resistome, where

97 antibiotic use during hospitalisation has been associated with increased relative
98 abundance of ARGs in the gut [12]. The potential for transition of ARGs during food
99 production, or the 'farm-to-fork' hypothesis, has been extensively discussed in the
100 literature [13]. Although evidence remains sparse [14, 15], direct exposure to livestock
101 has been linked to an increase in the number of ARG within the human gut [16].
102 Furthermore, other environmental or lifestyle factors have also been linked to gut
103 resistome variation [17]. For example, significant gut resistome associations with travel
104 [18] and pet ownership [19] suggest that a multitude of factors could be at play.

105

106 Despite this, the factors shaping the human gut ARG reservoir are still not well
107 understood. Exploring country-specific environmental variation allows insight into
108 environmental parameters involved in this process [8, 10, 20]. In addition, previous
109 work has demonstrated that the gut microbiome could also be influenced by host
110 genetics [21, 22], with even stronger influence observed when considering gut
111 microbiota functionality [23]. Therefore, it is plausible that host genetic impacts may
112 also affect the abundance of bacteria that carry ARGs, as well as the potential to
113 transfer ARGs in the gut community.

114

115 In this study, we hypothesised that both host genetic and environmental factors
116 influence the human gut ARG reservoir. By profiling the ARP in a sample of 250
117 healthy female volunteers from the TwinsUK cohort, we evaluated the role of host
118 genetic and environmental impacts on the resistome using a twin study design. We
119 then explored resistome associations with specific environmental factors and health
120 status in shaping the human gut ARG reservoir.

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124 **Methods**

125 Samples

126 We used published gut metagenomic profiles of 250 female twins from the TwinsUK
127 cohort of mean age 61 (range 36-80 years of age). The sample contained 35
128 monozygotic (MZ) and 92 dizygotic (DZ) twin pairs with an average body mass index
129 (BMI) of 25.8 ± 4.61 . Sample collection and sequencing methods have previously been

130 described [23], with on average 74 million non-human high-quality Illumina HiSeq
131 paired-end reads of a read length 100 bp (insert size 350 bp) per sample. Sequence
132 data quality control, gene catalogue build, gene abundance estimation, and taxonomic
133 assignment have previously been described in this dataset [23]. Briefly, the published
134 gene catalogue consisted of 11,446,577 non-redundant genes, at which relative gene
135 abundances were estimated [23] using previously described methods [24, 25].
136 Taxonomic annotation has previously been described in this sample [23], and utilised
137 taxonomic assignments from the IGC gene catalogue [26] and application of the same
138 pipeline [25, 25] for taxonomic assignment of the additional genes reported in this
139 sample [23]. The relative abundance of a taxon is calculated from the relative
140 abundance of its genes, considering only signals with at least 10 genes from a taxon.

141

142 *Antibiotic Resistance Potential*

143 Gut resistomes were profiled using the antibiotic resistance potential (ARP) approach
144 [10]. The ARP is defined as the average microbial genome fraction encoding ARGs
145 for a particular antibiotic or class of antibiotics, across all bacteria in the gut
146 microbiome sample, based on known taxonomy of the ARGs (here considered at the
147 genus level, with each genus represented by its average ARG carriage within the
148 ProGenomes database) [27]. The approach uses the above described gene
149 catalogue, published relative gene abundances and catalogue amino acid sequences
150 to assess ARG abundance in the sample and subsequently takes into account their
151 taxonomic composition to generate the ARP. For ARP estimation amino acid
152 sequences were translated from the gene sequences, selecting the frame resulting in
153 the longest uninterrupted protein, and where for the majority of sequences (80%) only
154 one specific frame was full length and was selected. The gene catalogue in this dataset
155 was the annotated for ARGs using CARD (version 2.0.1) [28] and ResFams [29],
156 assigning ResFams hits only to sequences without a CARD hit and integrating both
157 types of annotation via the Antibiotic Resistance Ontology (ARO). This resulted in a
158 gene catalogue annotated with ARG family membership and thus total gene
159 abundances per ARG family. Together with projections on expected ARG abundance
160 from taxonomic composition of each sample, ARPs were then computed. The ARP is
161 a measure of antibiotic resistance gene abundance relative to the amount of sample
162 material stemming from taxa known to carry such resistance genes. The measure
163 aims to decouple ARGs increases following from taxonomic composition change only,

164 compared to changes resulting from selection within taxonomic groups for higher ARG
165 carriage. Thus, findings of altered raw ARG abundance versus altered ARP
166 abundance represent different scenarios each leading to altered resistance capacity
167 in microbial ecosystems – ARG shift in the absence of ARP shift reflects changes
168 driven by larger-scale taxonomic composition shift with accompanying changes in
169 ARG abundance, whereas ARP shift may indicate a shift within taxa to more resistant
170 varieties, including by direct propagation of resistance genes, copy number alterations,
171 mobile element transmission, strain replacement or other scenarios. ARP were
172 estimated for 339 profiles that clustered and represented resistance to 39 specific
173 types of antibiotics or classes of antibiotics, many of which were highly correlated.
174 Altogether, estimates were obtained for 41 different variables, spanning 39 types of
175 antibiotics or classes of antibiotics, one antibiotic resistance mechanism represented
176 as a proxy class (efflux pumps), and the overall total of resistance genes within an
177 individual. Pair-wise correlations were estimated across the 41 variables, with multiple
178 highly correlated profiles (**Supplementary Figure 1**). Therefore, a single ARP was
179 chosen to represent each cluster of highly correlated of ARPs (pair-wise Spearman
180 rho > 0.9), selecting the most prevalent profile as the representative per correlated
181 cluster (**Supplementary Figure 1**). ARP profiles were then corrected for potential
182 covariates in a linear mixed effects regression to generate the ARP residuals that were
183 included in the majority of downstream analyses. Covariates included BMI, age, and
184 α-diversity as fixed effects, and family and zygosity as random effects.

185
186

187 *Twin modelling: ARP heritability and environment effects*

188 Twin-based heritability of ARP variables was calculated by fitting the ACE model to
189 ARP residuals using the ‘OpenMx’ package in R version 3.6.1. The model assesses
190 the relative contribution of additive genetic effects (A), common environment (C), and
191 environment unique to an individual (E), towards the variance of a phenotype of
192 interest, here a specific ARP residual profile
193 (http://openmx.ssri.psu.edu/docs/OpenMx/2.3.1/GeneticEpi_Path.html). The
194 significance of the A component was based on the difference between the fit of the
195 ACE and the CE models to evaluate if inclusion of A fit the data better than use of C
196 and E alone.

197

198 Association study

199 To follow up twin-based results of host environmental influences on ARPs, we carried
200 out association analyses comparing inter-individual variability in each ARP profile to a
201 series of host environmental variables. Host environmental variables included factors
202 related to health and host environment, such as lifestyle and diet factors, and
203 medication use. We identified 24 health markers that included 21 conditions that were
204 reported in at least 10 of the 250 twins, as well as further variables such as number of
205 days spent in a hospital, DEXA measures of visceral fat mass, and estimated frailty
206 [30] and neuroticism scores [31] (**Supplementary table 1**). Next, a total of 32
207 environmental factors were selected and divided in three categories: distal
208 environment (5), diet (14) and medication use (12). Information related to diet, lifestyle,
209 medication use, and health status were collected through questionnaires sent to the
210 volunteers and time matched with the date of sample collection. Dietary intakes were
211 estimated via food frequency questionnaire (FFQ) data, collected following Epic-
212 Norfolk guidelines [32], and used to construct the Healthy Eating Index (HEI) 2010
213 [33], previously validated within this cohort as a means of capturing dietary variance
214 [34]. The index of multiple deprivation (IMD), a composite measure of area-level
215 deprivation, was downloaded from government websites and used to derive within-
216 population quintiles as described previously [35]. Environmental data were not always
217 available for the 250 twins and details of the sample size for each variable can be
218 found in **Supplementary table 1**.

219 To evaluate the association between each individual ARP and the environmental
220 variable of interest we used a linear mixed effects regression model (lme4 package in
221 R version 3.6.1). Unadjusted ARPs were fit as the response variable, the
222 environmental or health variable was the predictor, and models were adjusted for BMI,
223 age, alpha diversity and family structure as previously described. Significance of the
224 results were evaluated by comparing the full model (including the variable of interest)
225 to a null model (excluding the variable) using a likelihood ratio test. Results were
226 adjusted for multiple testing using the false discovery rate (FDR 5%).

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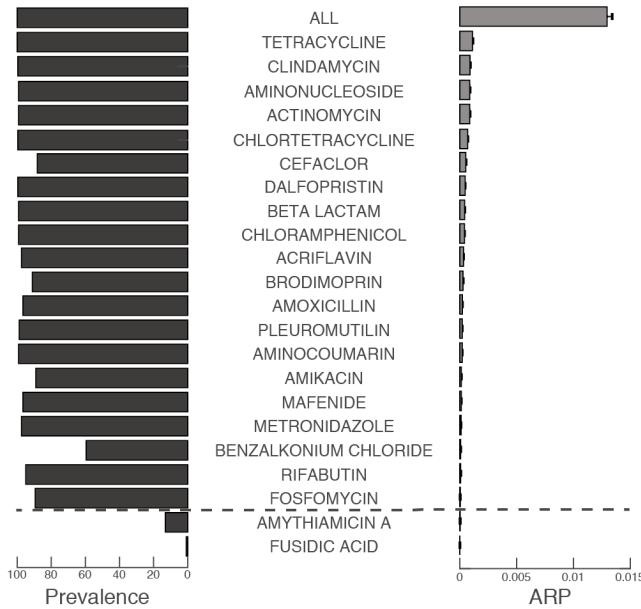
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231 **Results**

232 *Profiling the gut resistome*

233 We explored gut metagenomic profiles of 250 healthy older (mean age 65) Caucasian
234 female twins from the TwinsUK cohort, including 35 MZ and 92 dizygotic DZ twin pairs.
235 The gut resistome in each individual was characterised using the antibiotic resistance
236 potential (ARP), a previously developed measure of ARG abundance relative to
237 abundance of their likely carrier taxa [10]. ARP profiles were estimated for 41
238 variables, which included antibiotics, antibiotic classes, and antibiotic resistance
239 mechanisms. Some of the ARP variables were highly correlated and therefore
240 replaced by the most prevalent profile as a representative of each cluster
241 (**Supplementary Figure 1**). Altogether, 23 ARP profiles were less correlated and
242 therefore considered as independent variables (Spearman rho < 0.9). The variables
243 assess potential of AR to specific antibiotics and classes of antibiotics, including ARP
244 for the total gut resistome estimated as the overall sum of ARPs within an individual,
245 or total ARP (ALL). Of the 23 ARPs, 21 were detected in over 90% of our sample and
246 were explored in subsequent analyses (**Figure 1**). Therefore, in most of our UK
247 population sample the gut communities could be considered as carriers of a large
248 proportion of well characterised ARGs (**Figure 1**). Tetracycline and clindamycin - two
249 broad spectrum antibiotics widely used in humans - were the ARPs detected at the
250 highest level in our sample (**Figure 1**). In contrast, ARPs to amythiamicin A and fusidic
251 acid were detected in less than 20% of the population sample and were excluded in
252 downstream analyses in this study.

253



254

255 **Figure 1:** Antibiotic resistance potential level and prevalence among the TwinsUK
256 cohort. Prevalence among the population is pictured on the left and mean ARP levels
257 on the right. ARPs below the dotted line are removed from subsequent analyses.

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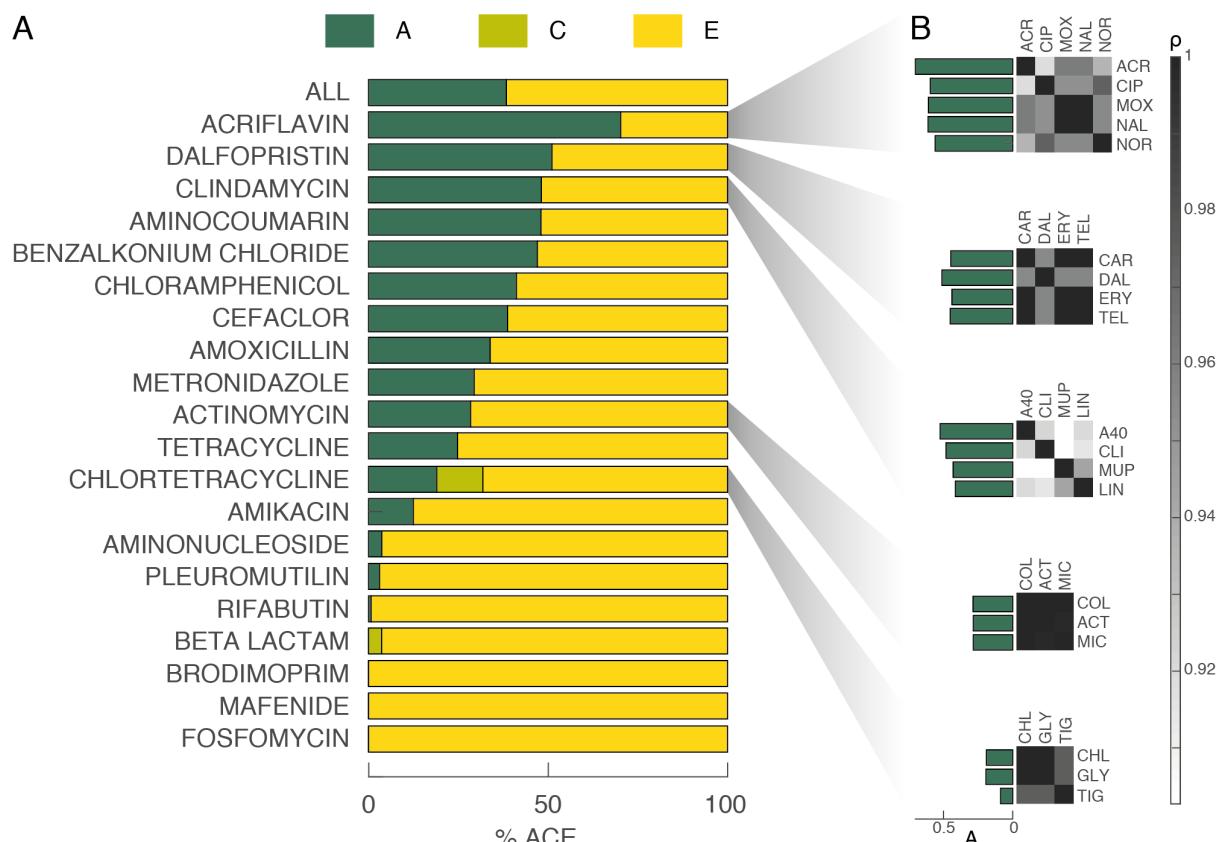
259 Host genetic influences on the gut resistome

260 Since our samples constitute only twin pairs, we carried out twin-based heritability
261 analyses of the ARP profiles. Using the ACE model, we estimated the proportion of
262 variation that is attributed to host genetic or environmental factors for each of the 21
263 ARP variables.

264 We observed that ARP profiles are predominantly under the influence of host
265 environmental factors (**Figure 2A, Supplementary table 2**). However, two ARP
266 profiles had strong evidence for heritability ($A > 50\%$), namely acriflavin ($A = 70\%$,
267 $95\% \text{ CI} = [36-85]\%$) and dalfopristin ($A = 51\%$, $95\% \text{ CI} = [6-72]\%$). Altogether, five
268 ARPs displayed a nominally significant fit of the heritability term in the twin model, and
269 these were acriflavin, dalfopristin, aminocoumarin ($A = 48\%$, $95\% \text{ CI} = [1-69]\%$) and
270 clindamycin ($A = 48\%$, $95\% \text{ CI} = [4-71]\%$), as well as total ARP (ALL, $A = 38\%$, $95\% \text{ CI} = [1-65]\%$). In total 12 ARPs (57% of profiles) had at least modest heritability
271 estimates over 20% ($A > 20\%$). The average ARP heritability across the 21 variables
272 was estimated to be over 25% ($A = 28.4\% \pm 21.4$, **Figure 2A**). The four ARPs
273 displaying greatest heritability estimates (acriflavin, dalfopristin, aminocoumarin,
274 clindamycin) were highly prevalent in our sample (>95% prevalence, **Figure 1**) and in

276 an independent gut metagenomic dataset from healthy Western Europeans ($\geq 50\%$ 277 prevalence of cluster components in Carr et al. 2020 [20], **Supplementary table 3**). 278 We also verified that highly correlated ARPs (Spearman rho > 0.9) displayed similar 279 levels of heritability estimates (**Figure 2B**). For instance, acriflavin that was the most 280 heritable ARP ($A = 70\%$) and was highly correlated with four other ARP measures 281 (ciprofloxacin, moxifloxacin, nalidixic acid and norfloxacin) that all displayed nominally 282 significant heritability estimates above 50%. 283 The twin model also allows the decomposition of the environmental variance into 284 components that can be attributed to each individual (E, or unique), or that are shared 285 within a twin pair (C, or common). In our data, the majority of the environmental 286 impacts were attributed to individual-specific effects, in line with previous observations 287 from 16S results [21].

288



289

290 **Figure 2:** Heritability of the human gut ARP. Heritability estimate results calculated 291 with the OpenMx ACE model. Full results are presented in **Supplementary table 2**. 292 ACR, acriflavin; CIP, ciprofloxacin; MOX, moxifloxacin; NAL, nalidixic acid; NOR, 293 norfloxacin; CAR, carbomycin; DAL, dalfopristin; ERY, erythromycin; TEL, 294 telithromycin; A40, antibiotic a40926; CLI, clindamycin; MUP, mupirocin; LIN, linezolid;

295 COL, colistin; ACT, actinomycin; MIC, microcin J25; CHL, chlortetracycline; GLY,
296 glycylcycline; TIG, tigecycline.

297

298 **Heritability of the gut resistome is only partially attributed to taxonomical heritability**

299 Previous studies conducted in the same cohort have showed that the relative
300 abundance of certain taxa in the gut can be heritable [21, 22]. Despite correcting for
301 genus abundance in the ARP calculation, as well as correcting for overall α -diversity
302 in the heritability analyses, it is plausible that the observed genetic contributions to
303 ARPs may be attributed to heritability of different components of the gut microbial
304 community that we may not have corrected for in full. To tackle this, we carried out
305 additional analyses with further corrections specifically for the ARPs that displayed
306 significant proportion of variance explained by host genetics ($P < 0.05$), namely:
307 acriflavin, aminocoumarin, dalfopristin and clindamycin; as well as the sum of total
308 ARPs (ALL).

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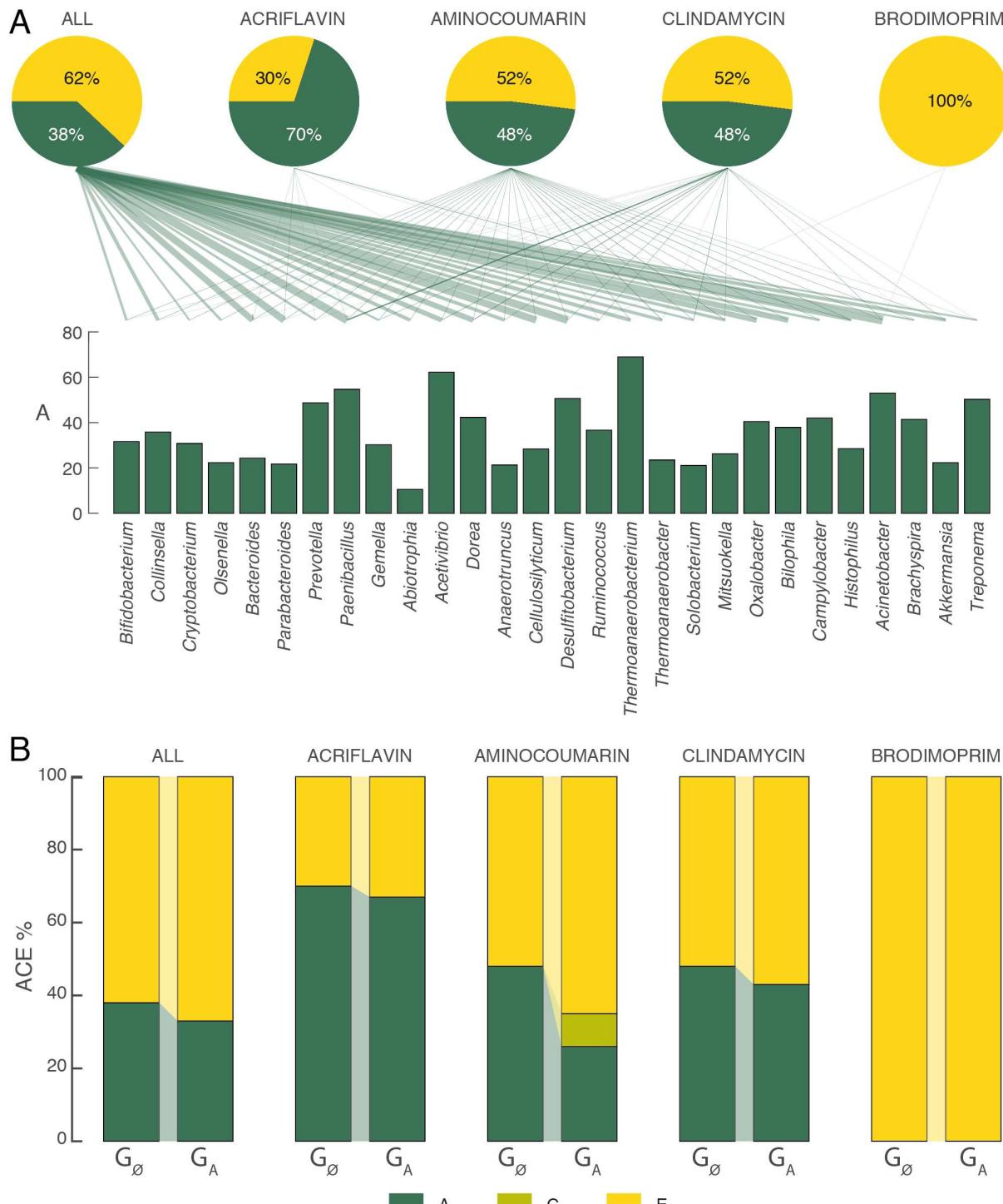
310 Some bacterial genera carry more ARGs on average per genome and will therefore
311 make a greater contribution to an ARP profile. We first evaluated if heritable ARGs
312 were carried by a large number of heritable genera ($A > 20\%$). Xie et al. (2016)
313 reported that in total 27 genera displayed at least moderate heritability ($A > 20\%$) using
314 the same dataset [23]. All 27 genera contribute to total ARP (ALL) and aminocoumarin
315 ARP, while only 19, 8, and 7 of these contributed to clindamycin, dalfopristin and
316 acriflavin ARPs, respectively (**Figure 3A, Supplementary table 4**). In contrast,
317 brodimopim, for which we estimated no heritable components ($A=0$), showed
318 contribution from only 2 of the 27 these moderately heritable genera.

319

320 To assess the impact of these genus-level observations on our ARP heritability results,
321 we regressed the four heritable ARPs as well as the sum of all ARPs and brodimoprim
322 (as a negative control) against their contributing heritable genera ($A>20\%$) and used
323 the residuals to re-estimate heritability. The heritability estimates of the sum of all
324 ARPs was reduced by 15% as a result of this correction (**Figure 3B, Supplementary**
325 **table 5**). For the four other ARPs, we observed a direct relationship between the level
326 of heritability reduction post correction and the number of heritable genera that
327 contributed to each ARP. However, although in all cases the heritability estimates were
328 attenuated after this correction, they still remained nominally significant. The

329 heritability estimates of aminocoumarin ARP, the ARP connected to the greatest
330 number of heritable genera (n = 27) dropped from 48% to 26%. On the other hand,
331 the clindamycin (19 genera), dalfopristin (8 genera), and acriflavin (7 genera) ARPs
332 heritability levels were reduced by only 11%, 0.01% and 4%, respectively, after
333 correction. As expected, the brodimoprim heritability estimate was unaffected by the
334 adjustment.

335



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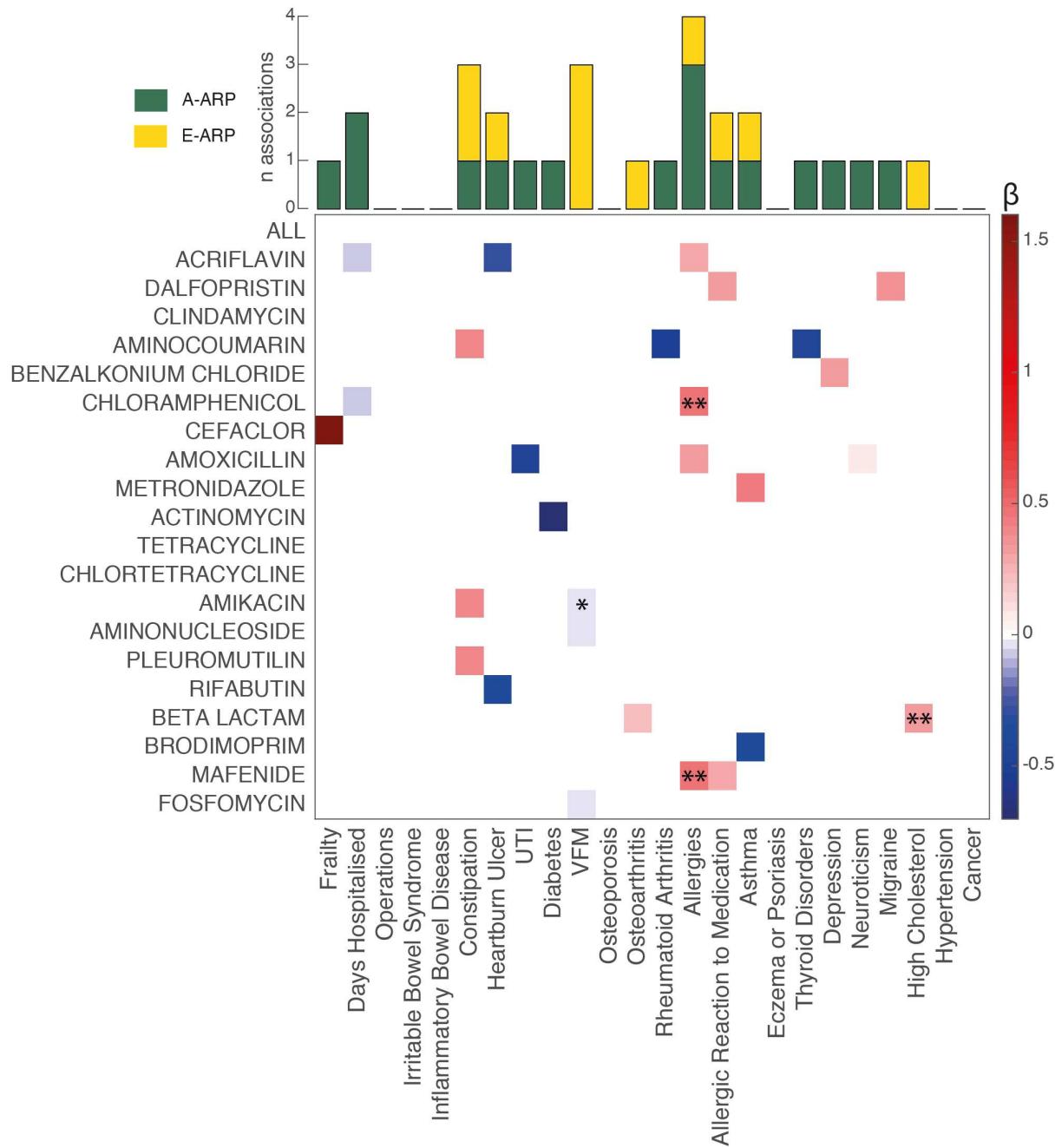
337 **Figure 3:** Impact of heritable taxonomy components on ARPs heritability. (A) Link
338 between heritable taxa and the three most heritable ARPs, total ARP (All) and a non-
339 heritable ARP (brodimoprim). Dark green bars represent A (proportion of variance of
340 the trait under genetic influence) estimates previously published for each of 27
341 heritable genera. The five ARPs represented on the top by pie charts representing
342 their heritability results are linked to genera with $A > 0.2$ at the bottom. The weight of
343 the link is proportional to the contribution weight of a genus to an ARP. (B) Heritability
344 estimate results for total ARP (All), acriflavin, dalfopristin, aminocoumarin, clindamycin
345 and brodimoprim before (G_\emptyset) and after correction for high A (G_A) bacterial genera
346 relative abundance.

347

348 *The gut resistome is poorly associated with host health status*

349 We next explored if gut resistome profiles are linked to health status of the host in our
350 predominantly healthy older female twin sample. We focused on 24 health traits
351 altogether, including 21 conditions that were reported in at least 10 of the 250 twins,
352 as well as number of days spent in a hospital, visceral fat mass (VFM) estimates, frailty
353 and neuroticism scores (**Supplementary table 1**) and explored their associations with
354 the 21 ARPs using linear mixed effect model adjusted for BMI, alpha diversity, age,
355 gender and family structure. None of the tested associations surpassed FDR at 5%
356 multiple testing correction overall, but allergy and high cholesterol were positively
357 associated with 3 ARPs at FDR 5% correction within health trait (**Figure 4**). Overall,
358 24 nominally significant associations were observed between 17 health traits and 18
359 ARPs. These included positive associations between allergy and constipation with 4
360 and 3 ARPs, respectively, as well as 3 negative associations between VFM and ARPs.
361 In total, 70% of the associations were observed with heritable ARPs. Only three traits
362 (VFM, osteoarthritis and high cholesterol) were associated exclusively with non-
363 heritable ARPs, while eight (frailty, days spent at hospital, UTI, diabetes, thyroid
364 disorders, depression, neuroticism and migraine) were associated exclusively with
365 heritable ARPs.

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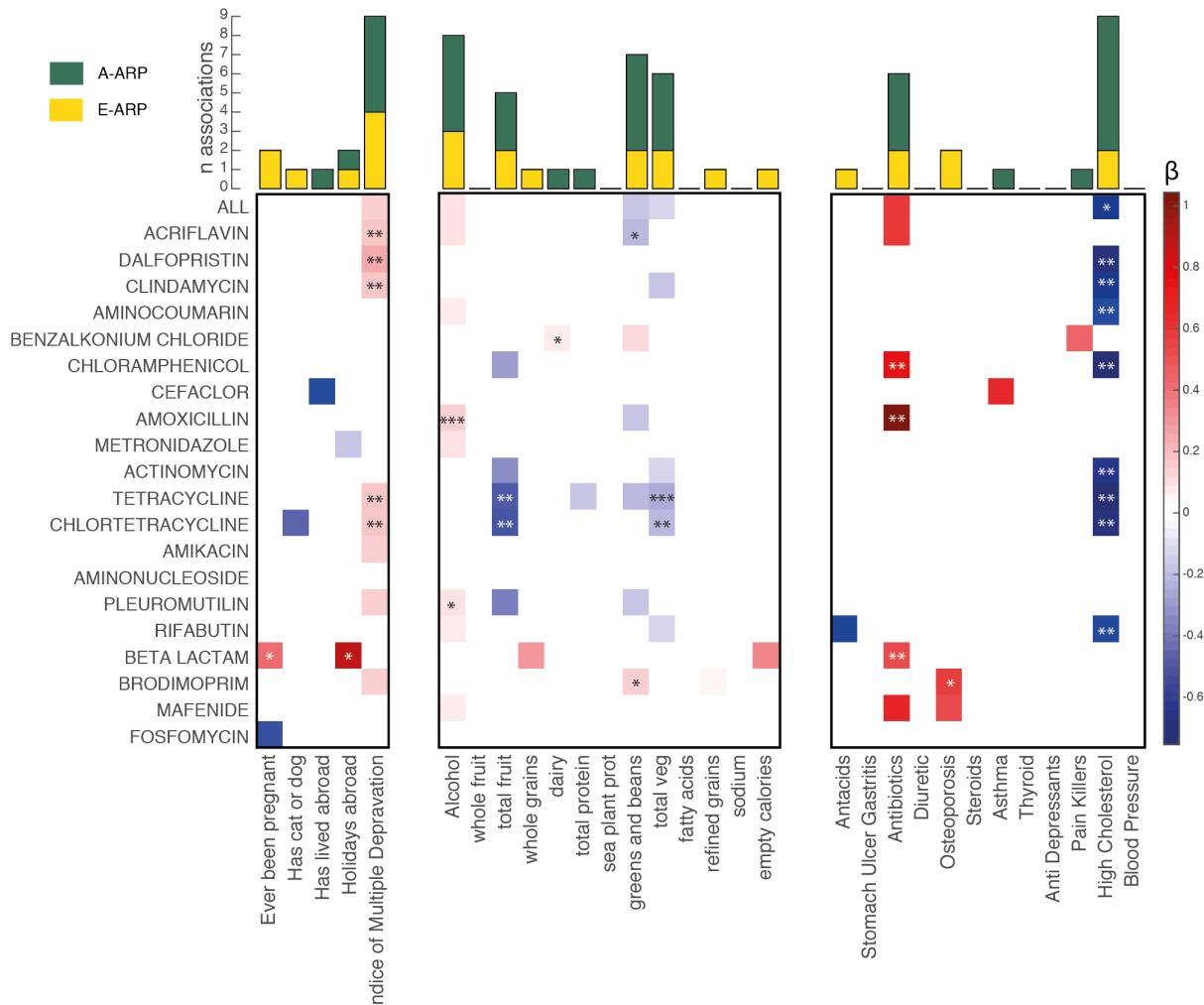


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368 **Figure 4:** Association between ARP profiles ranked based on their level of heritability
369 and disease. Nominally significant ($P < 0.05$) associations are colour-coded with blue
370 colours representing negative associations and red colours representing positive
371 ones. * $P < 0.01$; ** $P < \text{FDR } 5\%$ for the health condition of interest. The bar graph on
372 the top of the heatmap represents the number of associations observed for each trait
373 with heritable ARPs ($A > 20\%$) in green and with non-heritable ARPs ($A < 20\%$) in
374 yellow. Full results are available in **Supplementary table 6**.
375
376 Host environmental factors are associated with the gut resistome

377 To evaluate host environmental effects on the gut resistome, we explored the
378 association between ARP variability and 31 host environmental variables, using the
379 linear models as described above (**Supplementary table 1**). Host environment factors
380 were divided into three categories: (i) distal environment, such as the index of multiple
381 deprivation (IMD), has ever lived abroad and contact with pets, (ii) diet, including
382 alcohol consumption and 12 domains calculated from FFQs to build the healthy eating
383 index (HEI), and (iii) medication used by at least 10 participants in the study. Two
384 associations surpassed multiple testing adjustment in the diet category (adjusted P-
385 value < 0.05) and a total of 66 nominally significant associations were observed
386 between 30 environmental factors and 21 ARPs (**Figure 5**).
387 Over half of the nominally significant associations were observed with diet variables
388 (31 associations, 54% of total). The most significant results that surpassed multiple
389 testing adjustment were obtained between alcohol consumption and amoxicillin ARP
390 ($\beta = 0.1561 \pm 0.0390$; $P = 9.58 \times 10^{-5}$), followed by an association between total
391 vegetables consumption and tetracycline ARP ($\beta = -0.3840 \pm 0.0987$; $P = 1.44 \times 10^{-4}$). A large number of nominally significant associations were also observed between
392 ARPs and consumption of greens and beans (associated with 7 ARPs, of which 2
393 displayed association P-values < 0.01), as well as total vegetables intake (associated
394 with 6 ARPs, of which 1 displayed association P-values < 0.002). Dairy consumption
395 displayed one positive association with benzalkonium chloride ARP ($\beta = 0.0702 \pm 0.0249$; $P = 0.0053$), and total protein consumption was negatively associated with
396 tetracycline ARP ($\beta = -0.1335 \pm 0.0633$; $P = 0.0380$). All associations observed
397 between alcohol consumption and ARPs were positive, while most of those observed
398 between fruit, vegetable or greens and beans consumption with ARPs were negative.
399 For distal environmental factors, there were no results after multiple testing correction,
400 but 15 nominally significant associations were observed, the majority of which were
401 positive associations with IMD ($n = 9$). Notably, three of the four most heritable ARPs
402 were positively associated with IMD (acriflavin: $\beta = 0.1983 \pm 0.0653$, $P = 0.0032$;
403 dalfopristin: $\beta = 0.2630 \pm 0.0715$, $P = 0.0005$; clindamycin: $\beta = 0.1799 \pm 0.0587$,
404 $P = 0.0032$), as well as sum of all ARPs ($\beta = 0.1509 \pm 0.0706$, $P = 0.0355$).
405 Furthermore, we also observed a positive association between beta lactam and
406 previous pregnancy or pregnancies ($\beta = 0.4613 \pm 0.1389$; $P = 0.001$), as well as
407 having lived abroad ($\beta = 0.9235 \pm 0.3081$; $P = 0.003$). Interestingly, this was the

410 only set of variables for which a majority of the associations (53%) were observed with
411 non-heritable ARPs.

412 In the medication category, there were no results after multiple testing correction, but
413 20 nominally significant associations were observed between use of 7 medications
414 and 21 ARPs. As expected, antibiotic consumption was positively associated with 6
415 ARPs including amoxicillin ($\beta = 1.0441 \pm 0.3179$; $P = 0.001$), beta lactam ($\beta =$
416 0.5785 ± 0.2028 ; $P = 0.005$) and chloramphenicol ($\beta = 0.8327 \pm 0.3019$; $P = 0.006$).
417 However, the highest number of associations (9 associations) were detected with high
418 cholesterol medication, and in all cases, these were negative associations. The
419 strongest association was observed between tetracycline ARP and high cholesterol
420 medication (effect size = -0.75 ± 0.22 ; $P = 0.001$). As statins are the most commonly
421 used drugs for high cholesterol, we checked if this signal could be attributed to use of
422 statins. Out of 50 volunteers on high cholesterol medication, 29 (58%) reported statin
423 use. We evaluated the association between ARPs and statin use (excluding volunteers
424 who used high cholesterol drugs other than statins; and using the same mixed effect
425 model as previously described) and observed that none of the 9 associations remained
426 nominally significant. However, for 5 out of 9 associations (ALL, chloramphenicol,
427 chlortetracycline, rifabutin and tetracycline) the direction of the associations remained
428 negative. Thus, it is not possible to exclude that fact that statins use may be the
429 underlying cause of these results, and this would need to be confirmed in a larger
430 study. No concordance was observed between the results obtained for medication use
431 and the corresponding associated condition, where these data were available (**Figure**
432 **4**).



433

434 **Figure 5:** Host environmental factors associate with gut ARP profiles. Association
 435 between ARP profiles ranked based on their level of heritability and environmental
 436 factors. Nominally significant ($P < 0.05$) association results are colour-coded on the
 437 heatmap with blue colours representing negative associations and red colours positive
 438 ones. The bar graph on the top of the heatmap represents the number of associations
 439 observed for each trait with heritable ARPs ($A > 20\%$) in green and with non-heritable
 440 ARPs ($A < 20\%$) in yellow. * $P < 0.01$; ** $P < \text{FDR } 5\%$ for individual environmental
 441 factors, *** $P < \text{FDR } 5\%$ within category (distal environment, diet and drugs). Full
 442 results are available in **Supplementary table 7**.

443

444 Discussion

445 We describe the gut resistome of a Caucasian predominantly healthy older female
 446 sample from the UK and aim to dissect the role of host genetic and environmental
 447 factors on shaping the antibiotic resistance reservoir. Most ARPs were prevalent in
 448 over 90% of the population sample, which was much higher than previously reported

449 [10, 11]. This difference is likely due to changes and improvement of the databases
450 used to characterise ARGs and indicates that the majority of the population is likely to
451 harbour many ARGs in the gut, with potential implications for risk of developing
452 resistance to antibiotic treatments in case of infection.

453

454 Our results confirm that the human gut resistome is mostly shaped by environmental
455 factors. Yet, we observed that on average over a quarter of ARP variance may be
456 under host genetic control. While some ARPs could be considered not heritable, many
457 (57% of ARPs) were over 20% heritable. The most heritable ARP, acriflavin, showed
458 very strong evidence of host genetic impacts with heritability of 70%. Acriflavin is a
459 topical antiseptic and this observation may be driven by the fact that most common
460 human skin diseases are also heritable [36]. ARP heritability was in line with previous
461 analysis of the TwinsUK microbiome demonstrating that the abundance of both
462 bacterial taxa and genes could be heritable [23]. On the other hand, our estimates of
463 ARPs heritability are greater than expectation based on previously reported host
464 genetic contribution to the taxonomic composition of the gut microbiota [21, 22, 23].
465 By correcting ARPs for the relative abundance of highly heritable genera that
466 contributed ARGs, we observed a proportion of the measured ARP heritability likely
467 reflects the heritability of the bacterial gene carriers. Nonetheless, this did not fully
468 eliminate the role of host genetics onto the ARP itself. Thus, these results suggest that
469 host genetic effects may not only shape the gut bacterial ecosystem and favour the
470 growth of specific bacterial taxa, but could also promote presence or absence of
471 specific gene functions such as antibiotic resistance within the gut.

472

473 The twin model results indicated that, as expected, the majority of antibiotic resistance
474 variation in our sample could be attributed to environmental factors unique to an
475 individual. To explore this further, we compared antibiotic resistance profiles to
476 multiple environmental exposures, lifestyle and health factors. Overall, the strongest
477 and most wide-spread associations were observed with dietary intake components
478 (especially alcohol intake and vegetable consumption), medication use (particularly
479 cholesterol lowering drugs such as statins), and socioeconomic status (SES) defined
480 by the IMD. Dietary components (predominantly alcohol, fruits, vegetables and legume
481 consumption) exhibited associations with multiple ARPs. Diet plays an important role
482 in shaping the gut microbiome [37, 38] that is then able to influence the resistome [39],

483 which may explain our observations. For instance, the HEI built using the dietary
484 components considered here has been strongly associated with the composition of
485 the gut microbiota [34]. Consumption of alcoholic beverages such as red wine has
486 also been associated with alteration of the gut microbiota diversity and composition
487 [40] and may contribute to our results of numerous positive associations observed
488 between alcohol consumption and ARPs in this study. Although positive ARP-
489 associations with alcohol intake was observed, ARP-associations observed with
490 vegetables as well as fruits and beans were all negative. These results could reflect
491 the importance of these foods, potentially through their high fibre content, in
492 modulating the composition of the gut microbiome at the taxonomic level [41, 42], thus
493 affecting the gut resistome. The observed effect of diet on ARPs may also contribute
494 to their associations with SES. Indeed, diet intake has been correlated with SES in
495 numerous studies [43, 44] and we observe here that 9 ARPs were positively
496 associated with IMD, of which 7 are also associated with one of diet items studied.
497 Yet, a recent study demonstrated that the associations detected between IMD and the
498 gut microbiome were not all affected by dietary intake [35]. This suggest that other
499 components of SES may contribute to shaping the gut resistome.

500
501 Beside the general effect of diet on the gut resistome, the spread of ARGs across the
502 human population could partly be attributed to the use of antibiotics in the food industry
503 described as the 'farm-to-fork' hypothesis [45]. Indeed, it was found that the total ARP
504 levels of a human gut within a country is directly proportional to the quantity of
505 antibiotics use in farms [10]. However, in our study, only two nominally significant
506 associations were observed between ARPs and protein intake suggesting that meat
507 consumption may not be the main driver of ARG transfer. We observed one positive
508 association between dairy consumption and benzalkonium chloride (BC) ARP. BC is
509 an agent that can be used as a disinfectant in the dairy industry, leading to the
510 development of BC resistant bacteria [46, 47, 48]. Furthermore, bacteria from farm
511 animals can be transferred to humans *via* fermented dairy products such as cheese
512 [49]. Together, this suggests that dairy consumption may also be relevant in terms of
513 transfer of ARG from animals to human and selective dietary alteration of the gut
514 microbiota composition at a taxonomic level may also play an important role in shaping
515 the gut resistome. Diet could also contribute to the observed heritability of ARPs as
516 food choices were also described as heritable [50].

517
518 We assessed the association between ARPs and medication use as many drugs affect
519 the composition of the gut microbiota [51, 52]. As expected, antibiotic consumption
520 was positively associated with 9 ARPs, as well as the sum of all ARPs, but these
521 results did not surpass multiple testing correction. A recent study following the gut
522 microbiome of 12 men post antibiotic treatment described an increasing trend in ARP
523 levels up to 6 months after exposure [53]. Nevertheless, most of the major effects were
524 observed within a 4 to 8 days window suggesting a time-limited effect of antibiotic
525 consumption on the gut resistome, which may explain our modest association results
526 [53]. The strongest associations were observed with amoxicillin and methicillin ARPs,
527 two commonly used antibiotics, for which resistance of human commensals have been
528 reported [54, 55]. Other drugs had no noticeable effects on the ARP profiles apart from
529 negative associations observed with drugs used to treat high cholesterol. The most
530 common cholesterol lowering drugs are statins, that have been reported to affect the
531 composition of the gut microbiota [51]. Statins have been proposed as potential 'AMR
532 breakers', molecules described as capable of re-sensitising bacteria resistant to
533 antibiotics [56, 57]. The observed associations between high cholesterol and ARPs
534 were not significant when considering statin use only, but the lower sample size in the
535 statin subset analyses reduced our power. However, 5 of the 9 associations remained
536 negative, in line with a potential effect of statins on the resistome. While the effect of
537 statins on ARPs would need to be confirmed in a larger sample, our data suggest that
538 other high cholesterol drugs may also be at play and should be studied in more depth.
539
540 ARPs were relatively weakly correlated to host health status variables, with only few
541 nominally significant associations observed with common diseases and health-related
542 phenotypes. Surprisingly, the number of urinary tract infections (UTIs) or days spent
543 in the hospital within the last year were negatively associated with ARP levels, despite
544 the fact that hospitals are thought to play a small, but significant role in ARG spread
545 [58, 59], and UTIs are generally eradicated by antibiotic treatment. This result may be
546 due to the small sample size in these analyses, with only 20 volunteers reporting at
547 least 3 UTI in their lifetime, and 19 with a hospital visit (of at least one day) within the
548 last year. On the other hand, autoimmune disorders such as allergy and rheumatoid
549 arthritis, were positively associated with ARPs, in line with our expectations. Both
550 diseases have been associated with alteration of the composition of the gut microbiota

551 [30, 60, 61]. While allergy has been associated with increased *Proteobacteria* that
552 carry a high number of ARGs in our dataset [62], rheumatoid arthritis has mostly been
553 linked to an increase in *Prevotella* [63, 64] that contains less ARGs than the average
554 genus. Interestingly, visceral fat mass was generally negatively associated with ARP
555 levels, which is in line with the negative trend described by Forslund et al. (2014)
556 between ARPs and BMI [11].

557

558 Although these results can improve our understanding of the many intrinsic and
559 extrinsic factors that shape the human gut resistome, this study has limitations. First,
560 although this is one of the largest studies of its kind so far, the sample size was
561 relatively limited and only suggestive associations were observed that would need to
562 be replicated in larger samples to lead to robust conclusions. Furthermore, causal
563 mechanisms could not be inferred due to the cross-sectional nature of the study. Most
564 of the phenotypes and environmental exposures that we explored were self-reported,
565 including diet. Ideally future work would explore these findings using objective
566 measures of environmental exposures and diet, and clinically validated phenotypes.
567 Finally, ARPs are an *in-silico* measure of potential for antibiotic resistance, and actual
568 resistance of the gut community would need to be further assessed *in vitro* or *in vivo*
569 to fully assess the impact of host genetic and environmental factors on the resistance
570 of the gut community to antibiotic treatment.

571

572 **Conclusions**

573 In summary, our results show that based on our UK female population sample, the
574 human gut can be considered as a reservoir for antibiotic resistance genes. We
575 demonstrated that while the gut resistome is mostly shaped by environmental factors,
576 over a quarter of its variance can be mapped to host genetics and this can only partly
577 be explained by the overall heritability of the gut microbiota composition. Although we
578 are still far from being able to conduct genome-wide association studies that will
579 enable us to understand the role of host or bacterial genetic architecture on the human
580 gut resistome, our results imply that, in the future, host genetic variation could be taken
581 into consideration when prescribing antibiotics. Additionally, we observed that the
582 composition of the human gut resistome is strongly linked to a multitude of
583 environmental factors, beyond antibiotic consumption. Indeed, diet was the

584 environmental component associated with the most ARPs, suggesting that food
585 production and composition may play a key role in the global ARG spread in addition
586 to its effects on the taxonomic composition of the gut microbiome. Altogether, our
587 results suggest that, as for many other therapies, antibiotic prescription should be
588 framed in a personalised context to maximise treatment success and help constrain
589 the spread of antibiotic resistance.

590

591 **List of abbreviations**

592 A, additive genetic effects; A40, antibiotic a40926; ACR, acriflavin; ACT, actinomycin;
593 AMR, antimicrobial resistance; ARG, antibiotic resistance genes; ARO, antibiotic
594 resistance ontology ; ARP, antibiotic resistance potential; BC, benzalkonium chloride;
595 BMI, body mass index; Bp, bade pair; C, common environment effects; CAR,
596 carbomycin; CHL, chlortetracycline; CI, confidence interval; CIP, ciprofloxacin; CLI,
597 clindamycin; COL, colistin; DAL, dalfopristin; DEXA, Dual-energy X-ray
598 absorptiometry; DNA, Deoxyribonucleic acid; DZ, dizygotic; E, unique environment
599 effects; ERY, erythromycin; FDR, false discovery rate; FFQ, food frequency
600 questionnaires; GLY, glycylcycline; HEI, healthy eating index; IMD, indices of multiple
601 depravation; LIN, linezolid; MIC, microcin J25; MOX, moxifloxacin; MUP, mupirocin;
602 MZ, monozygotic; NAL, nalidixic acid; NOR, norfloxacin; RUC, rural urban
603 classification; SES, socioeconomic status; TEL, telithromycin; TIG, tigecycline; UK,
604 United Kingdome; UTI, urinary tract infection; VFM, visceral fat mass.

605

606

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824

825 **Ethics approval and consent to participate**

826 Ethical approval was granted by the National Research Ethics Service London-
827 Westminster, the St Thomas' Hospital Research Ethics Committee (EC04/015 and
828 07/H0802/84). Informed consent was obtained from all volunteer participants.

829

830 **Consent for publication**

831 Not Applicable

832

833 **Availability of data and materials**

834 The metagenomic shotgun sequencing data for the 250 samples after removal of
835 human sequences reported in this paper are available on the European Bioinformatic
836 Institute (EBI) repository under the following accession number ERP010708. All other
837 phenotypical information's may be available upon request to the department of Twin
838 Research at King's College London (<http://www.twinsuk.ac.uk/data-access/accessmanagement/>).

840

841 **Competing interests**

842 T.D.S is a scientific founder of Zoe Global Ltd. All other authors declare no potential
843 conflicts of interest.

844

845 **Funding**

846 The authors thanks collaborative grant from the Healthy Diet for a Healthy Life Joint
847 Programming Initiative (JPI administered by the MRC UK, MR/N030125/1). The
848 TwinsUK microbiota project was funded by the National Institute of Health (NIH) RO1
849 DK093595, DP2 OD007444. TwinsUK is funded by the Wellcome Trust, Medical
850 Research Council, European Union, The CDRF, The Denise Coates Foundation, the
851 National Institute for Health Research (NIHR)-funded BioResource, Clinical Research
852 Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS
853 Foundation Trust in partnership with King's College London. T.C.M. has received a
854 personal award by the Philippe Foundation Inc. D.L.M. is supported by the Centre for
855 Host-Microbiome Interactions, King's College London, funded by the Biotechnology
856 and Biological Sciences Research Council (BBSRC) grant BB/M009513/1. V.R.C. is
857 supported by The Alan Turing Institute under the Engineering and Physical Sciences
858 Research Council (EPSRC) grant EP/N510129/1.

859

860 **Authors' contributions**

861 J.T.B. conceptualised the study. J.T.B. and S.K.F. supervised the analysis. C.I.LR led
862 the analysis. S.K.F, R.C.E.B, T.C.M, J.C.F, R.C, V.R.C., D.M., C.J.S, and T.D.S.
863 contributed data and analysis inputs. C.I.LR, and J.T.B. wrote the manuscript. All
864 authors reviewed and approved the manuscript.

865

866 **Acknowledgements**

867 We acknowledge the study participants from the TwinsUK cohort. We acknowledge
868 support provided by the JPI HDHL funded DINAMIC consortium (<http://www.jpi-dinamic.wzw.tum.de/>), administered by the MRC UK, MR/N030125/1) and JPI HDHL
869 funded DIMENSION consortium (administered by the BBSRC UK, BB/S020845/1).
870 TwinsUK is funded by the Wellcome Trust, Medical Research Council, European
871 Union, the National Institute for Health Research (NIHR)-funded BioResource, Clinical
872 Research Facility and Biomedical Research Centre based at Guy's and St Thomas'
873 NHS Foundation Trust in partnership with King's College London.

875

876 **Supplementary material**

877

878 **Supplementary Figure 1:** ARPs are highly correlated. Results of the spearman
879 correlation between 41 independent ARPs. ARP clusters are highlighted by the
880 dashed boxes. ARPs selected for the analysis conducted in this study are indicated in
881 bold.

882

883 **Supplementary table 1:** Summary statistics of the cohorts and variables used in the
884 study.

885

886 **Supplementary table 2:** Full results from the ACE heritability analysis. A, proportion
887 of variance explained by host genetics; C, proportion of variance explained by
888 common environment; E, proportion of variance explained by environment unique to
889 an individual; CI_up, upper 95% confidence interval; CI_low, lower 95% confidence
890 interval; P, p-value.

891

892 **Supplementary table 3:** Prevalence of the most heritable ARPs in other European
893 population.

894

895 **Supplementary table 4:** Weight of taxa contribution to acriflavin, aminocoumarin,
896 clindamycin, daflopristin, brodimoprim and total (ALL) ARPs. ACE estimates obtained
897 in the Xie et al. publication are also presented [23].

898

899 **Supplementary table 5:** Effects of taxonomy on the heritability of acriflavin,
900 aminocoumarin, clindamycin, daflopristin, brodimoprim and total (ALL) ARPs.

901

902 **Supplementary table 6:** Association results between ARPs and health parameters
903 obtained using a mixed effect linear model where ARPs were a response and BMI,
904 age, gender and alpha diversity were considered as fixed effects and family structure
905 as a random effect.

906

907 **Supplementary table 7:** Association results between ARPs and environmental
908 factors obtained using a mixed effect linear model where ARPs were a response and
909 BMI, age, gender and alpha diversity were considered as fixed effects and family
910 structure as a random effect.

911