



**HAL**  
open science

**Gut microbiome diversity and composition in individuals with and without extended-spectrum  $\beta$ -lactamase-Producing enterobacterales carriage: a matched case-control study in infectious diseases department**

Anders Boyd, Mariam El Dani, Roula Ajrouche, Vanessa Demontant, Justine Cheval, Karine Lacombe, Guillaume Cosson, Christophe Rodriguez, Jean-Michel Pawlotsky, Paul-Louis Woerther, et al.

► **To cite this version:**

Anders Boyd, Mariam El Dani, Roula Ajrouche, Vanessa Demontant, Justine Cheval, et al.. Gut microbiome diversity and composition in individuals with and without extended-spectrum  $\beta$ -lactamase-Producing enterobacterales carriage: a matched case-control study in infectious diseases department. *Clinical Microbiology and Infection*, In press, 10.1016/j.cmi.2024.03.016 . hal-04539691

**HAL Id: hal-04539691**

**<https://hal.sorbonne-universite.fr/hal-04539691>**

Submitted on 5 Jul 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Gut Microbiome Diversity and Composition in Individuals With and Without Extended-**  
2 **Spectrum  $\beta$ -Lactamase-Producing *Enterobacterales* Carriage: a Matched Case-Control Study in**  
3 **infectious diseases department**

4  
5 Anders Boyd\*<sup>1,2,3</sup>, Mariam El Dani\*<sup>1,4</sup>, Roula Ajrouche<sup>4,5</sup>, Vanessa Demontant<sup>6</sup>, Justine Cheval<sup>6</sup>,  
6 Karine Lacombe<sup>1,7</sup>, Guillaume Cosson<sup>7</sup>, Christophe Rodriguez<sup>8,9</sup>, Jean-Michel Pawlotsky<sup>8,9</sup>, Paul-  
7 Louis Woerther<sup>6,8,10</sup>, Laure Surgers<sup>1,7</sup>

8 \*These authors contributed equally.

9  
10 **Institutional affiliations**

11 <sup>1</sup> Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie et de Santé Publique, Paris,  
12 France.

13 <sup>2</sup> Stichting HIV Monitoring, Amsterdam, the Netherlands.

14 <sup>3</sup> Public Health Service of Amsterdam, Infectious Diseases, Amsterdam, the Netherlands.

15 <sup>4</sup> Clinical and Epidemiological Research Laboratory, Faculty of Pharmacy, Lebanese University,  
16 Hadat, Lebanon.

17 <sup>5</sup> Institut National de Santé Publique, d'Épidémiologie Clinique et de Toxicologie-Liban (INSPECT-  
18 LB), Beirut, Lebanon.

19 <sup>6</sup> NGS Platform, Henri Mondor Hospital, AP-HP, and IMRB Institute, University of Paris-Est-Créteil,  
20 Créteil, France.

21 <sup>7</sup> GHU APHP. Sorbonne Université, Service des Maladies Infectieuses et Tropicales, Hôpital Saint-  
22 Antoine, Paris, France.

23 <sup>8</sup> Département de Microbiologie, Hôpitaux Universitaires Henri Mondor, Assistance Publique  
24 Hôpitaux de Paris (AP-HP), Université Paris-Est-Créteil, Créteil, France.

25 <sup>9</sup> INSERM U955, Team «Viruses, Hepatology, Cancer», Créteil, France.

26 <sup>10</sup> Université Paris-Est-Créteil (UPEC), EA 7380 Dynamic, Ecole nationale vétérinaire d'Alfort, USC  
27 Anses, Créteil, France.

28

29 **Corresponding Author**

30 • **Full name:** Laure Surgers

31 • **Primary Affiliation :** Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie  
32 et de Santé Publique, Paris, France, Service des maladies infectieuses et tropicales, AP-HP,  
33 GHU Sorbonne Université

34 **Email address:** laure.surgers@aphp.fr

35 **Authors**

36 • **Full name:** Anders Boyd

37 **Primary Affiliation :** Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie  
38 et de Santé Publique, Paris, France

39 **Email address:** a.c.boyd@amsterdamumc.nl

40 • **Full name:** Roula Ajrouche

41 **Primary Affiliation:** Clinical and Epidemiological Research Laboratory, Faculty of  
42 Pharmacy, Lebanese University, Hadat, Lebanon

43 **Email address:** roula.ajrouche@ul.edu.lb

44 • **Full name:** Vanessa Demontant

45 **Primary Affiliation:** NGS Platform, Henri Mondor Hospital, AP-HP, and IMRB Institute,  
46 University of Paris-Est-Créteil, Créteil, France.

47 **Email address:** vanessa.demontant@aphp.fr

48 • **Full name:** Justine Cheval

49 **Primary Affiliation:** NGS Platform, Henri Mondor Hospital, AP-HP, and IMRB Institute,  
50 University of Paris-Est-Créteil, Créteil, France

51 **Email address:** justine.cheval@aphp.fr

52 • **Full name:** Karine Lacombe

53 **Primary Affiliation:** Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie et  
54 de Santé Publique, Paris, France

55 **Email address:** karine.lacombe2@aphp.fr

- 56 • **Full name:** Guillaume Cosson
- 57 **Primary Affiliation:** GHU APHP. Sorbonne Université, Service des Maladies Infectieuses et
- 58 Tropicales, Hôpital Saint-Antoine, Paris, France.
- 59 **Email address:** guillaume.cosson@aphp.fr
- 60 • **Full name:** Christophe Rodriguez
- 61 **Primary Affiliation:** Département de Microbiologie, Hôpitaux Universitaires Henri Mondor,
- 62 Assistance Publique Hôpitaux de Paris (AP-HP), Université Paris-Est-Créteil, Créteil, France
- 63 **Email address:** christophe.rodriguez@aphp.fr
- 64 • **Full name:** Jean-Michel Pawlotsky
- 65 **Primary Affiliation:** Département de Microbiologie, Hôpitaux Universitaires Henri Mondor,
- 66 Assistance Publique Hôpitaux de Paris (AP-HP), Université Paris-Est-Créteil, Créteil, France
- 67 **Email address:** jean-michel.pawlotsky@aphp.fr
- 68 • **Full name:** Paul-Louis Woerther
- 69 **Primary Affiliation:** Département de Microbiologie, Hôpitaux Universitaires Henri Mondor,
- 70 Assistance Publique Hôpitaux de Paris (AP-HP), Université Paris-Est-Créteil, Créteil, France
- 71 **Email address:** paul-louis.woerther@aphp.fr
- 72 • **Full name:** Laure Surgers
- 73 **Primary Affiliation:** Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie et
- 74 de Santé Publique, Paris, France
- 75 **Email address:** laure.surgers@aphp.fr

76 **ABSTRACT**

77

78 **Background:** Little is known on the effect of gut microbial and Extended-Spectrum  $\beta$ -Lactamase-  
79 Producing *Enterobacterales* (ESBL-E) carriage, particularly in the general population. The aim of this  
80 study was to identify microbiota signatures uniquely correlated with ESBL-E carriage.

81 **Methods:** We conducted a case-control study among individuals seeking care at the Sexual Health  
82 Clinic or Department of Infectious and Tropical Diseases at Saint-Antoine Hospital, Paris, France.  
83 Using coarsened exact matching, 176 participants with ESBL-carriage (i.e., cases) were matched 1:1  
84 to those without ESBL-carriage (i.e., controls) based on sexual group, ESBL-E prevalence of countries  
85 traveled in <12 months, number of sexual partners in <6 months, geographic origin, and any antibiotic  
86 use in <6 months. 16S rRNA gene amplicon sequencing was used to generate differential abundances  
87 at the genus level and measures of  $\alpha$ - and  $\beta$ -diversity.

88 **Results:** Participants were mostly men (83.2%,  $n=293/352$ ) and had a median age of 33 years  
89 (IQR=27-44). Nine genera were found associated with ESBL-E carriage (Figure 2C): *Proteus*  
90 ( $p<0.0001$ ), *Carnobacterium* ( $p<0.0001$ ), *Enterorhabdus* ( $p=0.0079$ ), *Catonella* ( $p=0.017$ ),  
91 *Dermacoccus* ( $p=0.017$ ), *Escherichia/Shigella* ( $p=0.021$ ), *Kocuria* ( $p=0.023$ ), *Bacillus* ( $p=0.040$ ), and  
92 *Filifactor* ( $p=0.043$ ); however, differences were no longer significant after Benjamini-Hochberg  
93 correction ( $q>0.05$ ). There were no differences between those with versus without ESBL-E carriage in  
94 measures of  $\alpha$ -diversity (Shannon Diversity Index,  $p=0.49$ ; Simpson Diversity Index,  $p=0.54$ ; and  
95 Chao1 Richness Estimator,  $p=0.16$ ) or  $\beta$ -diversity (Bray-Curtis dissimilarity index,  $p=0.42$ ).

96 **Conclusion:** In this large carefully controlled study, there is lacking evidence that gut microbial  
97 composition and diversity is any different between individuals with and without ESBL-E carriage.

98

99 **Keywords:** ESBL- producing *Escherichia coli*, gut microbiome, colonization resistance, 16S  
100 sequencing, antibiotic resistance

101 **BACKGROUND**

102

103 The intestinal microbiota is a complex community of microorganisms, which is believed to play a  
104 broad range of physiological functions essential for human health. Among the most explored  
105 functions, colonization resistance (CR) represents the capacity of the intestinal microbiota to prevent  
106 colonization by exogenous aerotolerant or aerobic bacteria, including *Enterobacteriales* [1]. While  
107 some of these organisms encompass beneficial commensal microbiota [2] others have a pathogenic  
108 potential [3]. Extended-Spectrum  $\beta$ -Lactamase-Producing *Enterobacteracerales* (ESBL-E),  
109 particularly *E. coli*, are considered by the World Health Organization among the 12 types of bacteria  
110 that pose the greatest threat to human health [4]. In this context, there is an increasing interest in  
111 understanding the role of the gut microbiome in ESBL-E carriage.

112

113 CR is the consequence of complex interactions that involve hundreds of species composing the  
114 intestinal microbiota [5]. CR results from many mechanisms that include competition for carbon and  
115 energy sources, antagonism mediated by small molecules, such as antimicrobial peptides between  
116 normal microbiota and competing species, inflammation of the microenvironment, with possible  
117 effects from yeasts, viruses or Archaea. As a consequence, any factor that could affect the species  
118 equilibrium could result in dysbiosis, and therefore influence the microbiota's function in CR [6,7,8].  
119 The most common factor responsible for this dysbiosis is exposure to antibiotics [9,10,11,12].

120

121 Studies aiming to link intestinal composition with the risk of ESBL-E colonization in the community  
122 setting are scarce [13,14,15,16,17] and have yielded heterogeneous results. In a previous cross-  
123 sectional study conducted at a sexually transmitted infection (STI) and human immunodeficiency virus  
124 (HIV) clinic in Paris, France, it was observed that 10.5% of 2157 participants carried ESBL-E [18].  
125 The prevalence of ESBL-E carriage in this study was notably higher as the number of sexual partners  
126 increased, suggesting that sexually active individuals are an important key population for harboring  
127 ESBL-E. Nevertheless, reasons other than sexual contact could explain the higher prevalence of  
128 ESBL-E carriage in this population and given the previous evidence, gut microbiota diversity and

129 composition could be a major driver of carriage. To this end, we aimed to identify microbiota  
130 signatures that may be associated with the presence of ESBL-E.

131

132

## 133 **METHODS**

134

### 135 **Study design and participants**

136

137 Participants were selected from the *Bactéries MultiRésistantes-Infections sexuellement transmissibles*  
138 (BMR-IST) study. The study design and procedures were described previously [18]. In brief, we  
139 included individuals who were  $\geq 18$  years old, were HIV negative (seeking care at the Sexual Health  
140 Clinic) or HIV positive (seeking care at the Department of Infectious and Tropical Diseases), and  
141 signed informed consent. This study was approved by the *Comité de protection des personnes Ouest II*  
142 ethics committee (2017-73), in accordance with the Helsinki Declaration.

143

144 After obtaining written informed consent, we asked participants to fill out a questionnaire on  
145 demographic characteristics, ESBL-E risk factors, and sexual behavior with the assistance of a study  
146 investigator. The choice of STI tests was at the physician's discretion. Participants provided a self-  
147 swab (Copan ESwab), which were later processed at the Microbiology Department of Saint-Antoine  
148 Hospital. We gave participants a self-swab (Copan ESwab<sup>TM</sup>) and asked them to collect a sample in  
149 the lavatory of the clinic by inserting the swab into the rectal canal. Only swabs with visible fecal  
150 material were included in bacteriological analysis.

151 Heart-brain broths supplemented with 10  $\mu\text{g/mL}$  cefotaxime were inoculated with rectal swabs,  
152 incubated for 18 hours at 37°C and plated on ESBL bioMérieux media, as previously described [18].  
153 Grown colonies were identified (MALDI-TOF, Bruker, Germany). In the BMR-IST study, to ensure  
154 that *Enterobacteracerales* exhibiting antibiotic resistance via indirect pathways (e.g., overproduction  
155 of AmpC) were not selected, all *Enterobacteracerales* isolates were subjected to a standard  
156 antibiogram using Mueller-Hinton agar (Biorad), according to recommendations from the European

157 Committee on Antimicrobial Susceptibility Testing (<https://www.eucast.org/>). Then, briefly, whole  
158 genomes were sequenced on these isolates using Illumina NextSeq and the NextEra XT sequence  
159 library (Illumina, San Diego, CA), and were then used to perform ESBL-E Multi-Locus Sequence  
160 Typing (MLST) [18].

161  
162 In this case-control study, we matched the individuals with ESBL-E carriage (i.e., cases) 1:1 to  
163 individuals without ESBL-E carriage (i.e., controls) using coarsened exact matching (details provided  
164 in the Supplementary Methods). The following matching criteria were used (Supplementary Methods):  
165 sexual group, ESBL-E prevalence of countries traveled in the previous 12 months, number of sexual  
166 partners in the previous 6 months [ $\log(n+1)$ -transformed], geographic origin, and any antibiotic use in  
167 the previous 6 months. These characteristics were selected *a priori* for their putative confounding role  
168 in the association between microbiota and ESBL-E carriage. This procedure was carried out using the  
169 ‘cem’ ado command in Stata.

170

## 171 **16S rRNA gene amplicon sequencing**

172

### 173 ***Extraction and controls***

174 Only swabs with visible fecal material were included in the bacteriological analysis. Stool swabs  
175 (Copan ESwab) obtained by rectal self-swabbing were placed in transport medium and vortexed for 15  
176 seconds. DNA-RNA extraction was performed using an unbiased procedure before performing  
177 targeted sequencing [19]. Briefly, pre-extraction combining bead beating, chemical cell disruption  
178 with detergent, and proteinase K lysis was followed by extraction using the DSP virus-pathogen kit on  
179 QiaSymphony (Qiagen, Hilden, Germany). In each run, a negative control and a positive control  
180 represented by a microbial community standard (Zymobionics®, Zymo Research, Irvine, CA, USA)  
181 were tested to evaluate the performance of the sequencing techniques for the detection of bacteria.

182

### 183 ***16S rRNA gene amplification and sequencing***



184 16S rRNA gene amplicon sequencing included the study of four amplicon libraries domain V3-V4  
185 (16S-V3V4) [20] of the bacterial 16S rRNA gene. As described previously, each amplicon was  
186 prepared from 5  $\mu$ L extract following the “16S Sequencing Library Preparation Protocol” provided by  
187 the manufacturer (Illumina, San Diego, CA, USA) [21]. For each library, the quality was evaluated  
188 using a D1000 ScreenTape on a TapeStation (Agilent, Santa Clara, CA, USA) and the quantity using  
189 the Quant-it dsDNA Assay kit (ThermoFischer, Waltham, MA, USA) on a Varioskan LUX  
190 (ThermoFisher, Waltham, Massachusetts, USA). All libraries were normalized to 4 nM, pooled, and  
191 denatured before pair-end sequencing (2\*250bp) using MiSeq Reagent Kit v2 (500-cycles) on a  
192 MiSeq device (Illumina, San Diego, CA, USA), according to the manufacturer’s instructions [22].

193

#### 194 **Bioinformatic analysis**

195

196 The analysis of bacterial 16S rRNA amplicon data was performed using the USEARCH v11.0.667  
197 software pipeline with default parameters and the rdp16s database v16 [23]. Details of this analysis are  
198 provided in the Supplementary Methods.

199

#### 200 **Statistical analysis**

201

202 As sample size depended on the availability of ESBL-E-positive samples from a parent study (i.e.,  
203 convenience sample), we did not perform any sample size calculation before analysis.

204

205 We calculated the mean  $\log_{10}$  difference in relative abundances at the phylum, family, and genus level  
206 between cases and controls using targeted maximum likelihood estimation with the ‘tmle’ package in  
207 R, while accounting for matched variables (Supplementary Methods) [24]. Variance of the difference  
208 was corrected for matched strata and estimated using machine learning techniques with the  
209 “SuperLearner” package. We tested whether differences were below or above the null and corrected p-  
210 values using the Benjamini-Hochberg procedure with the ‘p.adjust’ function.

211

212 We calculated the  $\alpha$ -diversity metrics Shannon diversity index, Simpson diversity index, and Chao1  
213 richness estimator. We calculated the median and interquartile range (IQR) of the 3 indices and  
214 compared them between cases and controls using the Kruskal-Wallis test.

215  
216 We measured  $\beta$ -diversity using the Bray-Curtis dissimilarity index from the relative abundance data.  
217 We visualized patterns of compositional similarity or dissimilarity between cases and controls using  
218 the non-metric multidimensional scaling (NMDS) ordination and Principal Coordinates Analysis  
219 (PCoA) methods [25]. The difference between cases and controls was tested using the PERMANOVA  
220 (Permutational Multivariate Analysis of Variance) test under a reduced model with terms added  
221 sequentially (first to last) and 1000 free permutations. We carried out these analyses using the “vegan”  
222 package in R. In sensitivity analysis, we ran these analyses using the Canberra and robust Aitchison  
223 methods to calculate dissimilarity.

224  
225 Statistical analyses were conducted using R statistical software (v4.3.1, Vienna, Austria) and Stata  
226 (v15.0, College Station, TX).

227

228

## 229 **RESULTS**

230

### 231 **Description of the study population**

232

233 In the parent study, 226 and 1931 participants were identified with and without intestinal carriage of  
234 ESBL-E, respectively. Participant flow for this study is outlined in Figure 1. In total, 176 cases and  
235 176 controls were included in analysis.

236

237 Participant characteristics are described in Table 1. Covariate patterns were similar between cases and  
238 controls (Supplementary Methods). Participants were mostly from Europe (86.1%,  $n=303/352$ ) and  
239 had a median age of 33 years (IQR=27-44). 78.4% of participants traveled outside France within the

240 past year ( $n=276/352$ ), and 22.7% reported using antibiotics within the past 6 months ( $n=80/352$ ). Two  
241 participants carried *Klebsiella pneumoniae*, one participant *Enterobacter cloacae* and all others  
242 *Escherichia coli*.

243

#### 244 **Composition of the gut microbiome between individuals with and without ESBL-E carriage**

245

246 The total number of paired-end reads obtained was 43,321,028 with an average of 98,622  
247 (SD=22,773) per sample. Results of the negative and positive controls are presented in Supplementary  
248 Figure 1.

249

250 The phyla *Firmicutes* (72.85%) was the most abundant, followed by *Actinobacteria* (10.10%),  
251 *Bacteroidetes* (10.5%) and *Proteobacteria* (0.70%). There were no differences between individuals  
252 with and without ESBL-E carriage in relative abundances of phyla (Figure 2A). Three families were  
253 found associated with ESBL-E carriage (Figure 2B): *Intrasporangiaceae* ( $p=0.0098$ ),  
254 *Flavobacteriaceae* ( $p=0.039$ ), and *Enterobacteriaceae* ( $p=0.048$ ). These differences were non-  
255 significant following the Benjamini Hochberg correction ( $q=0.69$ ,  $0.99$  and  $0.99$ , respectively). Nine  
256 genera were found associated with ESBL-E carriage (Figure 2C): *Proteus* ( $p<0.0001$ ),  
257 *Carnobacterium* ( $p<0.0001$ ), *Enterorhabdus* ( $p=0.0079$ ), *Catonella* ( $p=0.017$ ), *Dermacoccus*  
258 ( $p=0.017$ ), *Escherichia/Shigella* ( $p=0.021$ ), *Kocuria* ( $p=0.023$ ), *Bacillus* ( $p=0.040$ ), and *Filifactor*  
259 ( $p=0.043$ ). All differences were no longer significant after using the Benjamini-Hochberg correction  
260 ( $q>0.05$ ).

261

#### 262 **Comparison of the gut microbiome diversity between individuals with and without ESBL-E** 263 **carriage**

264

265 Results from analysis on  $\alpha$ -diversity are shown in Figure 3. The median Shannon diversity index  
266 (Figure 3A), Simpson diversity index (Figure 3B) and Chao1 richness index (Figure 3C) were no  
267 different between individuals with and without ESBL-E.

268

269 Results from analysis on  $\beta$ -diversity are shown in Figure 4. One participant with outlying values was  
270 removed from this analysis. Both the NMDS ordination (Figure 4A) and PCoA plots (Figure 4B)  
271 demonstrated no difference in Bray-Curtis dissimilarities between individuals with and without ESBL-  
272 E carriage (PERMANOVA,  $p=0.25$ ). Results were comparable when using the Canberra and robust  
273 Aitchison methods to calculate dissimilarity (Supplementary Figures 2 and 3, respectively).

274

275

## 276 **DISCUSSION**

277

278 In the present study, the gut microbial diversity and composition was similar between individuals with  
279 and without ESBL-E carriage. To the best of our knowledge, this is the largest study to explore the gut  
280 microbiome, namely in terms of diversity and composition, in a specific population comprising  
281 predominately younger, healthy individuals.

282

283 Previous studies have clearly demonstrated higher prevalence of ESBL-E carriage in older individuals,  
284 those coming from or traveling to regions with high ESBL-E prevalence, and those with recent  
285 antibiotic use or increased sexual contacts [26, 27]. These factors must then be appropriately  
286 considered when examining differences in gut microbiota between those with and without ESBL-E  
287 carriage. Indeed, our matched case-control study design provides rigorous matching for factors  
288 potentially modifying microbiota composition as well as risk factors for ESBL-E carriage, which was  
289 lacking in previous studies [13,14,15]. A recently published study found that ESBL-producing  
290 *Escherichia coli* carriage in dogs is associated with a distinct microbiome and resistome composition  
291 without adjustment for potential microbiota-modulating confounding factors (i.e., age, breed, diet,  
292 hygienic measures, antibiotic use) [28]. One study conducted in an Amerindian community was  
293 matched for antibiotic exposure within the year preceding sampling, and revealed an increased  
294 abundance of members of two anaerobic taxa, belonging to the genera *Desulfovibrio*  
295 (*Desulfovibrionaceae*) and *Oscillospira* (*Ruminococcaceae*) in the microbiota of non-carrier controls

296 [16]. These differences were not corroborated in our study or another recent, more well-designed,  
297 case-control study from the Netherlands, which also found similarity in diversity parameters and in  
298 relative abundance between ESBL-positive and ESBL-negative groups at the bacterial species-level  
299 [17].

300

301 Historically, ESBL-E dissemination was more common in hospital settings. Over-time, emergence of  
302 ESBL-E infections has been more frequently reported in community settings. In the United States, an  
303 increase of 53% in the incidence of ESBL-E infections, between 2012 and 2017, has been largely  
304 attributed to community-onset cases [29]. Growing evidence suggests that the successful spread of  
305 ESBL encoding genes in the community setting owes to the ability of plasmids, notably IncF, to adapt  
306 to *E. coli* species [30]. Many of these strains also contained ESBL-encoding genes on pre-existing  
307 plasmids before newly acquiring other ESBL-containing plasmids [31]. Our results, together with  
308 those obtained by Ducarmon et al. [17] clearly indicate that ESBL-E circulating in the community are  
309 well adapted to the human intestinal microbiota and seem unaffected by the CR. Instead, ESBL-E  
310 dissemination may be driven by specific lineages, as demonstrated by Connor et al. in a gnotobiotic  
311 mouse model where a multidrug-resistant (MDR) ST131 *E. coli* was found capable of out-competing  
312 and displacing non-MDR *E. coli* from the gut, in absence of antibiotic exposure [32]. According to  
313 these authors, these high capacities to colonize human gut could be associated with increased diversity  
314 in carbohydrate metabolism genes of the MDR strains, as suggested by functional pangenomic  
315 analysis they performed.

316

317 The role of SCFAs in conferring colonization resistance against ESBL-E has been examined  
318 extensively. In a study conducted in mice, the total level of SCFAs production was found to be  
319 correlated to a decrease in *Enterobacterales* colonization in the intestinal lumen [33]. Another study  
320 conducted in 144 residents living in two different nursing homes found that the gut microbiome of  
321 individuals with ESBL-E carriage was depleted in butyrate-producing species compared to individuals  
322 without ESBL-E carriage, yet this comparison was not adjusted for microbiota-modulating factors  
323 [13]. In our study, the abundance of anaerobic bacteria identified as producers of SCFAs

324 (*Lachnospiraceae*, *Ruminococcaceae* families and *Butyrivibrio*, *Roseburia*, *Veillonella*, *Coprococcus*,  
325 *Megasphaera*, *Eubacterium*, *Faecalibacterium*, *Ruminococcus*, *Blautia* genera) was similar between  
326 individuals with and without ESBL-E carriage [34,35].

327

328 The findings need to be interpreted within the context of our study population. Individuals were  
329 seeking care for diverse reasons, possible because of increased sexual activity and risk of STIs for  
330 those without HIV, or routine care for those with HIV. The study population also mostly comprised  
331 men, mainly because of the low prevalence of women with ESBL-E carriage and the fact that women  
332 consult sexual health clinics less frequently than men in France [18,36]. Few participants were also  
333 from regions outside Europe. Although these characteristics were part of the matching criteria, the  
334 limited numbers of women and those born in a region outside of Europe do not allow us to determine  
335 whether the lack of association holds in these populations.

336

337 There are certain limitations of our study. First, since we used rectal swabs, there might have been  
338 some individuals without ESBL-E carriage who were in fact with carriage (i.e., false-negative). The  
339 extent of this misclassification depends on bacterial loads, which were unknown in this study.

340 However, this limitation is shared across all studies employing this commonly-used method. Second,  
341 there were no data on the use of proton pump inhibitors and antidiabetics, which could have  
342 additionally confounded the association between gut microbiota and ESBL-E prevalence [37,38].

343 Nevertheless, given that our study's population is comprised of mostly young participants, these  
344 medications would have been unlikely prescribed. Second, longitudinal data on gut microbiota were  
345 not collected, which could provide information on the possible changes of microbiota when ESBL-E  
346 carriage either develops or clears. Furthermore, our study was not designed to describe the functional  
347 pathways involved in colonization resistance. The use of advanced techniques in microbiological  
348 analysis, such as metagenomics, metatranscriptomics, metaproteomics and metabolomics, could have  
349 provided a more comprehensive understanding of functional profiling of microbial communities [39].

350

351

352 **CONCLUSION**

353

354 In conclusion, our results showed that the gut microbiome diversity and composition did not differ  
355 between individuals with and without ESBL-E carriage. Future longitudinal studies should examine  
356 the duration of ESBL-E carriage in community settings and factors from the intestinal microbiome that  
357 could influence the persistence or clearance of ESBL-E.

358

359

360 **SUPPLEMENTARY INFORMATION**

361

362 **Additional file 1:** Supplementary materials.

363

364

365 **ABBREVIATIONS**

366

367 **ESBL-E:** Extended-Spectrum  $\beta$ -Lactamase-Producing *Enterobacterales*; **CR:** Colonization  
368 resistance; **SCFAs:** Short Chain Fatty Acids; **HIV:** Human Immunodeficiency Virus; **STI:** Sexually  
369 Transmitted Infection; **BMR-IST:** Bactéries MultiRésistantes-Infections Sexuellement  
370 Transmissibles; **MSM:** Men who have Sex with Men; **MSW:** Men who exclusively have Sex with  
371 Women; **WSM:** Women who have Sex with Men; **PrEP:** Pre-exposure prophylaxis for HIV; **DOM:**  
372 Département Outre-Mer; **TOM:** Territoire Outre-Mer; **OTU:** Operational Taxonomic Unit; **IQR:**  
373 Interquartile Range; **NMDS:** Non-Metric Multidimensional Scaling; **PCoA:** Principal Coordinates  
374 Analysis; **PERMANOVA:** Permutational Multivariate Analysis of Variance; **MDR:** multidrug-  
375 resistant.

376

377 **DECLARATIONS**

378

379 **Ethics approval and consent to participate**

380

381 The “Comité de protection des personnes Ouest II” ethics committee (2017-73) approved the study.

382 The study was carried out in accordance with the Helsinki Declaration. All patients signed informed

383 consent for participation.

384

### 385 **Consent for publication**

386

387 Not applicable.

388

### 389 **Availability of data and material**

390

391 The microbiome dataset comprising of raw 16S rRNA sequences supporting the conclusions of this

392 article were deposited under the National Center for Biotechnology Information BioProject

393 PRJNA1024378 (<https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1024378>). Due to the

394 sensitive nature of participant data, metadata cannot be made publically available. Nevertheless, these

395 data are available upon reasonable request to the author with approval from the scientific committee of

396 the study and appropriate data transfer agreements.

397

### 398 **Competing interests**

399

400 The authors declare that they have no competing interests.

401

### 402 **Funding**

403

404 This work was supported by Agence Nationale de Recherches sur le Sida et les Hépatites Virales

405 (ANRS) (grant ECTZ62340).

406

### 407 **Authors' contributions**



408

409 L.S. coordinated the study and oversaw data analysis, and data interpretation and performed critical  
410 editing and review of the manuscript. A.B. participated to study design, oversaw data analysis and  
411 performed critical editing and review of the manuscript. P.L.W. and R.A. contributed to data  
412 interpretation and performed critical editing and review of the manuscript. M.E.D performed statistical  
413 analysis, created figures, interpreted data and drafted the initial version of the manuscript. J.C. and  
414 V.D. performed microbiological analyses. J.M.P. and K.L. contributed to study design and performed  
415 critical review of the manuscript. C.R. contributed to data interpretation and drafted parts of the  
416 manuscript. G.C. contributed to data interpretation. All authors contributed significantly to the  
417 manuscript and approved the final version.

418

#### 419 **Acknowledgements**

420

421 We would like to deeply thank all participants to BMR-IST study and Thibault Chiarabini, Nadia  
422 Valin and Valérie Lalande for their help in this study.

423 **REFERENCES**

- 424 1. Le Guern R, Stabler S, Gosset P, Pichavant M, Grandjean T, Faure E, et al. Colonization resistance  
425 against multi-drug-resistant bacteria: a narrative review. *J Hosp Infect.* 2021 Dec;118:48–58.
- 426 2. Tenailon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal *Escherichia*  
427 *coli*. *Nat Rev Microbiol.* 2010 Mar;8(3):207–17.
- 428 3. Donnenberg MS. Enterobacteriaceae. In: Mandell, Douglas, and Bennett's Principles and Practice  
429 of Infectious Diseases [Internet]. Elsevier; 2015 [cited 2023 May 17]. p. 2503-2517.e5. Available  
430 from: <https://linkinghub.elsevier.com/retrieve/pii/B9781455748013002204>
- 431 4. Willyard C. The drug-resistant bacteria that pose the greatest health threats. *Nature.* 2017  
432 Mar;543(7643):15–15.
- 433 5. Sorbara MT, Pamer EG. Interbacterial mechanisms of colonization resistance and the strategies  
434 pathogens use to overcome them. *Mucosal Immunol.* 2019 Jan;12(1):1–9.
- 435 6. Vollaard EJ, Clasener HA. Colonization resistance. *Antimicrob Agents Chemother.* 1994  
436 Mar;38(3):409–14.
- 437 7. Khan I, Bai Y, Zha L, Ullah N, Ullah H, Shah SRH, et al. Mechanism of the Gut Microbiota  
438 Colonization Resistance and Enteric Pathogen Infection. *Front Cell Infect Microbiol.* 2021;11:716299.
- 439 8. Ducarmon QR, Zwitter RD, Hornung BVH, Van Schaik W, Young VB, Kuijper EJ. Gut  
440 Microbiota and Colonization Resistance against Bacterial Enteric Infection. *Microbiol Mol Biol Rev.*  
441 2019 Aug 21;83(3):e00007-19.
- 442 9. Woerther PL, Lepeule R, Burdet C, Decousser JW, Ruppé É, Barbier F. Carbapenems and  
443 alternative  $\beta$ -lactams for the treatment of infections due to extended-spectrum  $\beta$ -lactamase-producing  
444 Enterobacteriaceae: What impact on intestinal colonisation resistance? *International Journal of*  
445 *Antimicrobial Agents.* 2018 Dec;52(6):762–70.
- 446 10. Chopyk J, Cobián Güemes AG, Ramirez-Sanchez C, Attai H, Ly M, Jones MB, et al.  
447 Common antibiotics, azithromycin and amoxicillin, affect gut metagenomics within a household.  
448 *BMC Microbiol.* 2023 Aug 2;23(1):206.

- 449 11. Abeles SR, Jones MB, Santiago-Rodriguez TM, Ly M, Klitgord N, Yooseph S, et al.  
450 Microbial diversity in individuals and their household contacts following typical antibiotic courses.  
451 *Microbiome*. 2016 Dec;4(1):39.
- 452 12. Elvers KT, Wilson VJ, Hammond A, Duncan L, Huntley AL, Hay AD, et al. Antibiotic-  
453 induced changes in the human gut microbiota for the most commonly prescribed antibiotics in primary  
454 care in the UK: a systematic review. *BMJ Open*. 2020 Sep;10(9):e035677.
- 455 13. Le Bastard Q, Chapelet G, Birgand G, Hillmann BM, Javaudin F, Hayatgheib N, et al. Gut  
456 microbiome signatures of nursing home residents carrying *Enterobacteria* producing extended-  
457 spectrum  $\beta$ -lactamases. *Antimicrob Resist Infect Control*. 2020 Dec;9(1):107.
- 458 14. Prevel R, Enaud R, Orioux A, Camino A, Sioniac P, M'Zali F, et al. Bridging gut microbiota  
459 composition with extended-spectrum beta-lactamase *Enterobacteriales* faecal carriage in critically ill  
460 patients (microbe cohort study). *Ann Intensive Care*. 2023 Apr 4;13(1):25.
- 461 15. Piewngam P, Quiñones M, Thirakittiwatthana W, Yungyuen T, Otto M, Kiratisin P.  
462 Composition of the intestinal microbiota in extended-spectrum  $\beta$ -lactamase-producing  
463 *Enterobacteriaceae* carriers and non-carriers in Thailand. *International Journal of Antimicrobial*  
464 *Agents*. 2019 Apr;53(4):435–41.
- 465 16. Gosalbes MJ, Vázquez-Castellanos JF, Angebault C, Woerther PL, Ruppé E, Ferrús ML, et al.  
466 Carriage of *Enterobacteria* Producing Extended-Spectrum  $\beta$ -Lactamases and Composition of the Gut  
467 Microbiota in an Amerindian Community. *Antimicrob Agents Chemother*. 2016 Jan;60(1):507–14.
- 468 17. Ducarmon QR, Zwitter RD, Willems RPJ, Verhoeven A, Nooij S, Van Der Klis FRM, et al.  
469 Gut colonisation by extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* and its association  
470 with the gut microbiome and metabolome in Dutch adults: a matched case-control study. *The Lancet*  
471 *Microbe*. 2022 Jun;3(6):e443–51.
- 472 18. Surgers L, Chiarabini T, Royer G, Rougier H, Mercier-Darty M, Decré D, et al. Evidence of  
473 Sexual Transmission of Extended-Spectrum  $\beta$ -Lactamase-Producing *Enterobacteriales*: A Cross-  
474 sectional and Prospective Study. *Clinical Infectious Diseases*. 2022 Oct 29;75(9):1556–64.

- 475 19. Angebault C, Payen M, Woerther PL, Rodriguez C, Botterel F. Combined bacterial and fungal  
476 targeted amplicon sequencing of respiratory samples: Does the DNA extraction method matter? PLoS  
477 One. 2020;15(4):e0232215.
- 478 20. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general  
479 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity  
480 studies. Nucleic Acids Res. 2013 Jan 7;41(1):e1.
- 481 21. Mounier R, Kapandji N, Gricourt G, Lobo D, Rodriguez C, Pons S, et al. Assessment of  
482 Bacterial Colonization of Intracranial Pressure Transducers: A Prospective Study. Neurocrit Care.  
483 2021 Jun;34(3):814–24.
- 484 22. 16S Metagenomic Sequencing Library Preparation 2015. Available from:  
485 <https://web.uri.edu/gsc/files/16s-metagenomic-library-prep-guide-15044223-b.pdf>
- 486 23. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics.  
487 2010 Oct 1;26(19):2460–1.
- 488 24. Van der Laan MJ, Rose S. Targeted Learning in Data Science: Causal Inference for Complex  
489 Longitudinal Studies. 2018. Switzerland: Springer.
- 490 25. Oksanen, Jari. (2008). VEGAN: AN INTRODUCTION TO ORDINATION.  
491 [https://www.researchgate.net/publication/238101961\\_VEGAN\\_AN\\_INTRODUCTION\\_TO\\_ORDINATION](https://www.researchgate.net/publication/238101961_VEGAN_AN_INTRODUCTION_TO_ORDINATION)  
492 ATION
- 493 26. van den Bunt G, van Pelt W, Hidalgo L, Scharringa J, de Greeff SC, Schürch AC, et al.  
494 Prevalence, risk factors and genetic characterisation of extended-spectrum beta-lactamase and  
495 carbapenemase-producing Enterobacteriaceae (ESBL-E and CPE): a community-based cross-sectional  
496 study, the Netherlands, 2014 to 2016. Euro Surveill. 2019 Oct;24(41):1800594.
- 497 27. Stabler S, Paccoud O, Duchesne L, Piot MA, Valin N, Decré D, et al. Prevalence of  
498 Antimicrobial Resistance and Infectious Diseases in a Hospitalised Migrant Population in Paris,  
499 France, a Retrospective Study. Int J Public Health. 2022 Dec 15;67:1604792.
- 500 28. Stege PB, Hordijk J, Sandholt AKS, Zomer AL, Viveen MC, Rogers MRC, et al. Gut  
501 Colonization by ESBL-Producing Escherichia coli in Dogs Is Associated with a Distinct Microbiome  
502 and Resistome Composition. Manning SD, editor. Microbiol Spectr. 2023 Aug 17;11(4):e00063-23.

- 503 29. Jernigan JA, Hatfield KM, Wolford H, Nelson RE, Olubajo B, Reddy SC, et al. Multidrug-  
504 Resistant Bacterial Infections in U.S. Hospitalized Patients, 2012–2017. *N Engl J Med*. 2020 Apr  
505 2;382(14):1309–19.
- 506 30. Li JJ, Spychala CN, Hu F, Sheng JF, Doi Y. Complete Nucleotide Sequences of *bla*<sub>CTX-M</sub> -  
507 Harboring IncF Plasmids from Community-Associated *Escherichia coli* Strains in the United States.  
508 *Antimicrob Agents Chemother*. 2015 Jun;59(6):3002–7.
- 509 31. Branger C, Ledda A, Billard-Pomares T, Doublet B, Fouteau S, Barbe V, et al. Extended-  
510 spectrum  $\beta$ -lactamase-encoding genes are spreading on a wide range of *Escherichia coli* plasmids  
511 existing prior to the use of third-generation cephalosporins. *Microb Genom*. 2018 Sep;4(9):e000203.
- 512 32. Connor CH, Zucoloto AZ, Munnoch JT, Yu IL, Corander J, Hoskisson PA, et al. Multidrug-  
513 resistant *E. coli* encoding high genetic diversity in carbohydrate metabolism genes displace  
514 commensal *E. coli* from the intestinal tract. *PLoS Biol*. 2023 Oct 17;21(10):e3002329.
- 515 33. Sorbara MT, Dubin K, Littmann ER, Moody TU, Fontana E, Seok R, et al. Inhibiting  
516 antibiotic-resistant Enterobacteriaceae by microbiota-mediated intracellular acidification. *Journal of*  
517 *Experimental Medicine*. 2019 Jan 7;216(1):84–98.
- 518 34. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host  
519 Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell*. 2016 Jun;165(6):1332–45.
- 520 35. Martin-Gallausiaux C, Marinelli L, Blottière HM, Larraufie P, Lapaque N. SCFA:  
521 mechanisms and functional importance in the gut. *Proc Nutr Soc*. 2021 Feb;80(1):37–49.
- 522 36. Pioche C, Ndeikoundam N, Sarr A, Cazein F, Bruyand M, Viriot D, et al. Activité de  
523 dépistage et diagnostic du VIH, des hépatites B et C, et des autres IST en CeGIDD, France, 2018. *Bull*  
524 *Epidémiol Hebd*. 2019;(31-32):625-33.
- 525 37. Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC, et al. Proton  
526 pump inhibitors alter the composition of the gut microbiota. *Gut*. 2016 May;65(5):749–56.
- 527 38. de la Cuesta-Zuluaga J, Mueller NT, Corrales-Agudelo V, Velásquez-Mejía EP, Carmona JA,  
528 Abad JM, et al. Metformin Is Associated With Higher Relative Abundance of Mucin-Degrading  
529 *Akkermansia muciniphila* and Several Short-Chain Fatty Acid-Producing Microbiota in the Gut.  
530 *Diabetes Care*. 2017 Jan;40(1):54–62.

531 39. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, et al. Best practices  
532 for analysing microbiomes. *Nat Rev Microbiol.* 2018 Jul;16(7):410–22.