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Charles Burdet, Thu Thuy Nguyen, Xavier Duval, Stéphanie Ferreira, Antoine Andremont, et al.. Impact of Antibiotic Gut Exposure on the Temporal Changes in Microbiome Diversity: Impact of moxifloxacin on gut microbiome. *Antimicrobial Agents and Chemotherapy*, 2019, 63 (10), pp.e00820-19. 10.1128/AAC.00820-19 . inserm-02362445

HAL Id: inserm-02362445

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1 **Title**

2 Impact of antibiotic gut exposure on the temporal changes in microbiome diversity

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10 **Keywords**

11 moxifloxacin; intestinal microbiome; diversity; dysbiosis; metagenomics; nonlinear mixed effect modelling

12 **Running title**

13 Impact of moxifloxacin on gut microbiome

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21 **Abstract**

22 Although the global deleterious impact of antibiotics on the intestinal microbiota is well known, the
23 temporal changes in microbial diversity during and after an antibiotic treatment are still poorly
24 characterized. We used plasma and fecal samples frequently collected during and up to one month after
25 treatment in 22 healthy volunteers assigned to a 5-day treatment by moxifloxacin (N=14) or no
26 intervention (N=8). Moxifloxacin concentrations were measured both in plasma and feces, and bacterial
27 diversity was determined in feces by 16S rRNA gene profiling and quantified using Shannon index and
28 number of operational taxonomic units (OTUs). Nonlinear mixed-effects models were used to relate drug
29 pharmacokinetics and bacterial diversity over time. Moxifloxacin reduced bacterial diversity in a
30 concentration-dependent manner, with a median maximal loss of 27.5% of the Shannon index (min;max:
31 17.5;27.7) and 47.4% of the OTUs number (30.4;48.3). As a consequence of both the long fecal half-life of
32 moxifloxacin and the susceptibility of the gut microbiota to moxifloxacin, bacterial diversity indices did not
33 return to their pre-treatment levels until days 16 and 21, respectively. Finally, the model characterized the
34 effect of moxifloxacin on bacterial diversity biomarkers, and provides a novel framework for analyzing
35 antibiotic effects on the intestinal microbiome.

36 **Background**

37 A large body of data describes the role of the intestinal microbiome in various host processes, including
38 metabolic, nutritional and immunological processes (1, 2) and in inhibiting the growth of potentially
39 pathogenic microorganisms within the intestines (3, 4). These insights have been unveiled by the growing
40 use of next generation sequencing techniques, making possible to investigate the impact of uncultivable
41 bacteria on health and disease. Among such methods, profiling of the 16S rRNA gene consists in
42 sequencing a subset of the 9 hypervariable regions of the bacterial genes encoding the 16S rRNA. Based
43 on their homology, the huge amount of sequences obtained can be clustered into operational taxonomic
44 units (OTUs), used for characterizing the composition of the bacterial community (5). This composition can
45 then be summarized for each sample using synthetic indices of diversity, such as the total number of
46 observed OTUs, which characterizes the richness from an ecological perspective, or the Shannon diversity
47 index (6), a composite index of the richness and the distribution of OTUs in the community. These indices of
48 within-sample diversity, also called “alpha diversity”, are now widely used for evaluating the relationships
49 between the microbiome and human health, and were recently shown to be highly predictive of mortality in
50 a hamster model of *C. difficile* infection (7). This suggests that such markers could be useful to measure the
51 impact of treatment on the microbiota, and possibly be predictive of clinical outcomes (8).

52 A major cause of microbiome disruption, called dysbiosis, is the administration of antibiotics (9-13). In that
53 respect, fluoroquinolones antibiotics have a particularly severe impact on the gut microbiome due to their
54 high concentrations achieved in feces (14). Through its disruptive effect on the resident bacterial flora,
55 antibiotic administration creates a large space that can be colonized by potentially resistant bacteria.
56 These bacteria may then be disseminated in the environment, making antibiotic-modified gut microbiota the
57 epicentre of bacterial resistance spread (10, 14, 15). In spite of the well-studied impact of repeated
58 administrations of antibiotics on the intestinal diversity (13), we still lack of a precise characterisation of the
59 association between drug concentrations, both in plasma and feces, and temporal changes in intestinal
60 microbiome (16). This can be done using mathematical models, but parameter estimation of these models
61 is hampered by the high level of both interindividual and intraindividual variability of bacterial diversity,
62 making it difficult to obtain reliable parameter estimates. This difficulty can be in part circumvented by the
63 use of mixed-effect models that specifically tease out the two sources of variability and allow to precisely

64 estimate parameters, as shown in the context of antibiotic pharmacokinetic/pharmacodynamic assessment
65 (17-19).

66 Here we aimed to employ the tools of mathematical and statistical modelling to analyze the relationship
67 between antibiotic gut exposure and temporal changes in gut microbiome, using the Shannon index and the
68 number of OTUs as synthetic markers of diversity. We focus our analysis on healthy volunteers treated by
69 moxifloxacin, a fluoroquinolone with high oral bioavailability and a high volume of distribution at steady-
70 state, which exhibits an excellent penetration in body tissues (20, 21) and whose elimination occurs
71 primarily through the intestinal route (22).

72 **Material and methods**

73 **Healthy volunteers and sample collection**

74 We used data from the CL-1002 trial (NCT02176005), a prospective, open label, randomized
75 clinical trial conducted in 2014 at the Clinical Investigation Center of the Bichat hospital, Paris (France). The
76 trial was approved by French Health Authorities and Independent Ethics Committee. Full details of the trial
77 have been reported elsewhere (23). We focused here on 22 subjects of this trial who received moxifloxacin
78 alone (n=14) or were not treated (negative control group, n=8).

79 Briefly, healthy volunteers over 18-year-old without exposure to antibiotics in the preceding 3
80 months were prospectively included after obtention of their informed consent. Subjects in the moxifloxacin
81 group received 400 mg of moxifloxacin orally, once a day for 5 days (from D1 to D5), and all subjects were
82 followed until D37. A total of 24 blood samples were collected in each moxifloxacin-treated volunteer on D1
83 (just before treatment administration and at 0.5h, 1h, 1.5h, 2h, 3h, 4h, 6h, 12h, and 24h post administration)
84 and at the last day of treatment, *i.e.* on D5 (just before treatment administration and at 0.5h, 1h, 1.5h, 2h,
85 3h, 4h, 6h, 12h, 24h, 32h, 48h, 56h, and 72h post administration). Thirteen fecal samples were collected in
86 all volunteers at screening (between D-21 and D-3), just before the first administration of moxifloxacin
87 (baseline), once a day from D2 to D9, and then at D12, D16, D37.

88 Moxifloxacin assays were performed using specifically developed and validated bioanalytical
89 methods (see Supplementary Methods). Total plasma moxifloxacin concentrations were determined by
90 reverse phase high-performance liquid chromatography coupled with fluorescence detection (lower limit of
91 quantification, 0.01 µg/mL). Fecal concentrations of free moxifloxacin were determined on the 11 samples

92 collected at: baseline, D2, D3, D4, D5, D6, D7, D8, D9, D12 and D16 by tandem mass spectrometry
93 detection (lower limit of quantification, 0.04 µg/g).

94 **Targeted metagenomic analysis of the intestinal microbiota**

95 The 7 fecal samples collected at: screening, baseline, D3, D6, D9, D16 and D37 were analysed by
96 16S rRNA gene profiling. Microbial DNA was extracted using an extraction protocol optimized at
97 GenoScreen, partially based on commercially available extraction kits (QIAamp DNA stool Kit, Qiagen,
98 Germany) with the addition of chemical and mechanical lysis steps.

99 The V3-V4 region of the 16S rRNA gene was then amplified using an optimized and standardized amplicon-
100 library preparation protocol (Metabio®[®], GenoScreen, Lille, France). Positive (Artificial Bacteria Community
101 comprising 17 different bacteria, ABCv2) and negative (sterile water) controls were also included. Briefly,
102 PCR reactions were performed using 5 ng of genomic DNA and in-house fusion barcoded primers (final
103 concentrations of 0.2 µM), with an annealing temperature of 50°C for 30 cycles. PCR products were purified
104 using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA), quantified according to
105 GenoScreen's protocol, and mixed in an equimolar amount. Sequencing was performed using 250-bp
106 paired-end sequencing chemistry on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at
107 GenoScreen.

108 Raw paired-end reads were then demultiplexed per sample and subjected to the following process:
109 (1) search and removal of both forward and reverse primer using CutAdapt, with no mismatches allowed in
110 the primers sequences; (2) quality-filtering using the PRINSEQ-lite PERL script (24), by truncating bases at
111 the 3' end with Phred quality score <30; (3) paired-end read assembly using FLASH (25), with a minimum
112 overlap of 30 bases and >97% overlap identity.

113 Diversity analysis was performed using the Metabio Online v2.0 pipeline (GenoScreen, Lille,
114 France) which is partially based on the QIIME software v1.9.1 (26). Following the steps of pre-processing,
115 chimera sequences were detected and eliminated (in-house method based on Usearch v6.1). Then,
116 clustering of similar sequences (97% identity threshold for an affiliation at the genus level on the V3-V4
117 regions of the 16S rRNA gene) was performed with Uclust v1.2.22q (27) through an open-reference OTU
118 picking process and complete-linkage method, finally creating groups of sequences or "Operational
119 Taxonomic Units" (OTUs). An OTU cleaning step corresponding to the elimination of singletons was

120 performed. Diversity in each fecal sample was estimated using 2 indices of alpha-diversity, the Shannon
121 diversity index and the number of observed OTUs.

122 **Modeling strategy**

123 First we developed a pharmacokinetic model of plasma moxifloxacin using total drug
124 concentrations Both one- and two- compartment models with first-order absorption and first-order
125 elimination were tested. The absorption delay of moxifloxacin in the central compartment was modelled
126 using either a lagtime or absorption transit compartments (28). As moxifloxacin was administered through
127 the oral route, we estimated an apparent volume of distribution after oral administration V/F .

128 Second, the model was extended to account for fecal pharmacokinetics, assuming a fixed fecal
129 weight of 200 g/day in all subjects, allowing us to reconstruct pharmacokinetic profiles in both plasma and
130 feces. Moxifloxacin elimination from the central compartment was divided into an intestinal elimination and
131 an extra-intestinal elimination, both being assumed to have first-order rates. Several structural models with
132 various numbers of elimination transit compartments between plasma and the lower gastrointestinal tract
133 were tested. As enteric recirculation had been reported for fluoroquinolones (20), we also tested several
134 models for moxifloxacin reabsorption, occurring either from one of the transit compartments between
135 plasma and the lower gastrointestinal tract or from the lower gastrointestinal tract to the plasma
136 compartment.

137 Third, a semi-mechanistic model was developed assuming that moxifloxacin impacted microbiome
138 diversity as follows (29). The evolution of the diversity index D in the absence of the drug was written as:

$$139 \quad \frac{dD}{dt} = R_{in} - k_{out} \times D \text{ (equation 1)}$$

140 where R_{in} is a zero-order constant for the production of the diversity, and k_{out} is a first-order rate constant
141 for elimination of diversity. At steady state, $\frac{dD}{dt} = 0$, and thus $D_0 = \frac{R_{in}}{k_{out}}$ in the absence of treatment. The
142 initiation of antibiotic treatment perturbs this equilibrium and can alter either lead to a reduction of R_{in} or to
143 an increase of k_{out} (k'_{out} , equation 2):

$$144 \quad k'_{out} = \left(1 + \frac{E_{max} \times C_f}{EC_{50} + C_f} \right) \times k_{out} \text{ (equation 2)}$$

145 with C_f being the fecal concentration of free moxifloxacin, E_{max} the maximal increase of k_{out} , and EC_{50} the
146 concentration leading to 50% of the maximal effect of moxifloxacin.

147 **Statistical methods**

148 We used nonlinear mixed effects models to analyse the evolution of pharmacokinetic and
149 pharmacodynamic data. Data from treated individuals were used for the pharmacokinetic analysis,
150 whereas data from both groups were used for the pharmacodynamic analysis (with fecal concentrations
151 assumed to be 0 in the control group, see equation 1). Intraindividual correlation of repeated data was
152 taken into account by random effects. Population parameters were estimated by likelihood maximization
153 using the stochastic approximation expectation maximisation algorithm (SAEM) (30), implemented in
154 MONOLIX v4.3.2 (Lixoft, Orsay, France, www.lixoft.eu). Data below the lower limit of quantification were
155 treated as left-censored data. Their contribution to the likelihood was computed as the probability that
156 these data are indeed below the lower limit of quantification(31). Estimates of the individual parameters
157 were computed as the mode of the *a posteriori* distribution and used to predict individual pharmacokinetic
158 and pharmacodynamic profiles for the following treatment regimens: moxifloxacin daily doses of 400 mg
159 and 800 mg for 5- and 10-day treatment duration. Model selection at each step of the model building was
160 performed using the Bayesian information criteria (BIC), and model evaluation was conducted by
161 investigating several goodness-of-fit plots. Full details on the statistical model and parameters estimation
162 are available in Supplementary Text S1.

163 **Measures of antibiotic impact on the microbiome**

164 The following parameters were computed for moxifloxacin-treated subjects using estimated
165 individual parameters (Supplementary Text S1): mean transit time between the central compartment and
166 the lower gastrointestinal tract, maximal loss of each bacterial diversity index in the intestinal microbiome
167 after the beginning of treatment (nadir), the time for which this maximal loss was achieved (time to nadir)
168 and the time at which each diversity index returned to 95% of its baseline value. The cumulated impact of
169 moxifloxacin on diversity was obtained by computing the area under the curve for each diversity index up
170 to 42 days after the beginning of treatment.

171 **Results**

172 The median age was 33.2 years (min; max 23.3; 59.5) and 8 were males (36.4%). Median weight
173 was 65.2 kg (56.7; 86.0). Full characteristics of included subjects can be found in (23).

174 **Pharmacokinetic analysis of moxifloxacin in plasma and feces**

175 A total of 322 values of total moxifloxacin plasma concentration and 138 values of fecal
176 concentration of free moxifloxacin were collected in the 14 treated patients (see Supplementary Figure S1).
177 Plasma concentrations were best described by a two-compartment model with first-order elimination and
178 first-order absorption using transit compartments to model the absorption delay (Figure 1). A compartmental
179 model including two transit compartments between plasma and the lower gastrointestinal tract best fitted
180 the fecal data, with enteric recirculation of moxifloxacin from the lower gastrointestinal tract to the central
181 compartment (Figure 1). The mathematical expressions of the final pharmacokinetic model are presented in
182 the Supplementary Text S2.

183 The evolution of moxifloxacin concentration in plasma and feces predicted by the model is presented in
184 Supplementary Figure S2 (panels A and B). Nearly all parameters of the pharmacokinetic model could be
185 estimated with a good precision (with r.s.e. below 30%), except for absorption related parameters k_{tr} and
186 k_a (Supplementary Table S1). The two rate-constant parameters for the absorption and the elimination
187 transit compartments models were estimated and not supposed to be identical (which would have led to an
188 increase in BIC). The model well characterized the evolution of both plasma and fecal concentrations of
189 moxifloxacin over time in the 14 treated healthy volunteers (Supplementary Figure S3). Other goodness-of-
190 fit plots for this pharmacokinetic model were satisfactory (Figure 2 and Supplementary Figure S4).

191 Of note, the fecal weight of P_f was assumed to be 200 g/d in all volunteers at all time. As what is
192 observed and fitted are the concentration in the feces, C_f , defined as A_f/P_f , a change in P_f results in a
193 similar change in A_f to keep C_f unchanged. As plasma pharmacokinetics (A_c and A_p) are not affected by
194 modification of the fecal weight, then k_{ct1} should increase by the same proportion. As elimination of
195 moxifloxacin from plasma is not affected by the fecal weight, the sum of k_{ct1} (intestinal elimination from
196 plasma) and k_e (extra-intestinal elimination from plasma) is constant. So when k_{ct1} increases, k_e
197 decreases: the first consequence of our assumption on the fecal weight is that it impacts the proportion of
198 the intestinal elimination of moxifloxacin. In addition, for equation (2) to be similar, k_{fc} should be divided by
199 that proportion. As the rate of elimination of moxifloxacin from the fecal compartment is not affected by the
200 fecal weight, the sum of k_{fc} , (reabsorption from the lower intestinal tract to the plasma) and k_f (elimination
201 of moxifloxacin from the lower intestinal tract) should be kept constant. Then, when k_{fc} decreases, k_f

202 increases: the second consequence of our assumption on the fecal weight is that it impacts the proportion
203 of moxifloxacin reabsorbed from the lower intestinal tract to the plasma.
204 We performed simulations with fecal weights of 100 g/day, 200 g/ day, 300 g/day and 400 g/day making the
205 described changes in the population parameters. The simulations resulted in identical concentration-time
206 profiles both in plasma and in feces. The proportions of moxifloxacin eliminated from the plasma by the
207 intestinal route was 94.3%, 88.8%, 83.2% and 77.7% for fecal weights of 100 g/day, 200 g/day, 300 g/day
208 and 400 g/day, respectively. The proportions of moxifloxacin reabsorbed from the lower intestinal tract to
209 the plasma was 49.7%, 24.9%, 16.6% and 12.4% for fecal weights of 100 g/day, 200 g/day, 300 g/day and
210 400 g/day, respectively.

211 The median (min; max) concentration of free moxifloxacin in feces observed 24 hours after the first
212 administration of moxifloxacin was 56.9 µg/g (21.7; 95.4), and increased up to 130.4 µg/g (79.2; 250.6) on
213 the 5th day of treatment. Median elimination half-lives from the central compartment and the lower
214 gastrointestinal tract were 0.53 day (0.43; 0.67) and 1.0 day (0.76; 1.49), respectively. The median transit
215 time of moxifloxacin between plasma and the lower gastrointestinal tract was 0.59 days (0.35; 1.23). The
216 median time for fecal concentrations of free moxifloxacin to be below the lower limit of quantification was
217 14.1 days (11.7; 19.3).

218 **Impact of drug concentrations on microbial diversity**

219 A total of 142 samples were analysed by 16S rRNA gene profiling (90 in subjects treated by
220 moxifloxacin and 52 in controls). Individual profiles of the Shannon index and number of OTUs are
221 presented in Supplementary Figure S5.

222 Both indices of bacterial diversity remained constant in absence of treatment, leading to an
223 estimated mean baseline value of 4.75 Shannon units (r.s.e., 2%) and 163 OTUs (r.s.e., 5%) for the
224 Shannon index and the number of OTUs, respectively (Supplementary Table S2). Initiation of moxifloxacin
225 led to a rapid drop in both diversity indices. This was attributed in our model to a drug concentration
226 dependent effect in the elimination rate of bacterial diversity from feces (Figure 1 and Supplementary Text
227 S2), with a rate increasing up to 38% (r.s.e. 14%) and 94% (r.s.e., 4%) for the Shannon and the number of
228 OTUs, respectively. Given the extended presence of moxifloxacin in feces and the susceptibility of bacterial
229 diversity to the drug (as measured by the parameter EC_{50} which was shown to be similar for both indices,

230 see methods), our model predicted that the diversity only slowly returned to pre-treatment values after
231 treatment cessation. Of note EC_{50} was estimated to 0.13 $\mu\text{g/g}$ of feces, and was associated with a large
232 interindividual variability (470%, see Supplementary Table S2). The model could well fit the evolution of
233 both the Shannon index and the number of OTUs in all individuals (Figure 3 and Supplementary Figures S6
234 and S7). The model prediction intervals are drawn in Supplementary Figure S2 (panels C and D). All
235 parameters were well estimated (Supplementary Table S2), except the drug EC_{50} , for which likelihood
236 profiling (see Supplementary Text S1) was used to calculate the 95% confidence interval ([0.01 $\mu\text{g/g}$; 2
237 $\mu\text{g/g}$], Supplementary Figure S8).

238 Pharmacodynamic indices derived from the model for the 14 subjects treated by moxifloxacin are
239 presented in Table 1 and Figure 4. A median loss of 0.6 units of Shannon index (0.3; 0.7) and 26 OTUs (11;
240 41) was achieved at 24 hours after the beginning of treatment. The maximal loss induced by moxifloxacin
241 was 1.2 Shannon units (0.8; 1.3) and 77 OTUs (47; 100), achieved 7.0 (5.1; 11.4) and 8.5 (5.5; 13.8) days
242 after treatment initiation, respectively. Results obtained for other simulated treatment regimens are
243 presented in the Supplementary Table S3.

244 **Discussion**

245 Here, we characterized for the first time the drug-concentration dependent impact of antibiotic
246 treatment on the temporal changes in bacterial diversity in the intestinal microbiome during and after
247 treatment initiation.

248 The pharmacokinetic analysis revealed that moxifloxacin concentrations in the lower gastrointestinal
249 tract rapidly reached a high plateau with median value of 130.4 $\mu\text{g/g}$ at day 5, and only slowly decreased
250 after treatment cessation, with a half-life of 1 day. This slow decrease in the lower gastrointestinal tract was
251 captured in our model assuming a reabsorption of moxifloxacin from the lower gastrointestinal tract to the
252 central compartment. Enteric recycling had been previously reported for moxifloxacin (20). Interestingly,
253 reabsorption from the gut to the central compartment did not seem to occur from the upper parts of the gut,
254 but rather from the lower gastrointestinal tract. This is consistent with the fact that moxifloxacin
255 concentrations within the colonic mucosa are greater than those measured in the small bowel mucosa (32).
256 This reabsorption process might explain the longlasting fecal excretion observed after treatment cessation,
257 with concentrations in the lower gastrointestinal tract greater than 2 $\mu\text{g/g}$ up to 5 days (2.9; 8.4) after the last

258 administration of moxifloxacin. As both indices of diversity were largely susceptible to moxifloxacin (with a
259 similar EC_{50} equal to $0.13 \mu\text{g/g}$), diversity was profoundly impacted up to 10 days after treatment cessation.
260 These slowly decreasing concentrations of moxifloxacin create a replication space for resistant bacteria,
261 consistent with the observation that the post treatment period is critical for the emergence of
262 fluoroquinolone-resistant strains in the human fecal flora (33). Despite moxifloxacin mechanism of action on
263 bacterial growth, in our model moxifloxacin appeared to increase the rate of elimination of bacterial
264 diversity, with a reduction of the Bayesian Information Criteria by 60 units).

265 Despite the complexity of the measures performed, relying on metagenomic analyses, semi-
266 mechanistic mathematical models provided good description of the temporal changes of the diversity
267 indices within the intestinal microbiome. Interestingly, the maximal effect of moxifloxacin on the two diversity
268 indices was different, leading to differences in the time needed to return to baseline values. This confirms
269 that the two indices do not exactly measure the same phenomena, and may be complementary to draw a
270 complete picture of the microbiome dysbiosis induced by an intervention.

271 Our approach shows that the complexity of the microbiome composition, along with the temporal impact of
272 treatment on it, may be advantageously characterized using semi-mechanistic mathematical models and
273 synthetic markers of microbiome diversity, such as the Shannon index and the number of OTUs. They can
274 then be used to calculate simple metrics, such as the cumulated loss of bacterial diversity, and may be
275 used to predict the impact of changes in experimental settings. This might prove to be very useful as
276 microbiome data on multiple days are usually difficult to obtain reliably in the clinical context. Model can
277 also be used to simulate the effect of different dosing regimens, such as increasing the daily dose to 800
278 mg or the treatment duration to 10 days. Given the high susceptibility of bacterial diversity to moxifloxacin,
279 we predicted that increasing the daily dose would result in only a minor additional effect on bacterial
280 diversity, as compared to the dosing regimen studied here. Doubling the treatment duration from 5 to 10
281 days would result in a 35% increase of the global impact of moxifloxacin on the bacterial diversity, as
282 measured by the AUC between day 0 and day 42 of the change of diversity indices from day 0. Further, the
283 use of a non-linear mixed effect model to estimate parameters, a statistical approach that advantageously
284 optimizes the information available by borrowing strength from the between-subject variability, allowed us to
285 precisely estimate parameters despite a limited number of samples per patient.

286 Our work has some limitations. The main one is that only healthy subjects were included. Model
287 parameters, in particular the interindividual variability of the pharmacodynamic parameters but also possibly
288 the gut microbiome, could differ in a clinical context. For instance, it has been reported that parameters
289 such as the body weight or the severity of the infection impact the pharmacokinetics of antibiotics, including
290 moxifloxacin (34-36). Next, the EC_{50} parameter could not be precisely estimated and was associated with a
291 large confidence interval, which prevented to extrapolate our findings for lower dosing regimens. Next, we
292 fixed the fecal daily weight to 200 g for all volunteers, whereas there is a high intra- and inter-individual
293 variability in the feces generation. This assumption affects the values on four elimination rate-constants
294 k_{ct1} , k_e , k_{fc} and k_f , whose values should therefore be considered with caution. Furthermore, assuming the
295 same values of fecal daily weight in all individuals at each sampling time, whereas it is known to vary, has
296 an impact on the estimated variabilities of those parameters and on the residual error. It does however not
297 have any impact on the pharmacodynamic model as it was written using fecal concentrations. Finally, we
298 restricted our analysis to a global measure of diversity and we did not model the specific evolution of OTUs
299 but this will require larger study population in order to account for the variability across patients and the high
300 dependency within OTU dynamics. Such models will also gain from using technologies capable to measure
301 absolute counts of OTUs and not only relative counts, such as coupling data from new generation
302 sequencing together with flow cytometric counts of bacterial cells (37).

303 In summary, we provided here a modelling framework to measure the impact of antibiotics on the
304 intestinal microbiome. Diversity indices obtained through next generation sequencing offer a simplified view
305 of the dynamics of the bacterial community within the microbiome, and mathematical modeling allowed to
306 precisely estimate several biomarkers to assess the global dysbiosis induced by moxifloxacin. This
307 encourages to extend these results to the clinical setting for estimating the unwanted effects of drugs on the
308 microbiome. Further, mathematical models make it possible to investigate the effect of various experimental
309 conditions on an outcome, and could be helpful to develop strategies aiming to reduce antibiotics impact on
310 the gut microbiota, *via* for instance locally released adsorbents, antibiotic-hydrolysing enzymes or optimized
311 dosing regimens of antibiotics.

312 **Acknowledgments**

313 The authors thank the Da Volterra Company for providing the data. We thank Jean de Gunzburg for fruitful
314 discussions.

315 Members of the DAV132-CL-1002 study group include: Ait-Ilaine B, Alavoine L, Duval X, Ecobichon JL,
316 Ilic-Habensus E, Laparra A, Nisus ME, Ralaimazava P, Raine S, Tubiana S, Vignali V, all from the Center
317 of Clinical Investigation 1425 at Bichat hospital, Paris, France;
318 Chachaty E, from the Institut Gustave Roussy, Villejuif, France

319 **Conflict of interest**

320 Antoine Andremont, Charles Burdet and France Mentré are consultants for the Da Volterra Company. Thu
321 Thuy Nguyen performed statistical work for the Da Volterra Company through a contract with INSERM
322 UMR 1137.

323 **Author Participation**

324 AA and FM designed the clinical trial. XD included participants. SF performed the metagenomic analysis.
325 CB, TTN, JG and FM performed the statistical analysis of the data. CB, TTN, JG and FM wrote the paper.
326 All authors agreed on the final version of the manuscript.

327 **Funding**

328 The randomized clinical trial was sponsored by Da Volterra (Paris) and funded in part by the European
329 Union Seventh Framework Programme (FP7-HEALTH-2011-single-stage) under grant agreement number
330 282004, EvoTAR.

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433

434

435 **Figures legends**

436 Figure 1. Final compartmental model for plasma and fecal moxifloxacin pharmacokinetics (red) and for
437 bacterial diversity indices (orange).

438 k_{tr} is the transfer rate between each compartment for the absorption delay; k_a is the absorption rate to the
439 central compartment; k_e is the extraintestinal elimination rate from the central compartment; k_{12} and k_{21}
440 are the transfer rates between the central compartment and the peripheral compartment; k_{ct1} is the
441 elimination rate from the central compartment to the intestinal tract; k_{fc} is the transfer rate between the
442 lower gastrointestinal tract and the central compartment; k_t is the transfer rate between the intestinal
443 transit compartments; k_f is the elimination rate from the lower gastrointestinal tract; R_{in} is the zero-order
444 constant for production of the diversity index; k_{out} is the first-order elimination rate of the diversity index
445 from the lower gastrointestinal tract.

446 C_f is the concentration of free moxifloxacin in the lower gastrointestinal tract; E_{max} is the maximal effect of
447 moxifloxacin on the elimination rate of the diversity index, and EC_{50} is the concentration of moxifloxacin
448 leading to 50% of the maximal effect.

449 Data were available for the 3 compartments with bold boxes. GIT, gastrointestinal tract.

450
451 Figure 2. Visual predictive checks for pharmacokinetic model. Plasma concentrations are depicted in the
452 panel A and fecal concentrations are depicted in the panel B. The blue and red lines are the observed
453 percentiles (10th, 50th, and 90th percentiles), the blue and red ribbons are the corresponding 95%
454 confidence intervals. The dashed black lines are predicted percentiles. Black points are the individual
455 observations.

456
457 Figure 3. Visual predictive checks for pharmacodynamic model. The Shannon index is depicted in the panel
458 A (moxifloxacin-treated subjects) and panel B (untreated subjects) and the number of OTUs is depicted in
459 the panel C (moxifloxacin-treated subjects) and panel D (untreated subjects). The blue and red lines are the
460 observed percentiles (10th, 50th, and 90th percentiles), the blue and red ribbons are the corresponding 95%

461 confidence intervals. The dashed black lines are predicted percentiles. Black points are the individual
462 observations.

463

464 Figure 4. Estimated impact of moxifloxacin on intestinal microbiome in the 14 subjects treated with
465 moxifloxacin. The impact was measured as the area under the curve (AUC) of the change of the Shannon
466 index (panel A) or number of OTUs (panel B) from baseline over time, between day 0 and day 42. The
467 AUC is a metric which allows a global view of antibiotics impact on the microbiota as it takes into account
468 both the extent and the duration of dysbiosis.

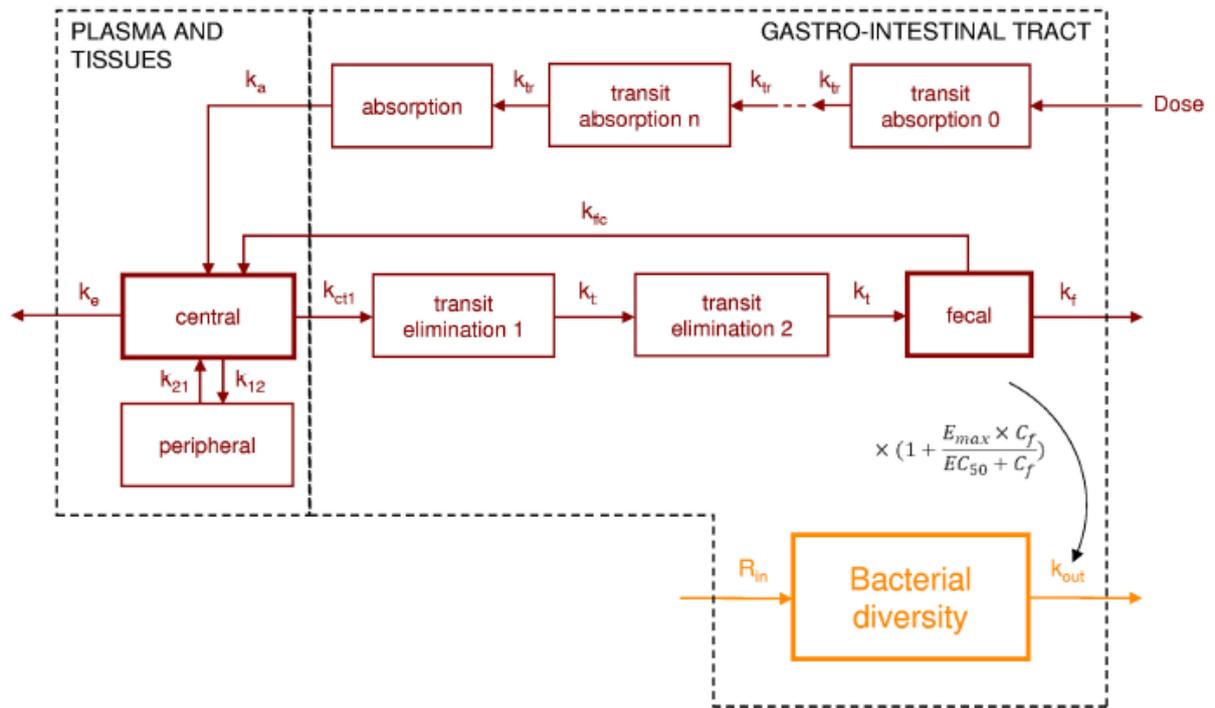


FIG1

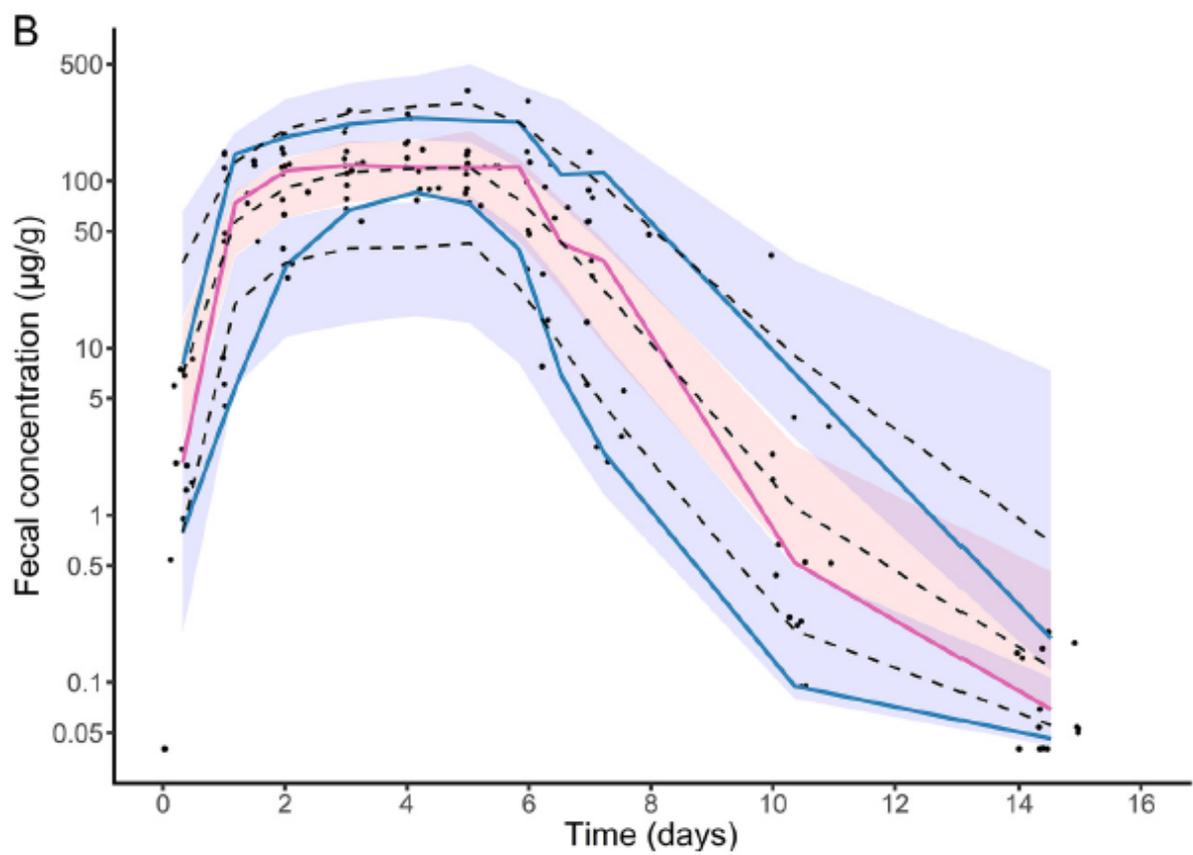
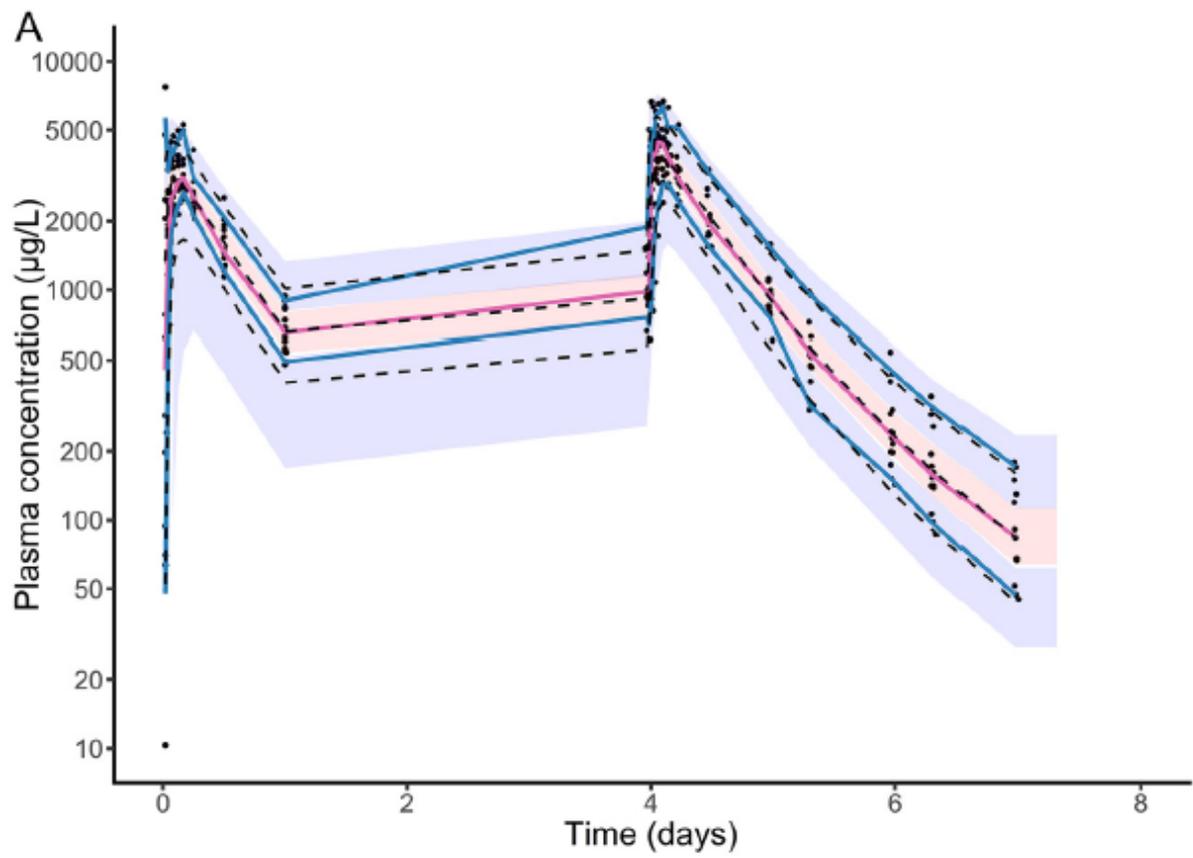


FIG2

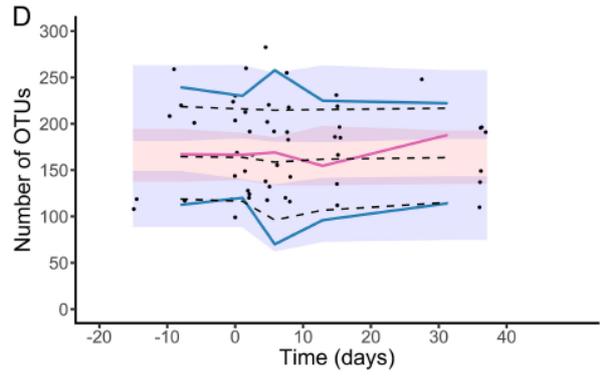
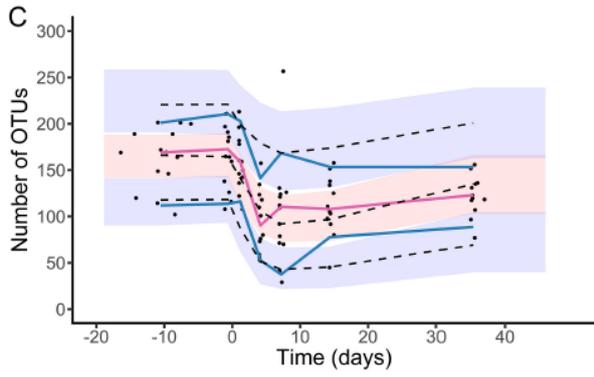
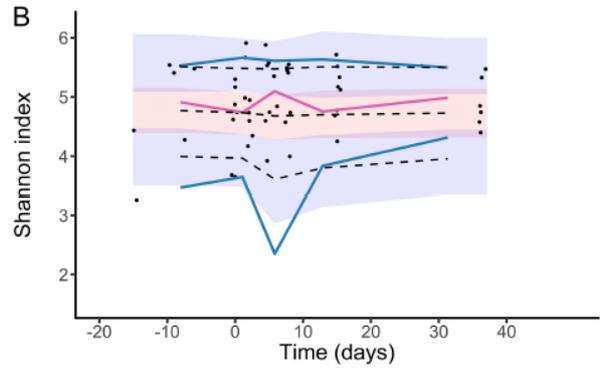
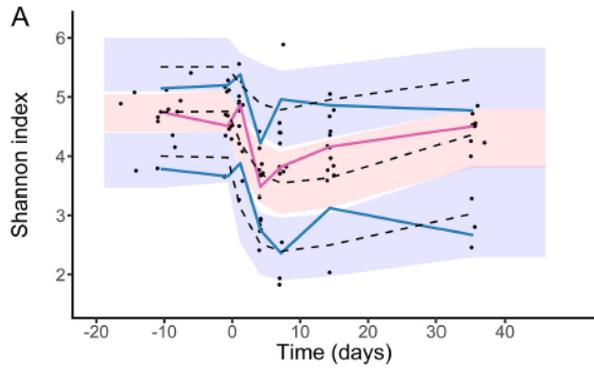


FIG3

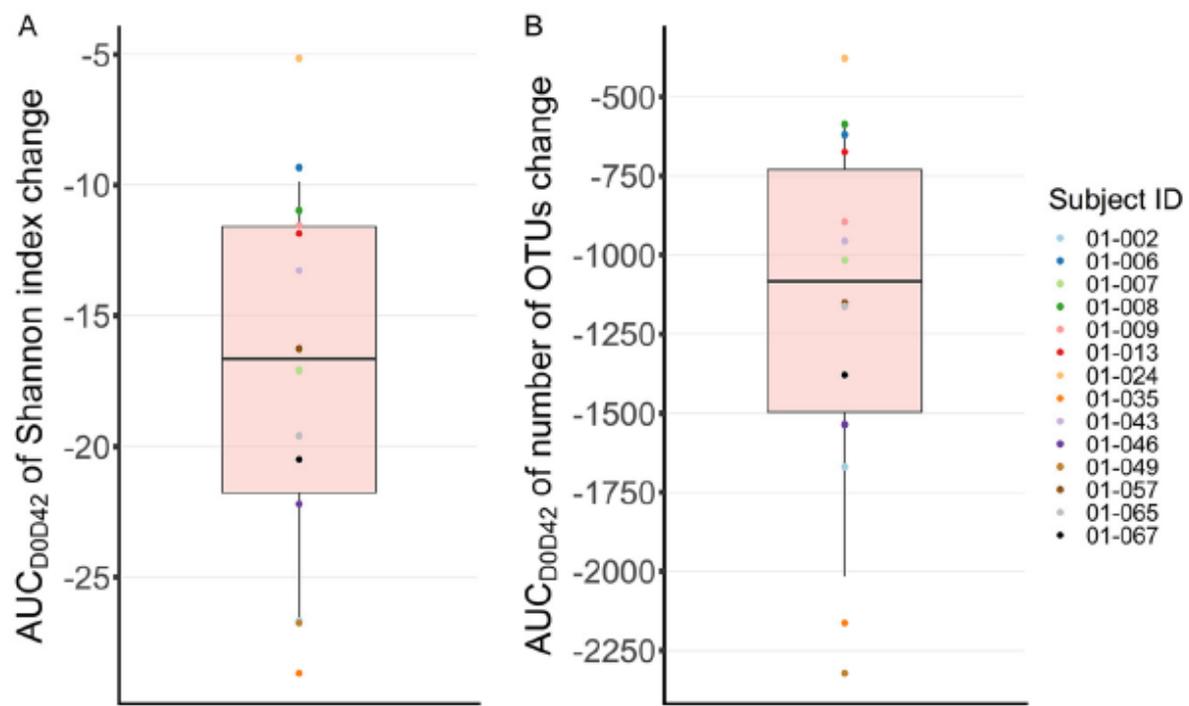


FIG4