

1 **Anthropogenic land use exerts selection pressures on the resistome of a wild rodent**

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## 50 **Abstract**

51 Antimicrobial resistance poses a significant global health challenge. However, the factors  
52 maintaining antimicrobial resistance genes (ARGs) in wildlife microbiomes remain unclear  
53 particularly in species inhabiting human-dominated landscapes. We analysed 875 gut  
54 metagenomes from wild house mice (*Mus musculus*) on German farms between 2016 and  
55 2022 to identify environmental and host determinants of ARG occurrence. Using joint species  
56 distribution models, we quantified the influence of landscape, climate and mouse associated  
57 characteristics on the occurrence of individual ARGs and on trait dependence among genes.  
58 Environmental variables and livestock farming intensity explained more than 21% of ARG  
59 variation, whereas host characteristics accounted for less than 10%. Analysis of ARG traits  
60 revealed that agricultural land use and exposure to livestock increased the occurrence of  
61 mobile ARGs. Pig density was strongly associated with integron-encoded sulfonamide  
62 resistance genes and genes conferring tetracycline and beta-lactam resistance. Consistent  
63 with these findings, mouse resistomes closely resembled those of livestock manure. Taken  
64 together, our results show that landscape conditions, particularly farming intensity, shape the  
65 distribution of specific ARGs and mobile ARGs in house mice microbiomes. Understanding  
66 the factors impacting ARGs prevalence in wildlife is crucial for determining transmission of  
67 antimicrobial resistant microorganisms from animal and environmental reservoirs.

68

## 69 **Background:**

70

71 Antimicrobial resistance (AMR) represents a major concern for global health responsible for  
72 an estimated 5 million deaths worldwide yearly<sup>1,2</sup>. In response to this crisis, the World Health  
73 organisation and the Food and Agriculture organisation established the “Global Action Plan  
74 on Antimicrobial Resistance” in 2015, aiming for a coordinated One Health approach including  
75 human, animal and environmental health sectors<sup>3</sup>. The complexity of transmission routes,  
76 origins and reservoirs within and between humans, farm animals, and their shared  
77 environments poses challenges for implementing effective control strategies<sup>4</sup>. In particular,  
78 our understanding of how different environmental factors impact the occurrence or potential  
79 transmission of antibiotic resistance genes (ARGs) in bacterial communities associated with  
80 wildlife hosts or the external environment, which both act as AMR reservoirs, is still limited<sup>5,6</sup>.

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82 The mechanisms of antimicrobial resistance are a natural phenomenon that have enabled  
83 bacteria to survive, persist and evolve within specific ecological contexts, which are  
84 determined by the dynamics of the microbial community<sup>7,8</sup>. The use of antimicrobial drugs in  
85 medical, agricultural and livestock production imposes additional evolutionary selection  
86 pressure on bacterial communities, driving the occurrence and transmission of ARGs<sup>9-13</sup>.  
87 Human activities have accelerated the horizontal spread of established ARGs to clinically  
88 relevant pathogens even from evolutionarily distant bacteria through mobile genetic  
89 elements<sup>14</sup>. Latent genes represent, in counterpart, a large collection of less studied genes  
90 conferring resistance that remain understudied at environmental bacterial communities and  
91 largely absent from databases<sup>15</sup>. Under antibiotic selection pressure, ARGs can also evolve  
92 from precursor genes, so called *proto-ARGs*, that have other functions within bacterial  
93 genomes and do not directly confer a resistance phenotype<sup>16-18</sup>. Thus, the resistome of a  
94 bacterial community is constituted by the metagenomic composition of established, latent and  
95 precursor genes, all of which may directly or indirectly contribute to resistance. The resistome  
96 varies substantially between environments, which represents important implications for the  
97 emergence and dissemination of resistance.

98

99 Metagenomic screening has extensively characterised the resistome of human gut,  
100 wastewater and livestock<sup>19–22</sup>. Such high throughput approaches have been instrumental to  
101 identifying socio-economic, genetic and environmental factors associated with resistance  
102 potential, and the dynamics of clinically relevant ARGs from environmental reservoirs to  
103 humans<sup>10,23–26</sup>. Although some wildlife species are recognised reservoirs of infectious  
104 diseases and antimicrobial resistance, systematic metagenomic screening of wildlife  
105 associated resistomes is still sporadic. This limits our understanding of background levels of  
106 resistance in species able to mobilise antimicrobial resistant bacteria over large geographical  
107 distances or to maintain local dissemination<sup>27,28</sup>. Therefore, the use of high-throughput  
108 approaches in wildlife species becomes crucial in two directions: first, to determine the basal  
109 resistome in their microbiomes and second to assess the impact of environmental drivers in  
110 resistance dynamics.

111

112 In human-dominated landscapes, generalist wildlife species able to move freely at the  
113 interface between natural and anthropogenic environments encounter diverse sources of  
114 ARGs, e.g. via foraging, and may act as “mobile links” for resistance dissemination<sup>29–31</sup>. While  
115 birds and large mammals have been highlighted as sentinels for resistance due to their  
116 capacity for long-distance movement, small mammals, particularly rodents, are key to  
117 understanding ARG transmission at local scales, and for distinguishing environmental effects  
118 in transmission and spread<sup>32–34</sup>. Rodents, such as rats (*Rattus norvegicus*, *R. rattus*) and  
119 house mice (*Mus musculus*), living near human settlements and domestic animals, as well as  
120 wild rodents that have never been exposed to antibiotics, are all known to carry ARGs and  
121 resistant bacteria in their gut<sup>35–37</sup>. The latter highlights both the role of “pest” species as  
122 carriers and disseminators of resistant bacteria and ARGs, and the widespread nature of  
123 antibiotic resistance in the ecosystem. In the context of antimicrobial resistance ecology, it is  
124 crucial to understand how bacteria carrying ARGs interact with their “total environment”, i.e.  
125 their direct host “environment” as well as the wider landscape context. Microbial communities  
126 and their functional potential including resistance genes are shaped by different host  
127 characteristics, e.g. different life-history strategies such as dispersal or philopatry or sex  
128 differences<sup>38</sup>. In this respect, it is also of importance how ecological dynamics within rodents’  
129 gut microbiota are impacted by external environmental changes and could be used to detect,  
130 predict and even control the emergence and spread of resistance threats.

131

132 Changes in the environment, land use and landscape structure are expected to influence  
133 wildlife and their associated microbial communities through different biotic and abiotic factors  
134 that surround them. Especially farms with high livestock density or extensive crops are sources  
135 of bacteria carrying ARGs and pollute other environments through manure, dust moved by  
136 wind, water or even wildlife<sup>39</sup>. Spatially-explicit integration of environmental factors along  
137 environmental gradients in a multidimensional way provides a framework to understand how  
138 microbial communities and their resistomes respond to environmental change and which  
139 factors contribute to the composition of ARG traits like mobility<sup>40</sup>. Nevertheless, the linkage  
140 from environment via host to microbiome to resistome has been rarely incorporated to  
141 investigate how abiotic environmental factors in an ecosystem shape the distribution and  
142 diversity of ARGs in “wild” microbial communities due to imperfect integration of ecological  
143 frameworks into microbiome research.

144

145 Here, we analysed the resistome of densely sampled wild house mice (*Mus musculus*)  
146 communities across transects spanning hundreds of kilometers in Germany. Under a  
147 spatially-explicit approach, we assessed whether the composition, potential mobility and  
148 selection of ARGs within gut microbial communities are associated with anthropogenic,  
149 environmental and climatic factors, and disentangled these effects from host-specific  
150 influences. Our study provides an integrative landscape scale perspective on the transmission  
151 routes and environmental drivers underlying ARG dynamics in mobile wildlife hosts.

152

## 153 **Material and Methods:**

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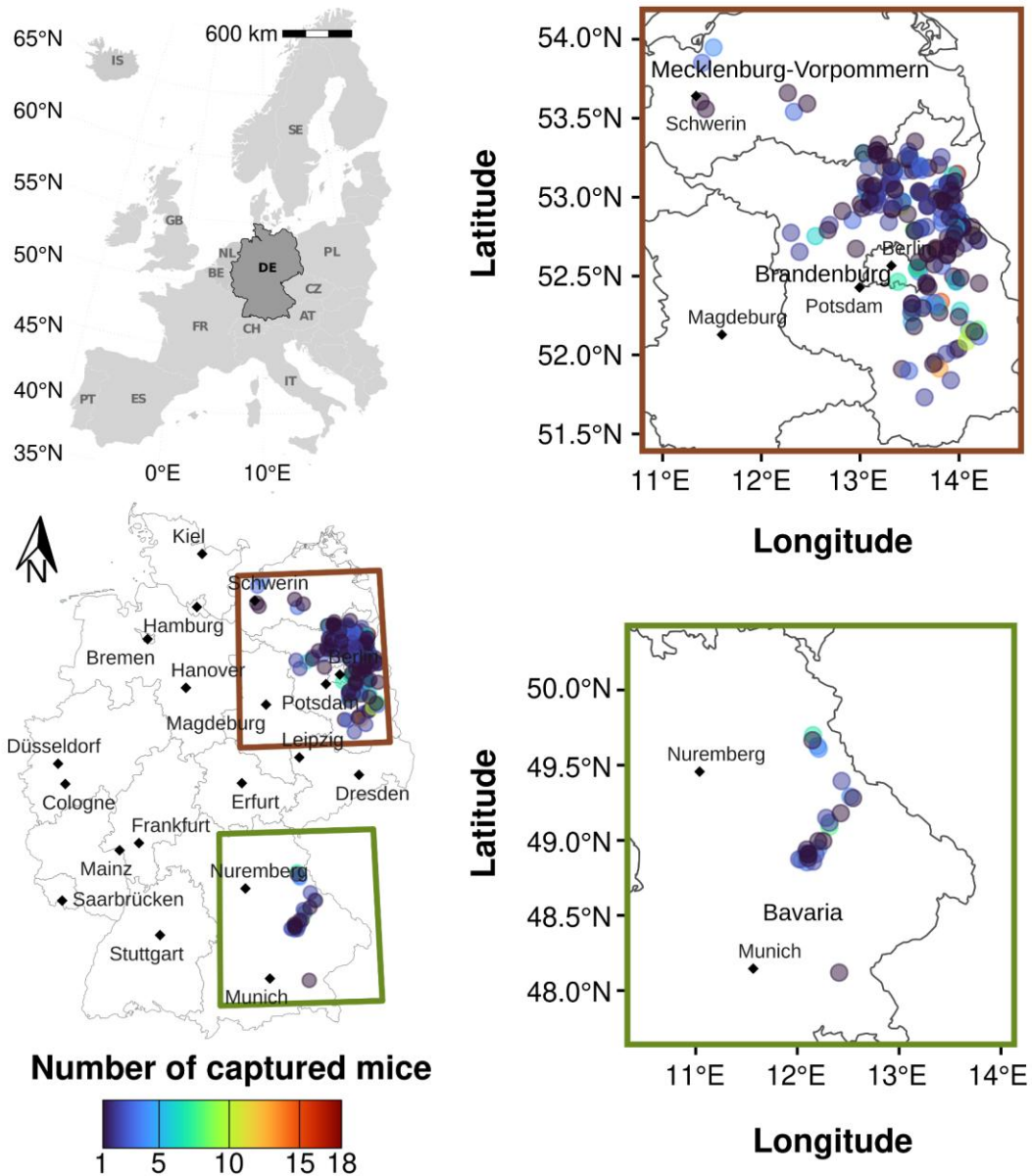
### 155 **Study area**

156 We conducted our study in Germany, central Europe, in the federal states of Berlin,  
157 Brandenburg, Bavaria and Mecklenburg-Vorpommern (Figure 1). Human population density  
158 in Germany is around 241 people/km<sup>2</sup>, with 77% of the population living in urban areas  
159 ([www.worldometers.info](http://www.worldometers.info)). The states selected for the study area are characterised by a large  
160 proportion of land dedicated to agriculture, from circa 70% in Mecklenburg-Vorpommern to  
161 45% in Brandenburg and Bavaria, and 4% in Berlin (<https://www.laiv-mv.de/Statistik/>,  
162 <https://www.statistikdaten.bayern.de/>, Amt Für Statistik Berlin-Brandenburg, 2025). Forested  
163 areas occupy 24%, 37%, 36% and 18% of the state surface, respectively. Within agriculture,  
164 a large proportion is dedicated to animal husbandry, with Germany being the main European  
165 producers of milk or pork ([www.bmleh.de](http://www.bmleh.de)). Crops and animal husbandry occur across the  
166 country, with different levels for each state. Poultry farming is present in all states, but it is  
167 predominant in Mecklenburg-Vorpommern. Cattle farms are found mainly in Bavaria and some  
168 areas in Brandenburg. Pig farming occurs in some locations in Bavaria and Mecklenburg-  
169 Vorpommern. In contrast, Berlin and Brandenburg produce mainly vegetables  
170 ([www.bmleh.de](http://www.bmleh.de)).

171

### 172 **Sample collection**

173 House mice (*Mus musculus*; hereafter: mouse) were captured alive from different private  
174 properties (farms) between 2015 to 2022, with only 2020 trapping missing due to COVID-19  
175 contact restrictions. After capture, mice were housed individually in cages overnight and  
176 euthanized by overdoses of isoflurane. Animal capture and handling was conducted under the  
177 animal experimentation licenses No. 2347/35/2014 and 2347/28/2021 issued by LAVG  
178 Brandenburg. All mice were dissected within 24 h after capture. The gastrointestinal colon  
179 content was collected, flash frozen in liquid nitrogen and later stored at -80 °C for DNA  
180 extraction at a later time<sup>41</sup>.



181

182 **Figure 1. Geographical distribution of farms where mice were captured.** The points colour  
183 indicates the number of mice captured at each farm location.

184

#### 185 **DNA extraction and sequencing**

186 Metagenomic DNA from colon content was extracted in two randomized batches. Samples  
187 collected between 2015 and 2018 (N= 679) using NucleoSpin®Soil kit (Macherey-Nagel  
188 GmbH & Co. KG, Düren, Germany) following the manufacturer's protocol with modification as  
189 reported in previous studies<sup>38,41</sup>. Samples from 2019, 2021 and 2022 (N= 196) were extracted  
190 using the ZymoBIOMICS-96 MagBead DNA Kit (Zymo Research Europe GmbH, Freiburg,  
191 Germany) in the automatized system TECAN Fluent® 780 NAP workstation following the  
192 manufacturer's instructions with minimum minimum modifications to the lysis step. In brief,  
193 750 µL ZymoBIOMICS Lysis Solution was added to ~100 mg of colon content and transferred  
194 to ZR Bashing Bead Lysis Tubes with beads (0.1 and 0.5 mm). The samples were  
195 mechanically disrupted using a PeQLab Precellys 24 (Bertin Corp., Rockville, MD, USA) for  
196 2×15 s at 5500 rpm. After 5 min rest, the cycle was repeated two more times for a total of three

197 cycles. The DNA was eluted with 50  $\mu$ L of ZymoBIOMICS DNase/RNase Free Water. Aliquots  
198 with at least 750 ng quantified using Qubit fluorometric quantification dsDNA Broad Range Kit  
199 (Thermo Fisher Scientific, Walham, USA) were shipped on dry ice for library preparation and  
200 sequencing at the Competence Centre for Genomic Analysis (Kiel, Germany). Sequencing  
201 libraries were prepared with the Illumina DNA prep kit following manufacturer's instructions.  
202 Shotgun sequencing was done on the Illumina NovaSeq6000 S4 platform (Illumina, San  
203 Diego, California, USA) using 300 cycles and v1.5 reagent kits.

204

### 205 **Bacterial taxonomy, ARG annotation, diversity and composition**

206 Paired-end shotgun sequencing reads were assessed for quality using FastQC v0.12.1 and  
207 processed using the NGLess pipeline v1.5<sup>42</sup>. Briefly, quality control consisted of trimming and  
208 filtering the reads with a minimum quality of 25, a minimum length of 45 b and removing the  
209 first 6 nucleotides. Two sequential decontamination steps were then performed. The first was  
210 to remove human contamination by filtering reads with a minimum match size of 45 and 90%  
211 identity to the reference human genome assembly GRCh38.p14. The second decontamination  
212 step aimed to remove host DNA by filtering reads mapped to the reference house mouse (*Mus*  
213 *musculus*) genome assembly GRCm39 - mm39 using the same criteria as for the human  
214 genome. Both reference genomes were masked for low complexity regions and regions  
215 mapping to the proGenomes3 gene catalogue<sup>43</sup>. The taxonomic profiling of decontaminated  
216 metagenomic reads was performed with mOTUs<sup>44</sup>. Samples with less than 1000 reads  
217 assigned to bacteria were removed from the analysis and only mOTUs with abundance above  
218 0.005% and 1% prevalence within each metagenome were included in the analysis.  
219 Antimicrobial resistance gene (ARG) annotation to summarise ARG occurrence and  
220 abundance in each sample was performed using Resistance Gene Identifier (RGI bwt) v5.2.1-  
221 2. Metagenomic reads were aligned to the Comprehensive Antibiotic Resistance Database  
222 (CARD) v3.1.4<sup>45</sup> using the bowtie2 aligner v2.4.5<sup>46</sup> and annotated following Antibiotic  
223 Resistance Ontology (ARO). For purposes of this study, only ARO IDs with more than 80%  
224 coverage to the reference were retained for further analysis and denoted as ARGs. ARG  
225 abundance was normalised to Fragments Per Kilobase of gene per Million mapped reads  
226 (FPKM) to control for differences in sequencing depth between samples and gene length  
227 differences. In addition, abundances were binned to ARG, AMR gene family and drug classes  
228 level. ARGs were manually regrouped based on the drugs to which they confer resistance.  
229 ARGs referring to penam, cephalosporin, carbapenem, cephamycin, penem and monobactam  
230 were grouped into the beta-lactam class. Those referring to macrolides, lincosamides and  
231 streptogramins were grouped into the MLS class. Those referring to more than one drug class  
232 were grouped into the multidrug class. The mobility of the ARGs was determined based on  
233 their genetic locations with MGEs by referencing CARD Data. The richness of ARGs observed  
234 for each sample was calculated as the total ARG detected in the dataset for a given sample,  
235 using Microbiome v1.26.0<sup>47</sup>. Principal component analysis (PCA) and permutational  
236 multivariate analysis of variance (PERMANOVA) were performed based on centred-log-ratio  
237 (CLR) transformed FPKM ARG abundances and 9999 permutations using 'adonis2' from  
238 package 'vegan' v2.6-475. PERMANOVA for both microbial and ARG profiles was run by =  
239 "margin" using year of collection, mouse sex, longitude, latitude and transect as geographical  
240 references, taxonomically assigned read counts and level of ARG count as predictors,  
241 stratifying by sequencing batch. Comparisons of ARG richness were conducted using a two-  
242 sided Mann–Whitney U-test with Bonferroni correction for multiple comparisons. The effect  
243 size  $r$  was calculated as the Z statistic of the MWU test divided by the square root of the  
244 sample size as implemented in the package rstatix v0.7.2. Significance was determined at a

245 p-value cut-off of 0.05 unless otherwise stated. All figures were created using the following  
246 packages: ggplot2 v3.5.1, ggsci v3.2.0, ggpubr v0.6.0, gridExtra v2.3 and RColorBrewer v1.1-  
247 3 with minor editions using Inkscape v0.92.5. Data handling was performed using pipelines  
248 compatible with tidyverse v2.0.0.

249

250 The bioinformatic analysis was performed on the High Performance Computing Cluster from  
251 the Max Delbrück Centrum, Berlin (Max-Cluster) equipped with 5.2k Cores – 46TB RAM.

252

### 253 **Retrieval of publicly available livestock manure metagenomes**

254 To assess the proximity of house mice derived resistomes to those from farm animals, we  
255 used two available datasets from livestock manure. One from the project EFFORT including  
256 pooled manure from pig, cattle and poultry herds in nine European countries<sup>25,48</sup>. The second  
257 from rural and urban pig and poultry farms in Ghana, which was included as a non-european  
258 reference<sup>49</sup>. A total of 639 fecal metagenomes were downloaded from the NCBI Sequence  
259 Read Archive (BioProjects PRJEB22062 and PRJEB62878). Both datasets were generated  
260 using Illumina platforms which ensure compatibility with data generated for our study. To avoid  
261 any bias in the comparisons, all metagenomes were processed and ARGs annotated in the  
262 same way as house mice derived metagenomes (see *Bacterial taxonomy and ARG annotation*  
263 *section*).

264

### 265 **ARG traits and explanatory variables**

266 We annotated detected ARGs based on four traits: i) Mobility (binary) indicates whether the  
267 ARG is associated with mobile genetic elements (MGE) as reported in the CARD database  
268 (FALSE: Chromosomally encoded ARGs; TRUE: ARG associated with mobile elements such  
269 as plasmids, integrons, transposons). ii) Localization (categorical) indicates the type of mobile  
270 genetic element in which the gene could be located. In our dataset, localization includes  
271 chromosome, plasmid, integron, transposon, pathogenicity island, multiple mobile genetic  
272 elements, and mixed when it could be either on the bacterial chromosome or in a MGE. Due  
273 to low its representation, multiple MGEs and pathogenicity islands were grouped together. iii)  
274 Drug class group (categorical) groups ARGs into broader drug class categories to increase  
275 statistical power due to the sparse representation of some individual classes. Grouping was  
276 based on similarity in chemical structure (Supplementary Table S1). iv) Resistance  
277 mechanism (categorical) is a functional mechanism by which the ARG confers resistance and  
278 is classified into antibiotic target alteration, antibiotic inactivation, efflux, target protection,  
279 target replacement, and reduced permeability. A subset of ARGs were analysed as clinically  
280 relevant based on the World Health Organization's list of medically important  
281 antimicrobials<sup>50</sup>(Supplementary Table S2).

282 We prepared a set of host-level and environmental explanatory variables hypothesised to  
283 influence the distribution of antibiotic resistance genes (ARGs) in house mice. As mouse host  
284 characteristics reflecting different life-histories, we used sex (female/male), the body condition  
285 (BC) of the mice calculated as the residuals of body weight and body length relationship and  
286 a mouse density proxy, calculated as the number of trapped individuals per site (*Ntrapped*).  
287 While mouse density may influence microbial exchange or exposure risk, the body condition  
288 might reflect disturbed microbiomes either as cause or consequence of bad health.

289 Environmental variables included proportions of different land cover features such as  
290 proportion of forest or agriculture in a 100m raster, accessibility of the farm calculated by

291 distances to linear transport features, farm animal density describing municipality-level  
292 livestock densities for cattle, pigs, and poultry as well as climatic data, averaged for the month  
293 of August in the respective sampling year.

294 All spatial variables were projected to a common coordinate reference system (ETRS89 /  
295 LAEA Europe, EPSG:3035, see source and original specifications of spatial layers in  
296 Supplementary Table S3), rasterised if needed and resampled to a 100 × 100 m resolution for  
297 spatial consistency. The proportion of agricultural areas was derived from the Corine Land  
298 Cover spatial layer class 2 by calculating the percentage of the land use class within each 100  
299 × 100 m grid cell. Distances to roads and paths were calculated by rasterizing the vector map  
300 to a 100 m resolution and calculating the distance to each linear road / path feature to the  
301 center grid cell coordinates. Values from these layers were then extracted for each sample  
302 based on their geographical location. Farm animal information was obtained from the Thünen  
303 agricultural atlas as number of livestock units per municipality<sup>51</sup>. We selected the three main  
304 livestock species in our study area (cattle, pigs and poultry) and transformed the number of  
305 heads of each species into densities to obtain a measure comparable across all sampling  
306 sites. The densities were obtained by dividing the number of heads by the total area of the  
307 corresponding municipality (Supplementary Figure S4).

308 To reduce multicollinearity among explanatory variables, we calculated pairwise Pearson  
309 correlation coefficients  $r$  and excluded one variable from each pair with  $|r| > 0.7$ , retaining the  
310 most ecologically informative predictors (Supplementary Figure S5).

## 311 **Data analysis**

312

### 313 *Factors affecting ARG distribution and abundance*

314

315 We applied two Joint Species Distribution Models (jSDM) within the Bayesian framework of  
316 Hierarchical Models of Species Communities (HMSC)<sup>52</sup> to model the occurrence (presence-  
317 absence) and the abundance of ARGs as a function of selected explanatory variables. Models  
318 were implemented using the *Hmsc* v 3.3-7<sup>53</sup>. The HMSC framework is ideal for the analysis of  
319 multiresponse data, as it allows for the inclusion of both fixed and random effects, as well as  
320 controlling for the spatial autocorrelation structure in the residuals, while analysing the data at  
321 the level of the taxa or the traits. Additionally, it also provides a measure of association  
322 between the different taxa after controlling for the effects of all explanatory variables, i.e.,  
323 positive or negative associations after accounting for environmental preferences.

324 The response matrix consisted of presence-absence or abundance data, respectively, for  
325 ARGs detected in individual mice sampled across multiple farms, municipalities, and regions.  
326 To assess how ARG trait–host–environment interactions shaped ARGs distributions, we also  
327 included a trait matrix comprising relevant ARG characteristics (see *ARG traits and*  
328 *explanatory variables*) in both models. To model binary ARG presence-absence data, we used  
329 a probit error distribution, and to model ARG abundance we log-transformed it ( $\log(x+1)$ ) to fit  
330 a Gaussian distribution. For the abundance models, we first filtered out ARGs with an overall  
331 prevalence lower than or equal to 5% to avoid chain convergence problems.

332 After the removal of correlated explanatory variables, we additively included the mouse  
333 characteristics (sex, body condition, mouse density), landcover (agricultural land, impervious

334 surface, tree cover density, small woody features), accessibility (distance to road, distance to  
335 path) farm animal density (cattle, pig and poultry) and climatic characteristics (precipitation  
336 and temperature) as explanatory variables into the model. To account for the nested and  
337 spatio-temporal structure of the study design, we specified three levels of random effects:  
338 Spatial autocorrelation was accounted for by including the XY-coordinates of farm locations  
339 as a spatially-explicit random effect. To account for local farm-level variability and nested  
340 effects of the sampling design with multiple mice per farm, we included *FarmID*. Lastly, we  
341 included *Year* as a temporal random effect to capture inter-annual variability.

342 For the ARG occurrence model, we ran four parallel Markov Chain Monte Carlo (MCMC)  
343 chains, with 1000 burn-in iterations, followed by 10,000 sampling iterations and a thinning  
344 interval of 10, resulting in 4000 posterior samples per parameter. For the ARG abundance  
345 model, we ran four parallel Markov Chain Monte Carlo (MCMC) chains, with 1000 burn-in  
346 iterations, followed by 15,000 sampling iterations and a thinning interval of 10, resulting in  
347 6000 posterior samples per parameter.

348 Model convergence was assessed using MCMC trace plots, Gelman-Rubin diagnostics, and  
349 effective sample sizes of a random subset of parameters. Model performance was evaluated  
350 using Tjur's  $R^2$ , or  $R^2$ , and the area under the receiver operating characteristic curve (AUC).  
351 To quantify the contributions of the host and environmental explanatory variables versus the  
352 nested and spatio-temporal structure of the data, we decomposed  $R^2$  into variance  
353 components associated with fixed effects and each random effect using the partitioning tools  
354 in the *Hmsc* v 3.3-7.

### 355 *Association of house mouse and livestock manure resistomes*

356 After preprocessing and ARG annotation, the resistomes of livestock manure and house  
357 mouse were merged into a single dataset for further analysis. The resulting dataset includes  
358 FPKM abundances for each ARG, along with country and host of origin and ARG ontology.  
359 The combined dataset was compiled using Phyloseq v1.48.0<sup>54</sup>. Resistome richness and  
360 dissimilarity were estimated as described for the house mouse dataset alone (*see Bacterial*  
361 *taxonomy and ARG annotation section*). The relative effect of the host of origin on differences  
362 in richness against the house mouse resistome was estimated by fitting a linear model with  
363 ARG richness as response and host and country as predictor variables using 'stats' v3.6.2.  
364 Estimated marginal means (EMMs) for each host, and pairwise comparisons between EMMs  
365 of mouse and livestock resistome richness were calculated using 'emmeans' v1.11.1. Fisher's  
366 exact test was used to determine the degree of intersection in ARGs detected between the  
367 resistomes of mice and livestock. Pairwise comparisons were performed between each  
368 livestock host and the mouse resistome, and the p-values were adjusted using Bonferroni  
369 correction for multiple comparisons. An Euler diagram to represent ARG overlaps between  
370 the resistome of different hosts was done with 'eulerr' v7.0.2. To determine whether the  
371 resistome composition of mice was more similar to that of a particular livestock host, a linear  
372 mixed-effects model was fitted using 'lme4' v1.1-37. The model used *1 - Aitchison* distance as  
373 measure of resistome similarity between pairs of samples. Only resistome similarities within  
374 mice and between mice and livestock hosts were used. Host pair and country pair were used  
375 as fixed effects, and the sample IDs of the pairs were included as random effects to control  
376 for pseudoreplication. As described for the richness model above, EMMs were calculated for  
377 each host pair, and the relative degree of resistome similarity was determined by calculating

378 pairwise comparisons between EMMs within the mouse and between mouse-livestock  
379 resistomes. The prevalence of each ARG in mice and livestock was estimated as the  
380 proportion of metagenomes from each host where the given gene was detected. Comparisons  
381 of host-based prevalence were used to determine the potential sources of genes between the  
382 livestock hosts and the mice. Genes were categorised as co-promoted if they were present in  
383  $\geq 10\%$  of samples from both mice and a given livestock host. Genes present only in  $\geq 10\%$  of  
384 samples from one type of host were categorised as host-specific. ARGs present in fewer than  
385 10% of samples from mice and a given livestock host were classified as non-promoted<sup>55</sup>.

386 All analyses were performed in R version 4.4.1 (R Core Team, 2024).

## 387 Results

388

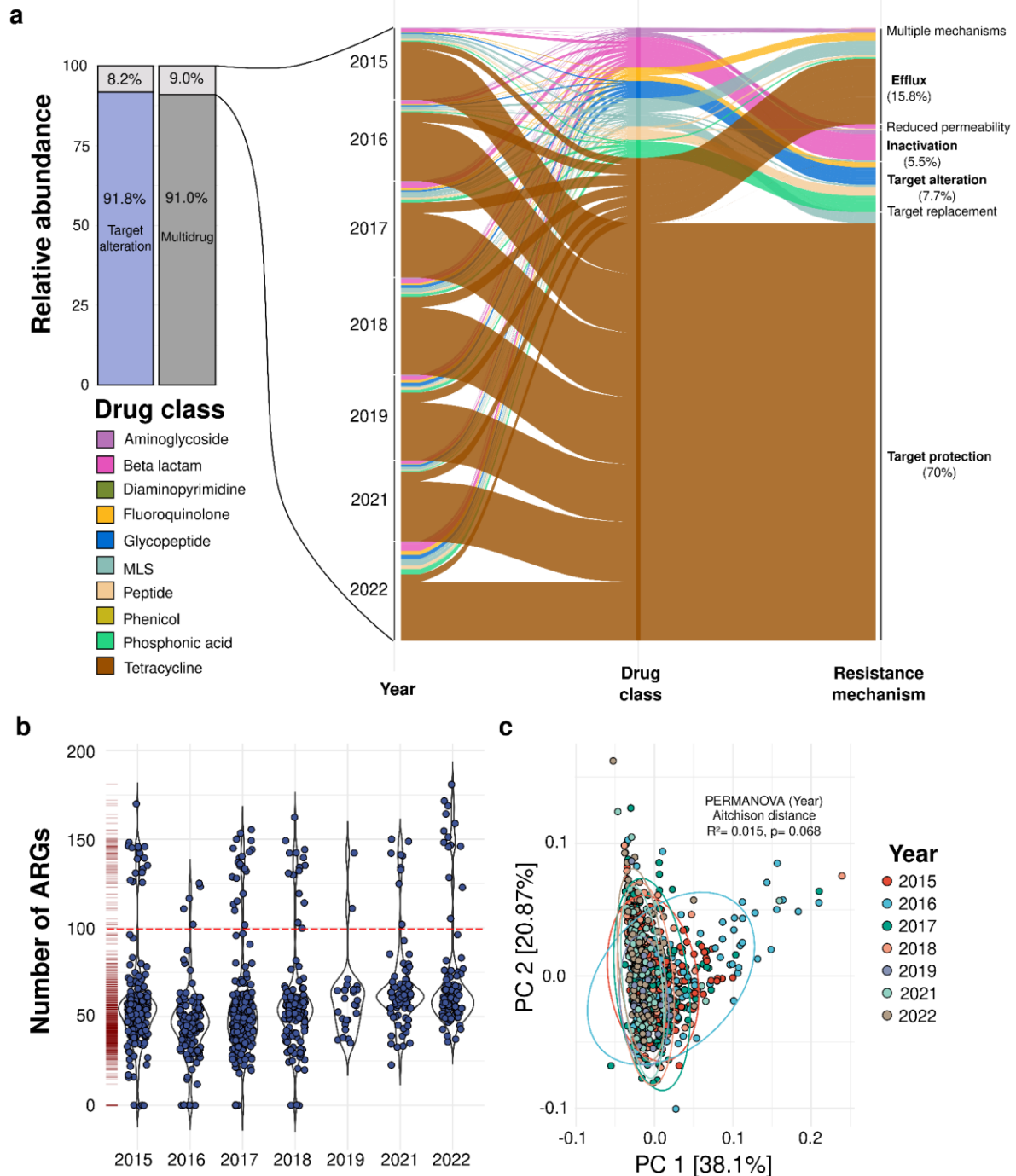
### 389 Composition of the house mouse resistome and microbiome

390 We detected 340 ARGs in the metagenomes of 875 house mice. The majority of the detected  
391 genes (178) potentially confers resistance to the ten relevant antibiotic classes that are of high  
392 clinical concern. However, these genes accounted for only 9% of the total abundance. The  
393 remaining genes corresponded to non-specific multidrug genes, including mutations in  
394 housekeeping genes (e.g. rRNA) and promoter regions, and members of different multidrug  
395 efflux pump families, accounting for up to 90% of the total gene abundance (Figure 2a). The  
396 most prevalent resistance mechanism is antibiotic target alteration (147 genes), which  
397 included 79 multidrug genes and 14 genes associated with other xenobiotics, such as  
398 disinfectants and antiseptics. Among the genes conferring resistance to relevant drug classes,  
399 those related to the target protection resistance mechanism for tetracyclines,  
400 fluoroquinolones, and MLS (macrolides, lincosamides, and streptogramins) antibiotics  
401 accounted for 70% of the abundance and were distributed across all years. Only 16% of the  
402 genes (55 genes) had prevalence higher than 50% and abundance above 2.5e6 FPKM across  
403 mouse metagenomes, including five conferring resistance to tetracycline and one to beta-  
404 lactam antibiotics (Supplementary Figure S6). About 23.5% (80 genes) were potentially  
405 associated with mobile genetic elements (MGEs). Most of the genes (260) were located on  
406 the main bacterial chromosome and correspond to multidrug or mutation in housekeeping  
407 genes associated with *Escherichia coli* variants. On average, each mouse metagenome  
408 contained 59.6 genes (Figure 2b), with few mice showing ARG richness higher than 100,  
409 which remained consistent over the years. During the sampling period of this study, we  
410 observed a significant increase in the number of genes detected towards the end; particularly,  
411 those from the last two years showed a strong increase. Those from 2016 generally had lower  
412 richness (Wilcoxon  $p$ -value adjusted  $< 0.01$ , effect size  $r > 0.2$ , Table 1). ARG composition was  
413 highly similar between mice; year explained only 2% of the variance and the composition  
414 remained consistent over time (PERMANOVA,  $p$ -value  $> 0.05$ ; Figure 2c).

415

416 A total of 606 bacterial mOTUs belonging to 50 different families were detected in the house  
417 mouse microbiomes. Each metagenomic sample contained an average of 212 mOTUs (range:  
418 25–374), and the composition remained homogeneous over the years, sexes, geographical  
419 locations, and levels of ARGs detected in the samples (PERMANOVA,  $p > 0.05$ ). Members of  
420 the *Lachnospiraceae* and *Muribaculaceae* families were highly prevalent (prevalence  $> 98\%$ )  
421 and represented the majority of abundance, at 25.8% [95% CI: 24.7–26.8] and 10.7% [95%  
422 CI: 9.99–11.5], respectively. Other bacterial families that could potentially mediate ARG  
423 transmission, such as *Enterobacteriaceae* and *Enterococcaceae*, had a prevalence below

424 50%, or were not detected, e.g. *Staphylococcaceae*. However, *Enterobacteriaceae*, and  
 425 particularly *Escherichia coli* abundance showed a strong correlation with the ARG richness  
 426 within each mouse resistome (Spearman's correlation,  $Rho > 0.5$ ,  $p < 0.001$ ) and partially  
 427 explained the difference in ARG levels in some resistomes (Supplementary Figure S7).  
 428



429  
 430 **Figure 2. General overview of ARGs in house mouse microbiomes**  
 431 **(a)** Average relative abundance of ARGs in all house mouse microbiomes from this study.  
 432 More than 90% of the abundance was related to multidrug genes (139 genes) and target  
 433 alteration genes (147 genes) as resistance mechanisms. Genes conferring resistance to the  
 434 ten relevant drug classes account for only 9% of the total abundance. Of these, the majority  
 435 were genes conferring resistance to beta-lactam antibiotics (46 genes), accounting for less

436 than 1% of the total abundance. Genes conferring resistance to tetracycline (18 genes)  
 437 represent 6% of the total abundance. Genes conferring resistance to tetracycline,  
 438 fluoroquinolone and MLS (macrolides, lincosamides and streptogramins) antibiotics, which are  
 439 related to the target protection mechanism, accounted for 70% of the abundance related  
 440 exclusively to the ten major antibiotic classes. Genes with efflux, target alteration or antibiotic  
 441 inactivation each represented more than 5% of the abundance related exclusively to the ten  
 442 major antibiotic classes. **(b)** ARG richness in house mice over the years. The number of ARGs  
 443 detected in 2015 and 2016 was significantly lower than at the end of the study period  
 444 (Wilcoxon, *p*-value adjusted <0.001). Most mice (*n* = 774) had an ARG richness of around 50  
 445 genes, while a smaller group (*n* = 85) had a higher number of genes detected. The dashed  
 446 red line defines a threshold of 100 ARGs detected. Each point represents a single mouse  
 447 metagenome. **(c)** PCA showing dissimilarity in ARG profiles between years of sampling. Each  
 448 point represents the ARG profile of a sample. Distances between points reflect biological gene  
 449 composition dissimilarity based on Aitchison distances. Points and ellipses are coloured by  
 450 year of sampling.

451

452 **Table 1. Differences in ARG richness in house mice at different years**

Year 1	Year 2	N 1	N 2	P-value adj.	Significance	Effect size
2015	2016	191	127	0.0002436	***	0.25
2015	2021	191	84	9.56E-05	****	0.28
2015	2022	191	104	2.84E-05	****	0.28
2016	2018	127	126	0.005061	**	0.23
2016	2019	127	26	0.011865	*	0.28
2016	2021	127	84	3.70E-12	****	0.51
2016	2022	127	104	8.55E-15	****	0.54
2017	2021	217	84	7.25E-08	****	0.34
2017	2022	217	104	4.10E-10	****	0.37
2018	2021	126	84	0.0003318	***	0.30
2018	2022	126	104	0.00013713	***	0.30

453 Only significant comparisons are shown

454

455 **Impact of environmental characteristics on the occurrence of ARG and compositional**  
456 **structure within house mouse microbiomes**

457 To determine the impact of environmental characteristics on the occurrence (presence-  
458 absence) of ARGs in house mice microbiomes, we employed joint Species Distribution Models  
459 (jSDM) for each of the ARG response, controlling for geographical and temporal proximity  
460 between samples and the farm identity and considering selected mouse-related  
461 characteristics, environmental factors. The jSDM on ARG presence-absence had an average  
462 effective sample size of over 800, which is indicative of robust parameter estimation and low  
463 autocorrelation. Additionally, Gelman-Rubin Diagnostic parameters for beta and gamma were  
464 higher than 1, indicating adequate model convergence. Overall, the model showed strong  
465 predictive performance (AUC= 0.91), identifying samples with high and low probability of  
466 specific ARG occurrence (Supplementary Figure S8). On average, 65% of the variance in  
467 ARG occurrence in the mouse gut bacterial microbiome was explained by random effects, and  
468 35% by explanatory variables (Figure 3a). The identity of each farm (Random: Farm ID) was  
469 the dominant source of explained variance, which indicates strong farm-specific effects not  
470 captured by environmental or host factors. Unmeasured ecological and farm management  
471 variables at the local level likely influenced the large variance explained by farm. Spatial  
472 proximity between samples led to similar ARG occurrence profiles, indicating local spread or  
473 spatially similar conditions not included in our models.

474

475 Mouse-related characteristics had a low impact in the occurrence of ARG. Mouse density,  
476 represented by the number of mice trapped at the farm site, explained 5%, while mouse  
477 condition and sex explained only 3%. Among the environmental parameters, land cover  
478 represented by agricultural lands, tree cover, small woody features and impervious surfaces  
479 explained about 9%. Farm accessibility, together with farm animal density (poultry, pig, cattle)  
480 and climatic variables explained each additional 6%.

481

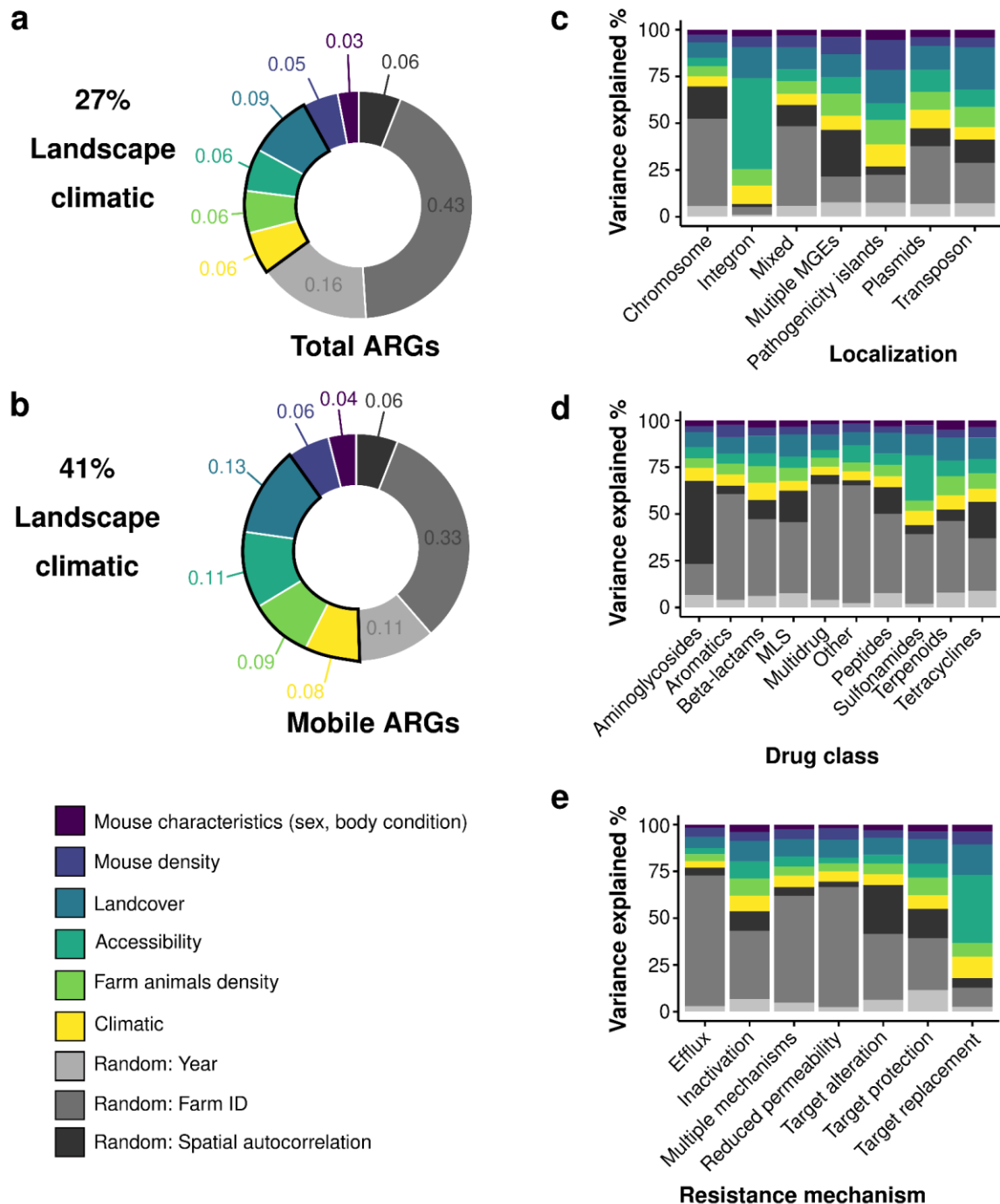
482 We used potential localization as a proxy of ARG mobility. Thus, when the model predicted  
483 the occurrence of only ARGs in any mobile genetic element, mouse characteristics and  
484 environmental factors explained more (51%) than the model for all genes combined (35%).  
485 Land cover, accessibility, farm-related variables and climate particularly had greater  
486 explanatory power for mobile genes, showing a potentially stronger link to management  
487 practices and dispersal pathways by explaining 41% together (Figure 3b). Mobile ARGs were  
488 less tied to specific farms (33% vs 43% for all genes) and more responsive to environmental  
489 characteristics, indicating greater potential for spread.

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495 **Figure 3. The relative contribution of mouse characteristics, environmental factors and**

496 **random effects to the variation in the occurrence of ARGs in the mouse gut**

497 **microbiome. (a)** Variance partitioning revealed that the farm of origin and the geographical

498 and temporal proximity between the samples (random effects) had a larger impact on the

499 occurrence of all the detected ARGs, accounting for over 60% of the total variation.

500 Environmental factors, including landscape characteristics and climatic factors, accounted for

501 an additional 27%. Mouse characteristics and density explained less than 10% **(b)** When these

502 effects were evaluated only for potentially mobile genes, the environmental factors increased

503 their explanatory contribution to 41% of the ARG occurrence, indicating that mobile genes

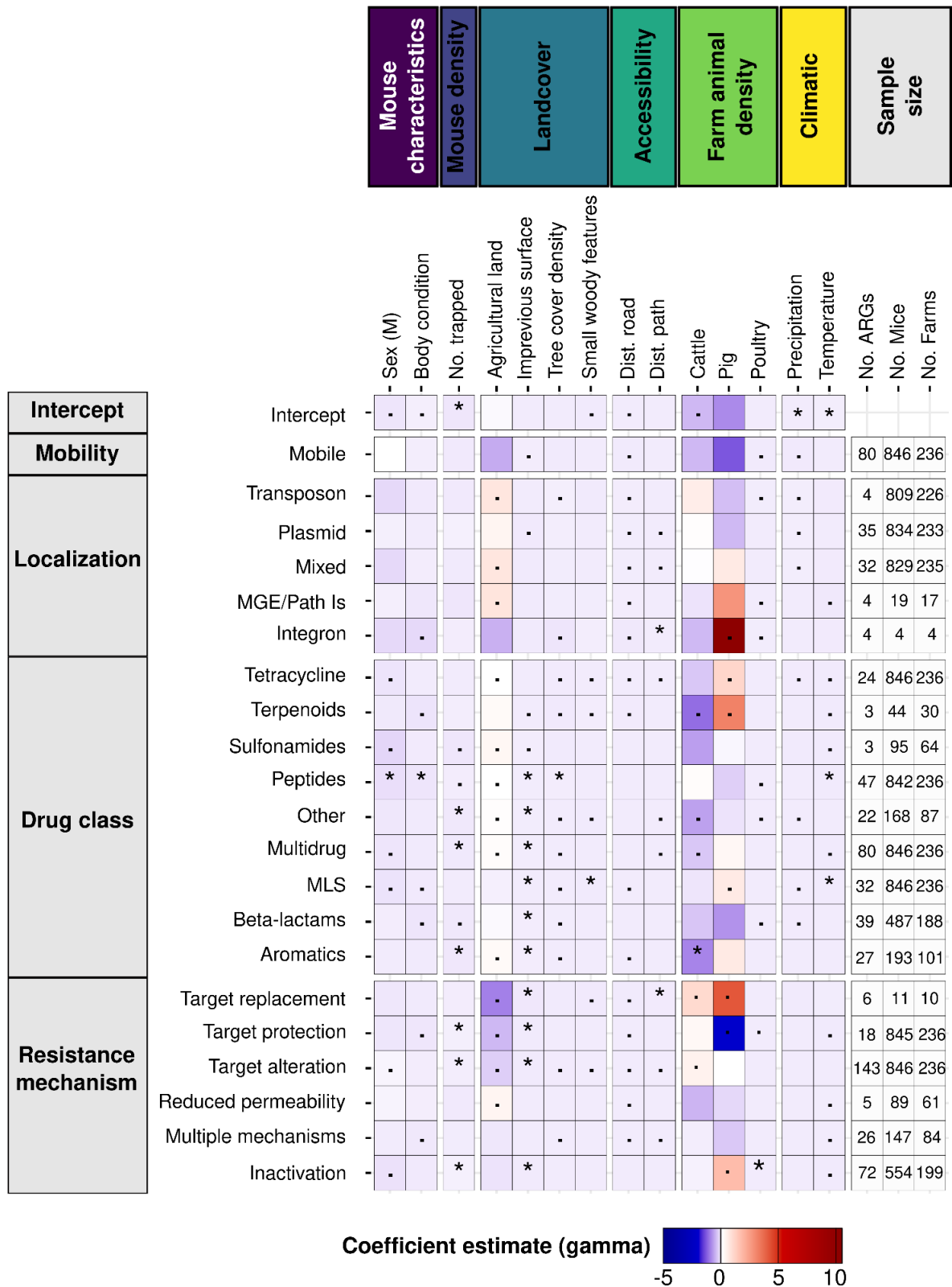
504 were less dependent on specific farm characteristics. **(c-e)** The variance partitioning averaged

505 for each ARG's traits shows the occurrence of genes with potential localization in integrons,

506 genes conferring resistance to sulfonamides and with target replacement mechanisms are  
507 more influenced by environmental factors.

508 Ecological and environmental factors explained a large portion of ARGs trait variance,  
509 especially for those potentially in mobile genetic elements, like plasmids, integrons, and  
510 transposons (Figure 3c). The spatial proximity was the most influential factor in drug class  
511 differences, especially for genes related to resistance to aminoglycosides, tetracyclines, and  
512 MLS (random: spatial autocorrelation > 20% variance explained) and indicated that they are  
513 more tied to farming practices. Genes conferring resistance to sulfonamides or beta-lactams  
514 showed a higher effect of mouse and environmental factors (> 30%) (Figure 3d). Genes  
515 corresponding to target replacement and inactivation resistance mechanisms were more  
516 influenced by environmental factors (> 35%) (Figure 3e).

517 To understand the influence of individual factors on the occurrence of different ARGs, we  
518 compared the gamma coefficient estimates of the jSDMs ( $\gamma$ ) to quantify the effect and  
519 directionality of each mouse-related, environmental or climatic factor on ARGs, averaged  
520 across their different traits (Figure 4). While most of the factors included in our models had a  
521 negative association with the occurrence of ARGs at different agglomeration levels, livestock  
522 density and agricultural land were two factors strongly associated overall, although not in all  
523 cases with support higher than 75%. We observed a positive association between the surface  
524 of agricultural land to genes related to aminoglycoside, MLS, and tetracycline inactivation that  
525 could be localised in mobile genetic elements. The presence of ARGs in integrons, as  
526 compared to chromosomal ARGs, was strongly and positively associated with higher pig  
527 density ( $\gamma > 10$ , support: 0.75). The genes detected in house mice with potential co-localisation  
528 in integrons associated with pig density were *sul1*, *aadA24* and *dfrA5*. These genes are linked  
529 to conferring resistance against sulfonamide, aminoglycosides and diaminopyrimidine  
530 antibiotics, respectively. The presence of ARGs conferring resistance to fluoroquinolone,  
531 phenicol and rifamycin (drug class: aromatics) showed a well supported but low decrease with  
532 the increase in cattle density ( $\gamma < 0$ , support: 0.95). Clinically and veterinary relevant ARGs  
533 conferring resistance to tetracycline were positively associated with high pig density ( $\gamma > 0$ ,  
534 support: 0.75), as well as other genes related to fusidane and pleuromutilin (drug class:  
535 terpenoids;  $\gamma > 0$ , support: 0.75). When we focused on the impact of genes agglomerated by  
536 resistance mechanisms, a high density of pigs and cattle was positively associated with genes  
537 involved in target replacement mechanisms, including *sul* and *dfr* genes, which could also be  
538 mobile ( $\gamma > 0$ , support: 0.75). While pig density was negatively associated with the occurrence  
539 of ARGs with target protection mechanisms ( $\gamma < -5$ , support: 0.75), it was positively associated  
540 with genes involved in antibiotic inactivation ( $\gamma > 5$ , support: 0.75). The latter included 40 genes  
541 for beta-lactamases, such as the AmpC type beta-lactamases *ampC1* and *ampH* (prevalence  
542 <10%), as well as the *CbIA-1* gene, which is highly prevalent in mouse resistomes  
543 (prevalence >50%). In addition to beta-lactamases, the group of genes involved in antibiotic  
544 inactivation influenced by pig density also included the *APH(3')-IIIa* gene, which is potentially  
545 mobile and confers resistance to aminoglycosides (9% prevalence). Particularly, the  
546 association between *CbIA-1* and pig density was better supported when we analysed the effect  
547 on a subset of clinically relevant genes (Supplementary Figure S9).  
548



549

550 **Figure 4. Effect of mouse-related, environmental, farm animals density and climatic**  
 551 **variables (columns) on ARG occurrence agglomerated according to their traits (rows).**

552 The heatmap shows the coefficient estimate (gamma) for the trait and a given explanatory  
 553 variable (jSDM). The higher the coefficient estimate, the higher the association of the variable  
 554 with the ARG trait. The intercept reference level for traits corresponds to ARGs that are non-

555 mobile, located on chromosomes, giving resistance towards the aminoglycoside drug class,  
556 and having an efflux resistance mechanism. Symbols in tile indicate the posterior probability  
557 of the estimate in the model: \* > 0.95 and · > 0.75.

558

### 559 **Effect of environmental characteristics on the ARG compositional structure within** 560 **house mice microbiomes**

561 We employed the jSDM on the ARG abundance after filtering for rare ARGs to determine the  
562 impact of the environmental factors on the compositional structure of ARGs in house mouse  
563 microbiomes. The abundance model included 161 ARGs and similarly to the occurrence  
564 based jSDM, the diagnostic parameters indicated adequate model convergence  
565 (Supplementary Figure S10). The variance in ARG abundance in the mousegut bacterial  
566 microbiome was explained on average almost totally by the random effects (88.1%), and  
567 relatively few by the fixed effects (11.9%), which is a much higher proportion compared to  
568 models predicting ARG occurrence. Similar to the occurrence model, farm spatial proximity  
569 (random effect: spatial autocorrelation) was the dominant source of explained variance. This  
570 means that spatial proximity of samples led to similar ARG abundance profiles, indicating  
571 spatially similar conditions or potential local spread not accounted for in our models. The  
572 landcover variables explained about 4%, followed by farm animal density, climatic variables  
573 and accessibility, each explaining 2%, and mouse-related characteristics each explained 1%.  
574 When focusing only on mobile ARGs, mouse-related, ecological and climatic variables  
575 explained about the same amount of variance in mobile genes (13.1%) compared to all genes  
576 (11.9%) (Supplementary Figure S11). Considering the little variance in ARG abundance  
577 explained these variables, we only detected few supported effects. The temperature showed  
578 a mild negative association with ARG abundance ( $\gamma < 0$ , support: 0.95). As in the occurrence-  
579 based jSDM, farm animal density did not show a well supported relationship with ARGs in  
580 transposons and plasmids ( $\gamma > 0$ , support < 0.75) (Supplementary Figure S12).

581

### 582 **Association of house mouse resistomes with livestock resistomes**

583 Given the association of the occurrence of specific ARGs and gene traits within the house  
584 mouse resistome with the density of farm animals, we assessed the similarity between  
585 resistomes of livestock to those of house mice in our study. We compared the resistomes of  
586 house mice to two previously published datasets: one including manure resistomes from pigs,  
587 cattle and chickens in nine European countries, and the other including resistomes of rural  
588 and urban pigs and poultry (chickens and turkeys) from Ghana, as a non-european reference.  
589 We detected 2,834 ARGs in total considering 269 pig, 238 chicken, 29 cattle and 17 turkey  
590 manure metagenomic samples in addition to our 859 house mousegut metagenomes. Overall,  
591 we observed that house mouse resistomes were less rich in ARGs than livestock resistomes  
592 (Wilcoxon  $p$ -value adjusted <0.01, effect size  $r > 0.2$ , Supplementary Table S13). To quantify  
593 the relative effect of these differences, we fitted a linear model with ARG richness as response.  
594 Estimated marginal means confirmed the differences between mice to the other host's  
595 resistomes. However, the difference between the resistomes of mice and pig manure was  
596 smaller than the one observed for the other hosts (Contrast<sub>pig-mouse</sub>: 173, SE [6.06], Figure 5a).

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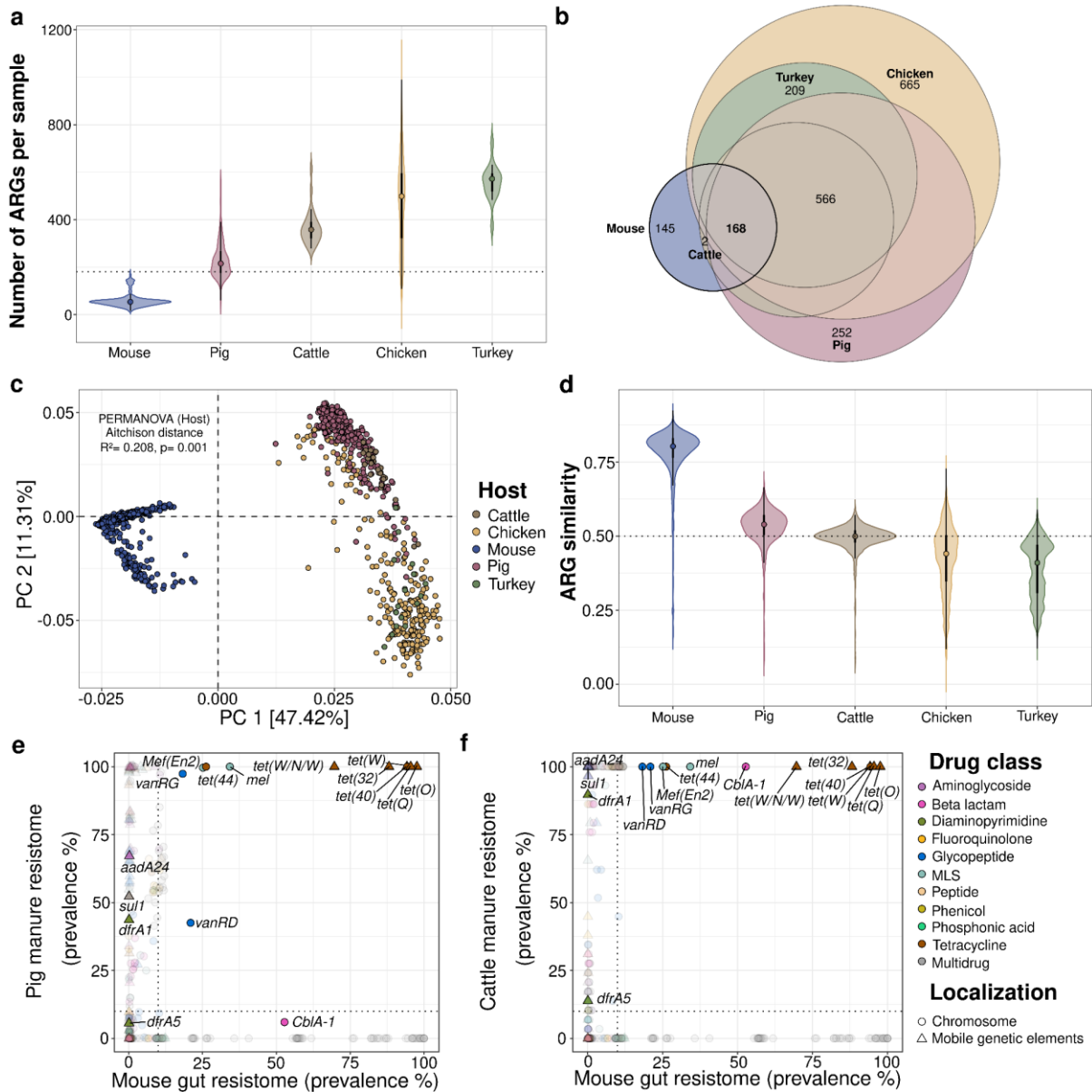
598 To determine the potential connectivity between house mouse resistomes and livestock  
599 manure, we calculate the number of ARGs shared between mice and each livestock host, as  
600 well as the composition similarity. Considering the total number of different ARGs detected  
601 (N= 2,834) in the distinct hosts, the resistomes of mice shared a small fraction of genes (n=  
602 168) with those of livestock manure and only had a significant overlap with cattle's manure

603 resistome (Fisher's exact test,  $n = 174$  shared genes,  $p$ -value adjusted  $< 0.01$ , Figure 5b). The  
604 house mouse resistome showed a distinctive ARG composition compared to the resistomes  
605 of livestock manure (PERMANOVA,  $R^2 = 0.21$ ,  $p$ -value  $< 0.01$ , Figure 5c). Resistome similarity  
606 was higher between resistomes of mice and pig and cattle manure than between resistomes  
607 from poultry hosts (contrast  $_{\text{pig-mouse}}$ :  $-0.264$ , SE  $[0.0098]$ , contrast  $_{\text{cattle-mouse}}$ :  $-0.310$ , SE  $[0.013]$ ,  
608 Figure 5d).

609  
610 We compared the gene prevalence from both pigs and cattle against the prevalence in house  
611 mice resistomes to determine genes that could be promoted by a specific host or in both host's  
612 resistomes. Those genes present in  $\geq 10\%$  of both mouse and livestock host metagenomes  
613 were classified as co-promoted, while genes with prevalence  $\geq 10\%$  in only one of the hosts  
614 were classified as host-specific-promoted. When mouse and pig manure prevalences were  
615 compared, we observed that 11 genes were co-promoted, particularly tetracycline resistance  
616 genes (*Tet*) associated with mobile genetic elements. In contrast, three genes encoded in  
617 integrons (*aadA24*, *sul1* and *dfra1*) were promoted in pig manure, but still detected in mice  
618 resistomes (Figure 5e). The gene *CblA-1* encoding a non-mobile beta-lactamase has higher  
619 prevalence in mice gut than in pig manure indicative of house mouse promotion. A similar  
620 picture arose from the comparison between mouse and cattle manure, with *tet* and integron  
621 associated genes being co-promoted, but with the difference that *CblA-1* is also co-promoted  
622 in both cattle manure and mice, suggesting the circulation of such genes between manure and  
623 house mice (Figure 5f).

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**Figure 5. Similarities between the house mouse resistome and livestock manure ARG reservoirs. (a)** ARG richness in house mice compared to livestock manure. The number of ARGs detected in house mice was significantly lower than in any other host's manure resistome (Wilcoxon,  $p$ -value adjusted  $<0.001$ ). Poultry manure had the highest ARG richness. Pig manure's resistomes had a smaller difference to mouse resistome richness than for the other hosts (Contrast  $_{\text{pig-mouse}}$ : 173, SE [6.06]). The dashed black line defines the maximum number of ARGs detected in house mice. Each point represents the median per host. Violin represents the distribution of richness per host's resistome. Solid boxes inside the violin indicate the interquartile range and whiskers extend to minimum and maximum values per host. **(b)** Euler diagram of shared ARGs between house mouse resistomes and different livestock manure resistomes; numbers next to the host labels indicate the number of unique genes for that particular host. A total of 168 genes were shared between the mouse resistome and the other hosts. The livestock resistomes shared 566 ARGs among them. **(c)** Principal component analysis (PCA) showing the dissimilarity in resistome composition among hosts. Dissimilarity is based on Aitchison distances between samples on centered log ratio transformed ARG abundance. Each dot represents an individual resistome. **(d)** Comparison

643 of ARG composition similarity between mouse resistomes and each of the other host's  
644 resistomes. ARG similarity was calculated as 1 - Aitchison distance between two samples.  
645 Similarity values of 1 represent identical ARG profiles, while those equal to zero indicate  
646 completely different resistomes. The similarity values between each mouse resistome and  
647 each host are presented. Pig and cattle manure's resistomes had a higher compositional  
648 similarity to mouse resistomes than to the poultry hosts (contrast<sub>pig-mouse</sub>: -0.264, SE [0.0098],  
649 contrast<sub>cattle-mouse</sub>: -0.310, SE [0.013]). The dashed black line indicates a similarity value of 0.5.  
650 Points represent the median resistome similarity between mouse and a given host. Violin  
651 represents the distribution of similarity to mice resistomes per host. Solid boxes inside the  
652 violin indicate the interquartile range and whiskers extend to minimum and maximum similarity  
653 values. **(e)** Prevalence of ARGs in the house mouse gut and pig manure metagenomes. While  
654 the gene *CblA-1* had higher prevalence in mouse guts than in pig manure indicative of house  
655 mouse promotion, three genes that can be localised in integrons (*aadA24*, *sul1* and *dfrA1*)  
656 were more prevalent in pig manure than in mice, indicating a promotion of those genes in pig  
657 manure. **(f)** Prevalence of ARGs in the house mouse gut and cattle manure metagenomes.  
658 The gene *CblA-1* prevalent in more than 50% of the mousegut metagenomes could be  
659 detected in all the cattle manure resistomes, similar to those genes that can be localised in  
660 integrons. For both environments, six *Tet* genes had a prevalence above 75% indicating co-  
661 promotion in such environments. In panels e and f, labels are shown for ARGs with prevalence  
662 higher than 25% in either of the hosts and those genes that could be localised in integrons.  
663 The shape indicates whether the gene can be localised in the chromosome (circles) or in  
664 mobile genetic elements (triangles) and thus suggest mobility potential. Dashed lines indicate  
665 a cutoff at 10% prevalence to highlight genes associated with one of the hosts or in both.

666

## 667 Discussion

668

669 Our study showed that the occurrence of antimicrobial resistance genes in the microbiomes  
670 of natural populations of generalist species, such as house mice, is strongly influenced by land  
671 use characteristics. Here, we provided a novel framework to assess how environmental  
672 gradients influence ARG composition and the potential interactions that facilitate gene mobility  
673 among microbial communities, by conceptualizing the ARGs as metacommunities within host-  
674 associated microbiomes sampled across transects within heterogeneous landscapes. We  
675 demonstrated that environmental variables, particularly livestock farming intensity, explained  
676 a substantial proportion of the variation in ARG occurrence, especially among genes carried  
677 by mobile genetic elements. Notably, high pig farming density was closely associated with  
678 integron-encoded sulfonamide resistance genes, as well as with genes conferring resistance  
679 to tetracyclines and beta-lactams. These findings demonstrate that specific ARGs in the  
680 resistomes of wild house mice are also found in livestock manure, highlighting the exchange  
681 of resistance genes between agricultural and natural ecosystems.

682

683 Our metagenomic surveillance revealed that the resistomes of house mice are primarily  
684 composed of genes of limited clinical or veterinary concern, largely involved in multidrug  
685 resistance mechanisms that are ubiquitous in bacterial genomes of both host-associated and  
686 environmental origin<sup>56,57</sup>. This pattern is consistent with our previous 16S-based predictions of  
687 chromosomally encoded ARGs in house mice<sup>58</sup> specifically, and with wild rodent resistomes  
688 in a broader sense<sup>59</sup>. However, we were able to detect genes potentially co-localised with  
689 mobile genetic elements. A previous study in wild house mice from urban environments  
690 reported a wide distribution of ARGs conferring resistance to macrolide–lincosamide–

691 streptogramin (MLS) antibiotics, fluoroquinolones, tetracyclines, and beta-lactams<sup>37</sup>. While our  
692 house mouse resistomes included some of these previously reported genes and others of high  
693 priority<sup>50</sup>, they were detected here at low abundance and prevalence compared to other  
694 environments like the gut and livestock microbiome or wastewater systems. One explanation  
695 for the low prevalence could be the reduced abundance or absence of their typical bacterial  
696 hosts. Families of commensal gut bacteria such as *Enterobacteriaceae*, *Enterococcaceae*,  
697 and *Staphylococcaceae*, which are usually involved in mediating ARG transmission within  
698 host-associated microbiomes<sup>22,60,61</sup>, were poorly represented in our house mouse samples. In  
699 other wild rodent species, culture-based analyses have revealed elevated levels of resistance  
700 among *Enterobacteriaceae*<sup>35</sup>. In contrast, wild cotton mice (*Peromyscus gossypinus*)  
701 inhabiting polluted environments with microbiomes dominated by *Desulfovibrionaceae*,  
702 *Pseudomonadaceae*, and *Helicobacteraceae* showed high abundance and prevalence of  
703 multidrug resistance genes, with only a few genes associated with last resort antibiotics<sup>62</sup>.  
704 Conversely, wild rodents from rural areas in Germany appeared to play only a minor role in  
705 the transmission of resistant *Escherichia coli* to the environment<sup>63</sup>, consistent with our broader  
706 characterization of the house mouse resistome. Together, these observations suggest that  
707 the resistomes of wild house mice, similar to those of other rodents, are dominated by  
708 environmentally ubiquitous ARGs. The house mouse ARG composition was largely shaped  
709 by the bacterial taxa present in their microbiomes, and both bacterial and ARG composition  
710 could reflect the degree of anthropogenic exposure and potential for gene exchange across  
711 ecological boundaries.

712  
713 Beyond microbial interactions within the host that may drive the selection of specific ARGs,  
714 we focused on identifying ecological drivers of the house mouse resistome. Given that both  
715 biotic and abiotic factors shape microbial communities through natural selection, and selective  
716 pressures may vary from habitat to habitat<sup>64</sup>, we hypothesized that rodents' gut microbial  
717 communities and associated genetic repertoires are shaped by exposure to diverse  
718 environmental conditions. We observed that spatial localization of each farm exerted a strong  
719 influence on the overall ARG composition. Ecological and environmental variables also  
720 explained a substantial proportion of the variability in ARG occurrence, exceeding the effects  
721 of host density and sex, consistent with previous observations in the human gut resistome<sup>19</sup>.  
722 These patterns likely reflect anthropogenic factors that promote antibiotic resistance in  
723 bacteria within the mouse gut. Environmental and climatic factors were influential for genes  
724 potentially co-localised on mobile genetic elements and therefore more likely to be transferred  
725 through horizontal gene transfer. This suggests that mobile genes may be promoted by  
726 changes in agricultural intensity or land management practices unlike chromosomal, non-  
727 mobile ARGs, which are less susceptible to external environmental pressures. In external  
728 environments such as soil and water, conventional agricultural management practices,  
729 including the use of manure as fertiliser, herbicides or compounds containing heavy metals,  
730 increase the prevalence of ARGs and mobility between bacteria by horizontal gene transfer<sup>65–</sup>  
731 <sup>68</sup>. We found that extensive agricultural land use was positively associated with potentially  
732 mobile genes, suggesting that those genes may be selected either through the direct impact  
733 of stress-inducing xenobiotics that reach wildlife via water and soil, or present in their  
734 microbiome through the invasion of bacteria carrying ARGs selected in the agricultural  
735 environment, as previously observed in humans<sup>69,70</sup>. Moreover, It has been shown that cross-  
736 species ARG transmission between livestock and wild mammals is mediated by their co-  
737 occurrence with mobile genetic elements<sup>71</sup>. The dissemination of ARGs between different  
738 environments, and between host-associated and environmental microbiomes increases with

739 the intensity of anthropogenic activities<sup>72</sup>. Thus, the influence of anthropogenic agricultural  
740 and farming practices are persistent on the house mouse resistome.

741

742 The use of antibiotics for food production has long been recognized as a strong contributor to  
743 antimicrobial resistance in the human microbiome, independently of clinical antibiotic  
744 consumption<sup>73–75</sup>. By modelling the effect of agricultural surface and livestock density on the  
745 mouse resistome, we aimed to test whether the effect of antibiotic use for food production can  
746 be quantifiable not only in humans but also in other species inhabiting human-impacted  
747 environments. Similar to agricultural practices, livestock density, particularly of pigs, was  
748 strongly associated with potentially mobile ARGs within house mouse microbes. The  
749 association between livestock density and mouse resistome can reflect two interconnected  
750 processes: dissemination of mobile ARGs through fecal pollution and epidemiological  
751 connectivity through host movement and interactions<sup>76</sup>. As livestock resistomes are  
752 considered “hotspots” of resistance genes, the dissemination of ARGs through manure occurs  
753 at a larger extent than from human, sewage and soil sources<sup>13,77</sup>. Pig manure is highly  
754 enriched in mobile ARGs and represents a relevant source for spreading ARGs to the  
755 environment<sup>78–81</sup>. The movement of mice between habitats and social interactions likely  
756 increase their exposure to sites contaminated with livestock faeces or with other rodents  
757 carrying ARGs<sup>34,82</sup>. Consistent with our results, other studies detected that wild rodents, and  
758 even larger species such as wild boar (*Sus scrofa*) from rural areas with high livestock density  
759 in Germany, carry *E. coli* resistant to antibiotics commonly administered in pigs, poultry and  
760 cattle suggesting livestock to wildlife transmission<sup>39,63</sup>. Overall, interactions between  
761 agricultural management practices, livestock density and mouse mobility may have significant  
762 impacts on the composition and mobility of resistomes in wild house mouse populations.

763

764 We observed a strong association of pig density to highly prevalent genes conferring  
765 resistance to tetracycline (*tet*), which could be traced in both the house mouse resistome and  
766 in pig and cattle resistomes from Germany and other European countries. Tetracyclines have  
767 been historically used in livestock husbandry in large-scale intensive farming to prevent  
768 diseases and promote animal growth<sup>83,84</sup>. In Germany, tetracyclines remain among the most  
769 widely administered antibiotics, together with beta-lactams<sup>85,86</sup>. Despite its regulation intended  
770 to minimise selection of resistant bacteria<sup>87</sup>, residues of tetracyclines and their bacterial  
771 transformation products persist in livestock manure and fertilisers at concentrations that still  
772 represent an ecological risk and have antibacterial activity<sup>88,89</sup>. Thus, *tet* genes are persistent  
773 and highly abundant in environments with fecal or manure pollution. These genes are  
774 considered part of the cross-environment core resistome and useful for the monitoring of the  
775 total resistome diversity in the environment<sup>15,90,91</sup>. In our study, we detected six *tet* genes that  
776 may reflect the impact of livestock in the house mouse resistome, as they were enriched in  
777 both environments. The transmission of these genes from livestock to wildlife likely results  
778 from their co-localisation on mobile genetic elements, which facilitates efficient spread through  
779 horizontal gene transfer across bacteria<sup>92,93</sup>. Tetracycline ARGs are known to be promiscuous  
780 and their capacity to transfer between phylogenetically distant bacterial hosts<sup>14</sup>. Our results  
781 indicate that house mouse resistomes are strongly impacted by livestock density, and that  
782 mobile *tet* genes represent good markers for tracking ARG transmission between farm  
783 environments and wildlife species.

784

785 Genes encoding for commensal beta-lactamases, like *CblA-1* and *su11* were additional  
786 markers of anthropogenic and livestock influence identified in the house mouse resistome.

787 The *sul1* gene, although not specifically promoted in house mouse resistomes, provides  
788 interesting insights into potential ways of resistance transmission. *sul1* is a mobile and highly  
789 prevalent gene in different environments, particularly human-impacted surface waters and  
790 wastewater commonly used for agricultural irrigation<sup>94,95</sup>. In riverine environments, animal  
791 feeding operations have been identified as a relevant source of *sul1*<sup>96</sup>, highlighting how this  
792 gene may serve as a marker of connections between farming activities, agricultural practices,  
793 and the dissemination to broader environments, including wildlife hosts such as house mice.  
794 We also detected that the *CblA-1* gene was highly promoted in house mouse resistomes.  
795 Although *CblA-1* is not a mobile gene and is restricted to the genus *Bacteroides*<sup>97,98</sup>, this *CblA-*  
796 *1* cephalosporinase is highly prevalent in the human gut microbiomes from Western countries  
797 and may exacerbate antimicrobial resistance infections if transferred to pathogens<sup>99</sup>. The  
798 presence of this gene likely reflects human or cattle fecal pollution, further supporting its role  
799 as an indicator of anthropogenic impact in wildlife resistomes. Taken together, genes as *sul1*  
800 and *CblA-1* can be complementary indicators of livestock-derived and anthropogenic pollution  
801 within the house mouse resistome.

802

803 Our findings reveal that antimicrobial resistance in wildlife is not an isolated phenomenon but  
804 a measurable reflection of anthropogenic activity across agricultural landscapes. We  
805 integrated metagenomic screening and ecological data to demonstrate the impact of the  
806 external environment on the resistome of natural populations of house mice, allowing the  
807 identification of specific genes that trace this impact. The transmission dynamics between  
808 livestock, environment and other mammal hosts, such as house mice, can be explained by  
809 the influx of selective agents and bacteria carrying ARGs from the farming environment into  
810 surrounding habitats, where ARGs could be maintained within wildlife microbiomes. Unlike  
811 other environments such as wastewater, polluted soil and the gut of humans and livestock,  
812 the house mouse resistome is characterised by several regulators that do not themselves  
813 confer a resistant phenotype and fewer of the high-priority genes identified by the WHO. In  
814 this respect, mice are not the most relevant carriers and are definitely not the most practical  
815 samples for surveillance purposes. However, our analysis clearly shows that their resistomes  
816 reflect anthropogenic impact, particularly with regard to genes carried by mobile genetic  
817 elements. Therefore, we identified mobility as an important trait for assessing how farm  
818 animals and agricultural practices influence the resistance profiles of natural house mice  
819 populations in surrounding environments. This study broadens our understanding of the  
820 transmission of antimicrobial resistance in human-impacted environments and supports the  
821 development of strategies to monitor the mobility and dissemination of ARGs and resistant  
822 bacteria into the environment and wildlife species.

823

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836

### 837 **Author contribution**

838 **MG:** methodology, investigation, formal analysis, writing - review editing; **AP:** methodology,  
839 investigation, data curation, formal analysis, writing - review editing; **EH:** conceptualisation,  
840 funding, supervision, resources, writing - review editing; **SKFS:** conceptualisation, funding,  
841 resources, writing - review editing; **SKS:** conceptualisation, funding, supervision, project  
842 administration, writing - review editing (equal); **SCMF:** methodology, investigation, writing -  
843 review editing; **VHJD:** conceptualisation, methodology, investigation, data curation, formal  
844 analysis, writing - review editing, funding, supervision

845

### 846 **Data availability**

847 All raw sequence data are deposited and available online at the European Nucleotide Archive  
848 with an accession number PRJEB102564. Additional data for the analysis and the code used  
849 for visualization and statistical analysis are available at  
850 [https://github.com/mgicquel/mus\\_musculus\\_de\\_amr](https://github.com/mgicquel/mus_musculus_de_amr) (non-static version under development)  
851 and will be archived at the EcoDyn github account and Zenodo upon publication.

852

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