


The intestinal microbiota predisposes to traveler's diarrhea and to the carriage of multidrug-resistant Enterobacteriaceae after traveling to tropical regions

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ABSTRACT

The risk of acquisition of multidrug-resistant Enterobacteriaceae (MRE) and of occurrence of diarrhea is high when traveling to tropical regions. The relationships between these phenomena and the composition of human gut microbiota have not yet been assessed. Here, we investigated the dynamics of changes of metabolically active microbiota by sequencing total RNA from fecal samples taken before and after travel to tropical regions. We included 43 subjects who could provide fecal samples before and after a travel to tropical regions. When found positive by culturing for any MRE after travel, the subjects sent an additional sample 1 month later. In all, 104 fecal samples were considered (43 before travel, 43 at return, 18 one month after travel). We extracted the whole RNA, performed retrotranscription and sequenced the cDNA (MiSeq 2x300bp). The reads were mapped to the reference operational taxonomic units (OTUs) and species/strains using the 16S Greengenes and 23S SILVA databases. We found that the occurrence of diarrhea during the travel was associated with a higher relative abundance of *Prevotella copri* before departure and after return. The composition of microbiota, before travel as well as at return, was not correlated with the acquisition of MRE. However, the clearance of MRE one month after return was linked to a specific pattern of bacterial species that was also found before and after return. In conclusion, we found specific OTUs associated to a higher risk of diarrhea during a stay in tropical regions and to a faster clearance of MRE after their acquisition.

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Introduction

Multidrug-resistant Enterobacteriaceae (MRE) that produce extended-spectrum beta-lactamases (ESBLs), plasmid-encoded AmpC-type cephalosporinases (pAmpC), and/or carbapenemases (CP) have been spreading in the community over the last two decades.¹ MRE represent a major public health issue, as a limited number of antibiotics remains active against these bacteria while very innovative antibiotics are expected to reach the market in the near future.² The spread of MRE

has been particularly intense in tropical regions, likely owing to poor hygiene conditions and uncontrolled antibiotic usage.³ Consequently, between 14% and 69% of travelers to tropical regions are reported to acquire MRE, depending on the cohort and specific destination.^{4,5} Besides, a high proportion of travelers to these destinations also report the occurrence of diarrhea (traveler's diarrhea) during their trip.^{4–8} Among travelers who acquire an MRE during a travel to tropical regions, the observed median carriage is short (≤ 1 month).^{5,6} Still, some of them experience long-

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 Supplemental data for this article can be accessed on the [publisher's website](#).

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term carriage of MRE, extending up to 1 year in 2.2–11% cases.^{5,6}

The capacity of the intestinal microbiota to resist long-term settlement of exogenous bacteria (including MRE) is referred to as colonization resistance^{9–11} which is mainly exerted by anaerobes.^{9,10} Antibiotics with high activity against anaerobes strongly affect the capacity of the microbiota to prevent colonization by exogenous microorganisms, and thus favor the acquisition and expansion of resistant bacteria.¹² Hence, the restoration of the intestinal microbiota through fecal material transplantation (FMT) has been shown to lower the intestinal concentrations of vancomycin-resistant enterococci (VRE),¹³ MRE¹⁴ and more globally, of antibiotic resistance genes.¹⁵ Moreover, the administration of a limited set of intestinal bacteria to mice colonized with VRE reduced the intestinal densities of VRE, suggesting that specific bacteria are involved in colonization resistance.¹⁶

To date, the link between the composition of the intestinal microbiota and the acquisition of MRE during travel and their clearance after return has not been assessed.

Here, we questioned whether the composition of the intestinal microbiota could be associated with the occurrence of diarrhea and the acquisition of MRE during travel and to the clearance of MRE after return. In particular, we analyzed the pre- and post-travel composition of metabolically active microbiota which has been previously shown to provide a better differentiation between ESBL-carriers and non-carriers.¹⁷

Methods

Population

The travelers' cohort originates from the VOYAG-R study (ClinicalTrials.gov n°NCT01526187) funded by the French Ministry of Health.^{6,18} From February 2012 to April 2013, subjects attending six international vaccination centers in the Paris area, prior to traveling to tropical regions, were asked to provide fecal samples before and after their trip. Only volunteers who had no detectable MRE in the feces taken before their departure, were asked to send a further stool sample within a week after their return. Travelers who revealed positive for MRE after their

return were asked to provide additional stool samples 1, 2, 3, 6 and 12 months after their return until MRE was no longer detected. Among the 574 included subjects, 292 (51%) acquired at least one MRE.⁶ From November 2012 to March 2013, travelers were provided a supplementary 50 mL RNALater® (Applied Biosystems, Villebon-sur-Yvette, France) containing vial before and after travel and one month after return when found positive for the acquisition of MRE. A total of 43 travelers were provided additional RNALater® containing pots, among whom 18 (41.8%) acquired an MRE (mostly ESBL-producing *Escherichia coli*) during their trip. The comparison between travelers providing additional samples stored in RNALater® (n = 43) and those who did not (n = 534) is described in the Supplementary Table 1. Of note, they experienced less diarrhea (9/43 [20.9%] vs 219/531 [41.7%], p-value <0.01), likely due to different travel destinations (e.g., none of RNALater® travelers went to Peru at that time, Supplementary Figure 8). All of them provided a stool sample one month after they returned, among whom six were still carrying an MRE. Thus, 104 samples were selected (43 before travel, 43 at return, and 18 one month after travel).

Since the number of individuals per visited country was too small to perform meaningful statistical analyses (Supplementary Figure 8), we grouped travelers according to the corresponding continental area of destination: Asia, Sub-Saharan Africa, and Latin America. In particular, of these 43 travelers 15 went to Asia, 15 to Sub-Saharan Africa, and 13 to Latin America.

RNA extraction from stool samples and sequencing

Total RNA (mostly made of ribosomal RNA, e.g., 16S, 23S, and 5S) was extracted for 104 stool samples using the RNeasy Plus Mini Kit (Qiagen, Gaithersburg, USA). The concentration of RNA obtained was measured by Qubit RNA BR Assay Kit or Qubit RNA HS Assay Kit (Life Technologies, Reinach, Switzerland). For simplicity, the terms 16S and 23S refer to 16S and 23S ribosomal RNAs, respectively. The integrity of RNA (ratio 16S/23S) was assessed by the RNA 6000 Nano Kit and RNA 6000 Pico Kit (Agilent Technologies, Plan-les-Ouates, Switzerland) on the Bioanalyzer 2100 system (Agilent Technologies,

Waldbronn, Germany). The detailed protocol can be found in the Supplementary Methods. One hundred nanograms of purified RNA in 10 μ L total volume were sent to LGC (Berlin, Germany) for (i) cDNA synthesis using NEBNext mRNA First/Second Strand Synthesis Module (New England Biolabs, Ipswich, USA), (ii) shotgun library preparation using NuGEN Ovation Rapid Library System (NuGEN, San Carlos, USA), and (iii) sequencing (2 \times 300 bp) of size-selected fragments (about 300–400 bp) using v3 Illumina chemistry and half the capacity of a MiSeq (Illumina, San Diego, USA) flow cell.

Bioinformatics and statistical analyses

Detailed bioinformatics and statistical methods are reported in Supplementary Materials. Briefly, paired reads were merged and quality-filtered using BBMerge 35.43 (<http://bbmap.sourceforge.net>) and Mothur v1.35.1,¹⁹ respectively. Operational taxonomic units (OTUs) and bacterial strains species were identified by mapping reads to 16S Greengenes²⁰ and 23S SILVA²¹ databases by USEARCH.²² The mean mapping rates of quality-filtered merged reads against the Greengenes 16S and SILVA 23S databases were 31.0% (median = 31.0%) and 42.8% (median = 43.2%), respectively. The fraction of non-ribosomal mapping reads was on average 26% (median = 25%). Statistical analyses were performed in PRIMER v6 (PRIMER-E Ltd, Plymouth, UK) and in the R software v3.2.3.

Results

Effect of traveling on microbiota composition

To test the hypothesis whether the modalities and the duration of traveling to tropical regions has an impact on microbiota composition, we analyzed the microbiota profiles at return of 39 travelers that were not administered antibiotics during their trip. Four subjects who took amoxicillin (n = 1), ciprofloxacin (n = 1) and nifuroxazide (n = 2) were not considered. Six subjects who took doxycycline were considered though as we did not detect any effect of doxycycline on the composition of the intestinal microbiota (Table 1). Likewise, we did not observe any significant influence on microbiota composition in relation to the visited region, the type of travel, duration of travel, to the use and the type of malaria prophylaxis (Table 1). However, the proportions of Enterobacteriaceae increased during the travel in all the 39 individuals (Supplementary Figure 3A).

Moreover, we analyzed microbiota profiles before departure, at return, and 1 month after the return of 17 travelers who acquired MRE during their journey and were not treated with antibiotics at return. We observed that the fecal samples taken at different time points (before departure, at return and one month after return) clustered by subject (global PERMANOVA test, p-value <0.0001; Supplementary Figure 5F-G), while travel did not have a significant impact on microbiota profiles (pairwise PERMANOVA tests, p-values ranged between 0.3 and 0.9).

Table 1. Summary of global PERMANOVA analyses performed on the cohort of 39 travelers at return.

Variables	Conditions	rRNA		
		subunit	Pseudo-F	p-value
Region	Sub-Saharan Africa/Latin America/Asia	16S	0.89	0.75
		23S	0.81	0.82
Type of travel	All-inclusive resort/Organized tour/visiting family/mix of all-inclusive resorts and organized tours/backpacking	16S	1.08	0.25
		23S	1.11	0.23
Duration of travel (*)	From 1 to 9 weeks	16S	1.04	0.35
		23S	0.82	0.71
Malaria prophylaxis	No/Yes	16S	0.79	0.86
		23S	0.76	0.80
Type of malaria prophylaxis	Atovaquone-proguanil/Chloroquine/Doxycycline/None	16S	0.95	0.57
		23S	0.92	0.63

(*) The test done was distance-based linear models (DistML; see Supplementary Methods).

Occurrence of diarrhea during the travel

In pre-travel microbiota of people who suffered from diarrhea during the travel ($n = 9$) the relative abundance of *Prevotella copri* species was >2-fold higher than in subjects who did not experience this condition ($n = 34$) (Wilcoxon rank sum test, p -value <0.05; Supplementary Figure 1G-H). Nevertheless, the overall composition of pre-travel microbiota was not significantly associated with the occurrence of diarrhea during travel (Table 2; Supplementary Figure 1E).

We, then, analyzed the intestinal microbiota profiles at return by excluding those four travelers who had taken antibiotics during their trip. Microbiota profiles from subjects who had diarrhea significantly differed from those who had not (Table 2; Figure 1(a)). The occurrence of diarrhea was associated with a microbiota significantly less rich and diverse at return as compared to the microbiota of individuals who did not experience this condition during the travel (Wilcoxon rank sum test, p -value <0.05; Figure 1(b)).

At return, people having experienced diarrhea during their travel presented increased proportions of Bacteroidetes and Proteobacteria phyla and decreased proportions of Firmicutes phylum compared to travelers without diarrhea (Figure 1(c)). The great majority of OTUs found to be differentially abundant after travel between people with and without diarrhea mapped to Enterobacteriaceae

Table 2. Summary of pairwise PERMANOVA tests performed at each travel time point (*).

Travel time point	Number of travelers	Variable	rRNA subunit	t	p-value
Before travel	43	Diarrhea	16S	1.05	0.25
		during travel	23S	1.1	0.18
		MRE	16S	1.09	0.15
		acquisition	23S	1.05	0.29
At return	39	Diarrhea	16S	1.44	0.003
		during travel	23S	1.8	0.0007
		MRE	16S	1.12	0.12
		acquisition	23S	1.13	0.15
One month after the return	17	Diarrhea	16S	1.04	0.3
		during travel	23S	0.97	0.6
		MRE carriage	16S	1.21	0.01
		at return	23S	1.16	0.07

(*) The number of samples analyzed varied among travel time points according to the use of antibiotics and MRE acquisition. Before travel, we have considered all the 43 travelers. At return, we analyzed only subjects who did not take antibiotics during the travel. One month after the return, we considered travelers who acquired MRE during travel and were not treated with antibiotics after the trip.

family, Clostridiales order, and the *P. copri* species (LEfSe and Wilcoxon rank sum test, p -value <0.05; Figure 1(d); Supplementary Figure 2A). In particular, the fecal samples taken at return from travelers who reported diarrhea showed a >2-fold higher proportion of Enterobacteriaceae and *Prevotella copri* and a <2-fold lower proportion of Clostridiales as compared to travelers without diarrhea. The relative abundance of *P. copri* was increased at return only in a fraction of travelers reporting diarrhea (Supplementary Figure 3B).

The results obtained for the samples collected at return from travel were also confirmed by 23S analyses (Supplementary Figure 2B-E). Most Enterobacteriaceae members associated with diarrhea mapped to 23S genes of *Escherichia-Shigella*, while in the 16S analysis the discriminating OTUs from Enterobacteriaceae were not classified at the genus level. Clostridiales included genera belonging to Ruminococcaceae (*Ruminococcus*), Bacteroidaceae (*Bacteroides*) and Lachnospiraceae (*Roseburia*; only for 16S dataset: *Lachnospira* and *Blautia*).

We then investigated whether diarrhea had protracted effects on gut bacteria composition one month after the return. Therefore, we analyzed samples from 17 travelers who did not take antibiotics during the first month following the return. We did not detect significant differences in the overall microbiota composition in relation to diarrhea (Table 2; Supplementary Figure 5A). Enterobacteriaceae relative abundance returned close to basal level in all 17 travelers but with statistical significance only when the analyses included the individuals who did not suffer from diarrhea (Wilcoxon signed rank test, p -value <0.05; Supplementary Figure 5C-D). One month after return, *Prevotella copri* abundance was still increased in travelers who had diarrhea (Supplementary Figure 3B) whereas Clostridiales members were more abundant in individuals without diarrhea (Supplementary Figure 4).

Acquisition and carriage of MRE

The acquisition of MRE was not associated with a specific microbiota profile neither before travel nor at return (Table 2; Supplementary Figures 1F and 5E).

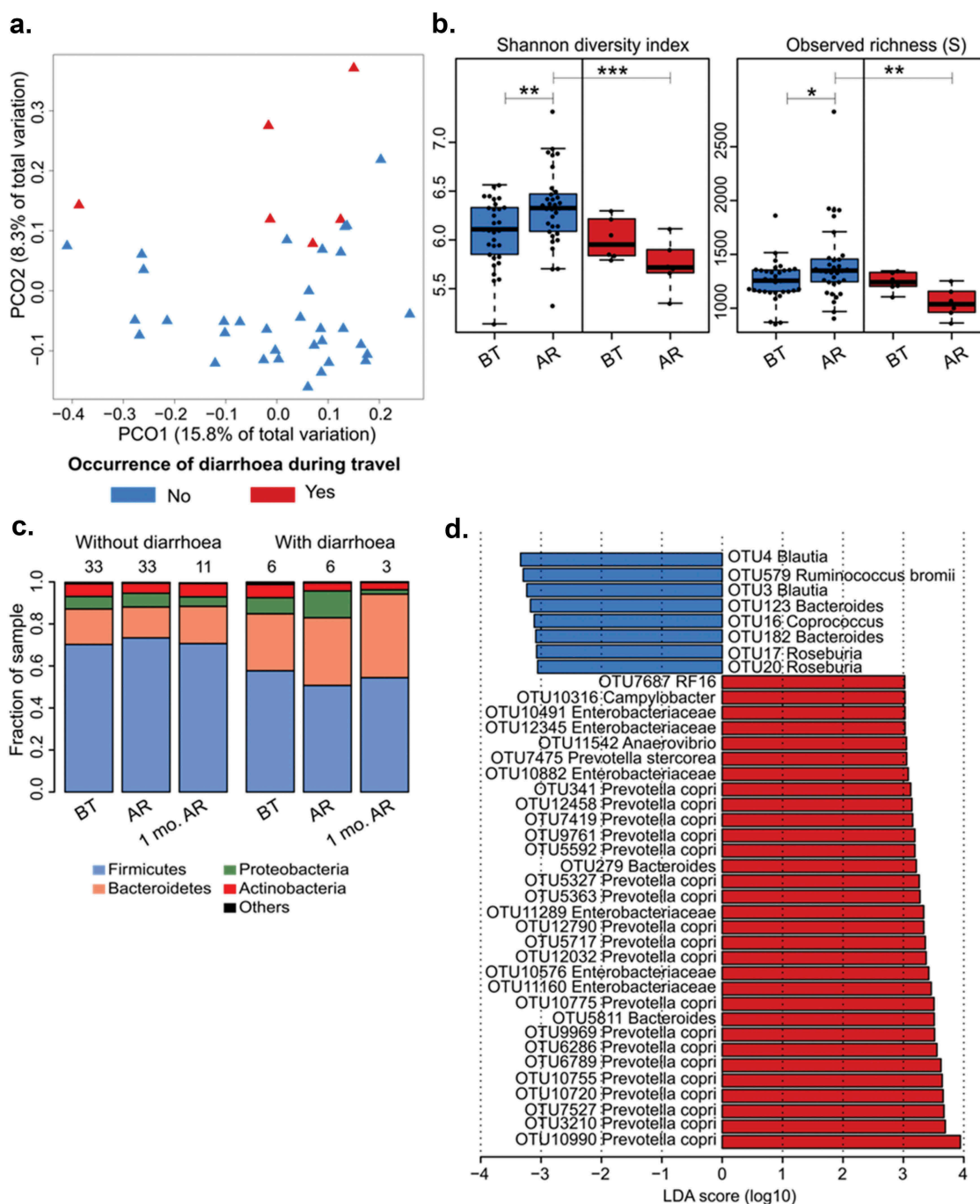


Figure 1. Microbiota changes with respect to the occurrence of diarrhea during the travel. (a) PCoA plot showing the distribution of samples taken at the return, from travelers who had (red triangles) or not (blue triangles) diarrhea during the travel. Of the 6 individuals who suffered from diarrhea 3 went to Asia, 2 to Sub-Saharan Africa and 1 to Latin America. Percentage of variation explained by the first two axes is indicated. (b) Boxplots and dot plots depicting the values and their corresponding distributions of ecological indexes, computed at the 16S OTU level, before travel (BT) and at return (AR), between travelers experiencing or not diarrhea. Stars correspond to the following p-values: * = <math>p < 0.05</math>; ** = <math>p < 0.01</math>; *** = <math>p < 0.001</math>. (c) Bar plots reporting the averaged relative abundance (/100) of phyla detected by mapping to 16S Greengenes database in travelers without diarrhea (n = 33 of which 11 were still MRE positive one month after the return) and those with (n = 6 of which 3 were still MRE positive one month after the return). Numbers at the top of the bars indicate the amount of samples for each travel time point/condition. (D) Bar plot reporting log₁₀-transformed LDA scores of OTUs selected by LEfSe analyses (p-value < 0.05). Cohorts of samples and colour label are the same as explained in (a). Only OTUs with the absolute value of LDA score (scaled in log₁₀) of at least 3 are represented. GreenGenes taxonomy identifiers for all OTUs are reported in Supplementary Table 4.

We also investigated the association of microbiota composition and the MRE carriage one month after return in the 17 travelers found MRE-positive at return and not treated with antibiotics (one subject took amoxicillin after return). In this case, the composition of the intestinal microbiota of the subjects whose samples became MRE-negative ($n = 11$) was significantly different from those whose samples remained MRE-positive ($n = 6$) (Table 2).

OTUs assigned to *Bifidobacterium adolescentis* (OTU6129) and to some Clostridiales (OTU61, OTU7384, OTU405, OTU8089) as well as *Bifidobacterium* strains detected by 23S analyses, were enriched in individuals who had cleared MRE one month after the return (LEfSe and Wilcoxon rank sum test, p -value < 0.05 ; Figure 2(a-b); Supplementary Figure 6A-B). Already before departure, the proportions of these bacteria were higher in MRE carriers who cleared their carriage one month after return than in those who were still positive (Figure 2(a); Supplementary Figure 6A). For most OTUs associated with MRE clearance, the species level taxonomy of *de novo* assembled 16S gene was not available (Supplementary Table 2).

Remarkably, within the subcohorts of 17 travelers, we found that the microbiota profiles of MRE cleared individuals were significantly different from persistent MRE carriers before departure and one month after return (Supplementary Table 3; Figure 2(c); Supplementary Figure 6C).

The observed species richness measured before travel was significantly lower in individuals who acquired MRE during the travel and remained MRE carriers one month after return than in those who cleared their carriage (Supplementary Figure 5B). We found that the relative abundance of several OTUs and strains/species mapping to Enterobacteriaceae significantly decreased more than 2 fold one month after the return, independently of MRE carriage (Supplementary Figure 7). Despite that, the abundance of Proteobacteria phylum, to which Enterobacteriaceae belong, did not change over time or in relation to MRE carriage measured one month after the return (Figure 2(d); Supplementary Figure 6D).

Discussion and conclusions

One of the main results of this study is that travelers who experienced diarrhea (regardless of the etiology) had a higher intestinal abundance of *P. copri*, before the travel, at return, and 1 month after, than those who did not. This suggests that subjects with higher relative abundance of *P. copri* could be at higher risk for diarrhea during travel. Interestingly, *P. copri* has popped up in various metagenomic studies as either a beneficial or pathogenic bacterium: it has been associated to rheumatoid arthritis,^{23,24} insulin resistance²⁵ but also to good health status^{26,27} and to an improved glucose homeostasis.²⁸ In relation to diarrhea, contradictory findings on the role of *P. copri* were documented. This species has been positively associated with parasitic diarrhea²⁹; however, it has also been reported to have a potential protective effect against diarrhea caused by enterotoxigenic *E. coli*.³⁰ Furthermore, *Prevotella* species are involved in the recovery from choleric diarrhea³¹ and have lower relative abundance in malnutrition-associated diarrhea.³² These contradictory findings could be explained by the high inter-strain and inter-individual genetic variations of this species,³³ suggesting that different strains have various functions, antigens and/or metabolites being either beneficial or deleterious for the host. Another characteristic of microbiota profiles of people suffering diarrhea is decreased species richness at return. Diminution of species richness upon diarrhea occurrence is also described in one study by Youmans and colleagues addressing by 16S profiling the intestinal microbiota of travelers returning to USA from Central America or India.³⁴ Oppositely, subjects suffering from a Norovirus-caused diarrhea had a surprisingly higher intestinal richness and diversity than diarrhea-free travelers. However, comparison of the results from Youmans *et al.* and this study are compromised by the low numbers of reads analyzed per sample (< 3000), the absence of pre-travel samples and the collection of samples from symptomatic subjects (while the subjects from VOYAG-R were sampled at distance from diarrheal symptoms).³⁴

We observed that the travelers who did not experience diarrhea during travel had a microbiota profile

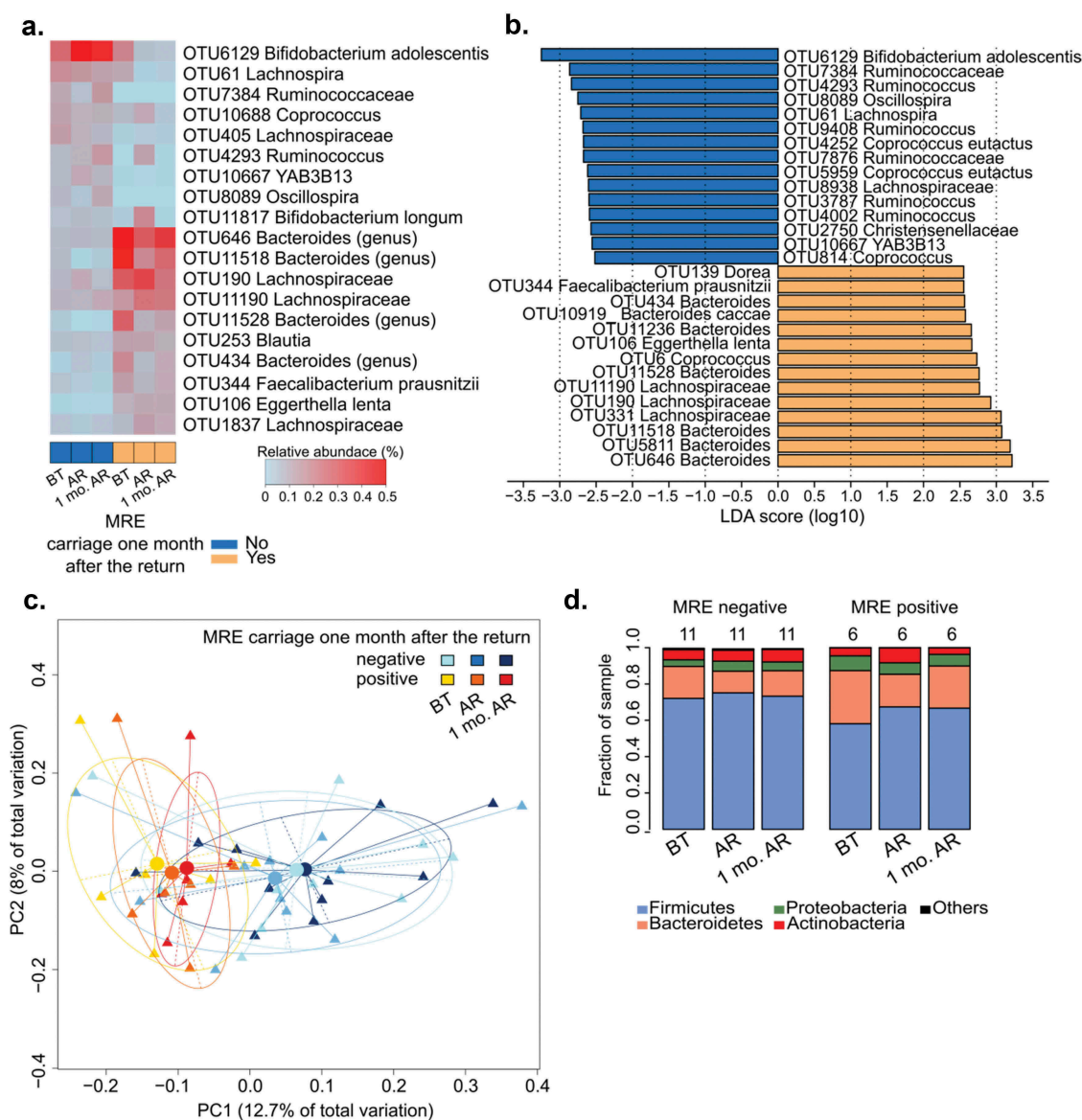


Figure 2. Comparison of travelers who acquired MRE and remained positive (n = 11) or became negative (n = 6) one month after return. (a) Heat map illustrating the mean relative abundance (expressed as percentage of total reads) of 19 OTUs in the three sampling points (BT, AR and 1 mo. AR = before travel, at return and one month after the return, respectively) of travelers MRE positive (yellow bottom bar) and MRE negative (blue bottom bar) one month after the return. OTUs were selected if the p-value was < 0.05 (Wilcoxon rank sum test), the fold change was of at least 2, and when the mean relative abundance was of at least 0.1% in one of the six represented conditions represented (i.e. BT, AR and 1 mo. AR of MRE-positives and of MRE-negatives one month after the return). Greengenes taxonomy identifiers for all OTUs are reported in Supplementary Table 4. (b) Barplot reporting log₁₀-transformed LDA scores from each OTUs resulting significant (p-value < 0.05) from LEfSe analyses on samples from travelers MRE positive (yellow) and MRE negative (blue) one month after the return. Only OTUs with an absolute value of LDA score (scaled in log₁₀) of at least 2.5 were retained for graphic representation. (c) PCoA plot showing the distribution of samples from time points of travelers MRE negative and MRE positive one month after return. PCO1 (12.7%) and PCO2 (8%) represent the degrees of variance of each axis. Samples are grouped in clusters delimited by ellipses. Represented centroids (spheres) capture the origin of each ellipsis. (d) Bar plots reporting the averaged relative abundance (/100) of phyla detected by mapping to 16S Greengenes database in travelers MRE negative (n = 11) and MRE positive travelers (n = 6) one month after return.

enriched in members of *Ruminococcus*, *Coprococcus*, and *Dorea*. Consistently with our findings, these genera were depleted in 16S microbiota profiles of individuals suffering from post-transplantation³⁵ and

nosocomial diarrhea, including *Clostridium difficile* infection.³⁶ Moreover, *Roseburia* and *Ruminococcus* species have been shown to prevent gut inflammation by strengthening gut barrier function in mice³⁷ and by

enhancing starch fermentation in humans,³⁸ respectively. In addition to an anti-inflammatory role, *Ruminococcus* species were reported to oppose *Vibrio cholerae* colonization in a mouse model by deregulating the expression of virulence factors via quorum sensing.³⁹ Subjects who did not experience diarrhea during the travel had a richer and more diverse microbiota after travel than before. However, 1 month after return, the diversity and richness for those individuals tended to decrease to a baseline (pre-travel) level. Increase in richness and diversity at return when diarrhea is not experienced could reflect the ingestion of new bacteria that are not met in France, and/or the consumption of food that could act as prebiotics for bacteria in the pre-travel sample.

Besides, we observed an increase of Enterobacteriaceae in all travelers, which was more significant in those subjects who experienced diarrhea during their trip. This could be expected as several diarrhea agents – *E. coli*, *Shigella* and *Salmonella* – belong to the Enterobacteriaceae family.⁴⁰ This increase was transient and the relative abundance of Enterobacteriaceae returned close to baseline one month after travel.

Another significant result of this study is the association between intestinal microbiota composition and clearance of MRE in healthy travelers. Indeed, we observed that a set of bacteria from the Clostridiales order was more abundant in travelers who had no detectable MRE one month after return than in those who remained MRE carriers. Moreover, this pattern was already observed before travel. In addition, among the subjects who acquired MRE during travel, bacterial richness and diversity at baseline (before travel) were lower in individuals who remained MRE carriers one month after travel than in those who did not.

Altogether, our findings support the concept that the intestinal microbiota affects MRE clearance. These results are in line with the observations performed in mice in which some specific OTUs were shown to be associated with the clearance of vancomycin-resistant enterococci¹⁶ – *Listeria monocytogenes*⁴¹ and *Clostridium difficile*⁴² – but also with the MRE clearance after fecal material transplantation.¹⁴ Nonetheless, these phenomena were observed after an alteration of the microbiota by antibiotics, whereas our observations were obtained from healthy, antibiotic-free travelers.

The main limitation of this study is that the number of samples is relatively low with regards to some variables such as MRE carriage after the return and the occurrence of diarrhea. We do not exclude that some travelers might have cleaned their gut already before return and therefore this has lowered the number of MRE positive travelers detected after the travel. To overcome the limitation concerning the low number of samples, we combined several bio-statistical and bioinformatics approaches which produced consistent results. Of note, we took advantage of using total RNA (rich in ribosomal RNA) to bypass the need for amplification of a specific region of the 16S gene (that leads to biases) and to allow a separate analysis on 16S and 23S taxonomic markers. On the other hand, with our approach, we did not provide insights into bacterial functions since mRNA enrichment steps were not carried out. Another limitation is that we could not precisely identify and characterize the bacterial species associated with the intestinal clearance of MRE despite our attempts to assemble the full 16S genes, hindering the realization of in vitro and in vivo experiments to demonstrate the precise role of these bacteria. Metagenomic sequencing that allows functional analysis could now be used on a similar setting to identify the genes associated with the clearance of MRE irrespectively of the taxonomy of their host. Besides, the etiological agents of traveler's diarrhea were not sought as it was outside of the VOYAG-R study's scope. Consequently, we could not link the composition of the microbiota to the presence of a specific pathogen. Still, the intestinal alteration due to traveler's diarrhea seems to be pathogen-independent.³⁴ Also, the loperamide intake (potentially involved in the acquisition of MRE⁴³) was not recorded in the VOYAG-R study. Finally, we did not consider patients taking antibiotics (other than doxycycline taken for malaria prophylaxis) during the travel and after they returned because we aimed at assessing the link between the composition of the intestinal microbiota and the acquisition/clearance of MRE and the occurrence of diarrhea. Nonetheless, excluding these travelers may prevent from extrapolating our observations to travelers who took antibiotics during the travel

or after they returned. Likewise, our subjects were French citizens traveling to tropical regions and our findings should be confirmed in subjects from other origins.

In conclusion, we showed that the composition of intestinal microbiota is associated with the risk of occurrence of diarrhea in healthy travelers and of carriage of MRE in those who acquired MRE during the travel. Our results call for further functional explorations of the interplay between the intestinal microbiota, traveler's diarrhea, and MRE carriage.

Data availability

Mothur-quality-filtered sequences were deposited as fastq files at the European Nucleotide Archive (ENA) under the project PRJEB24843. Prior to sequences' submission, reads assigned to human genome (vGRCh38.p10) by CLARK (v1.2.3.2)⁴⁴ were removed. CLARK taxonomic classification was performed at the phylum level (Chordata).

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Author contributions

ER, LAL, SM, CE, and AA conceived and designed the study. ER, JS and VL supervised the study. The VOYAG-R study group performed the princeps's study. MG and SL performed RNA extraction. ER, VL, SL designed the statistical analyses. SL, VL, and ER analyzed the data. NG helped with read pre-processing. SL, VL, and ER wrote the manuscript. SL assembled all the Figures. JS, CE, and SM revised the manuscript. All authors read and approved the final manuscript.

Disclosure of Potential Conflicts of Interest

All authors have no potential conflicts of interest.

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