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Gut Microbiota Dynamics in Travelers Returning from India Colonized with Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae*: a Longitudinal Study

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Abbreviations: extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESC-R-Ent), multidrug-resistant (MDR), sequence types (STs), extended-spectrum β -lactamase (ESBL), Shannon Diversity Index (SDI), variance adjusted weighted UniFrac (VAW-UniFrac), operational taxonomic units (OTU), Principal Coordinate Analysis (PCoA), Non-metric multidimensional scaling (NMDS), MANOVA (Multivariate analysis of variance), false discovery rate (FDR), European Nucleotide Archive (ENA).

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ABSTRACT

Background: Intestinal colonization by extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESC-R-Ent) has been attributed to travel to high prevalence countries. However, the dynamics of the microbiota changes during ESC-R-Ent colonization and whether there is a particular bacterial composition which is associated with subsequent colonization is unknown.

Methods: Forty healthy volunteers living in Switzerland underwent screening before and after a trip to India, and also 3, 6 and 12 months after traveling. Culture-based ESC-R-Ent screening and microbiota analysis based on 16S rRNA amplicon sequencing were performed at all time points.

Results: Prevalence of ESC-R-Ent colonization before traveling was 10% (n=4), whereas it increased to 76% (n=31) after the trip. Based on bacterial diversity analyses of the gut microbiota, there were few but significant differences for colonized versus non-colonized individuals. However, an alternative, cluster based analysis revealed that individuals remained in the same cluster over time indicating that neither traveling nor ESC-R-Ent colonization significantly influences bacterial composition. Moreover, none of the found microbiota clusters were significantly associated with subsequent risk of ESC-R-Ent colonization.

Conclusion: Based on their microbiota patterns, every volunteer was at the same risk of ESC-R-Ent colonization while travelling to India. Therefore, other risk factors for ESC-R-Ent colonization are responsible for this phenomenon.

1. INTRODUCTION

Intestinal colonization by extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESC-R-Ent) is a very well-known phenomenon frequently observed in travelers returning from countries like India with a high prevalence of these multidrug-resistant (MDR) bacteria (1-3). Therefore, it is not surprising that increasing rates of intestinal carriage are reported worldwide (1, 2).

Although in healthy people this colonization status is thought to be transient, the continuous importation of ESC-R-Ent in low prevalence countries significantly contributes to the spread and expansion of these pathogens. For instance, in Switzerland (a low prevalence country for MDR bacteria) the overall prevalence of ESC-R-Ent among in- and outpatients in the period 2004-2011 jumped from 1% to 5.8%, especially in community patients (from 0.9% to 5.3%) (4).

Aside from the specific destination of the journey, other risk factors have been associated with the acquisition of ESC-R-Ent in the gut, namely antibiotic intake and travelers' diarrhea (5). With regard to the antibiotic use, it is assumed that it affects bacterial diversity and enables colonization with MDR pathogens (6); however, different antibiotics may have a different effect on the microbiota leading to different consequences (7).

Upon arrival and stay at a new destination, a host is faced with many new conditions, such as stress, dietary shifts, and exposure to MDR pathogens present in both environment and food chain (5). These factors will exert different influences on the intestinal bacterial community, as demonstrated observing microbiota shifts after travelers' diarrhea (8) and in *in vivo* studies using country-specific diets (9). Moreover, it has been shown that people from different countries also exhibit a country specific human intestinal microbiota (10).

Given that ESC-R-Ent are known to have particular adaptations to specific host microenvironments (e.g., different metabolic capacities), these can have particular advantages that might lead to better intestinal colonization and persistence (11). Nevertheless, studies

with a longitudinal design assessing the potential effects of MDR bacteria over time in the microbiome are lacking.

In the present study, we aimed to assess the post-trip temporal variations of gut microbiota in healthy adults traveling to India to elucidate the effects of ESC-R-Ent colonization on these individuals. In particular, we tried understanding whether specific microbiota patterns may translate in an increased risk of being colonized by these MDR pathogens.

2. MATERIALS AND METHODS

2.1. Volunteer Recruitment and Study Design

Between December 2014 and September 2015, forty adult healthy volunteers living in Switzerland and traveling to India were recruited at the travel clinic of the Swiss Tropical and Public Health Institute (Basel, Switzerland) where they signed an informed consent. Subjects were instructed to self-collect native stools in sterile plastic containers and send them to the Institute for Infectious Diseases (IFIK) of the University of Bern (Bern, Switzerland). Five distinct time points were defined for sample collection for each individual: within one week before travel (T_0), within one week after travel (T_{1w}), 3 (M3), 6 (M6), and 12 months (M12) after returning from the journey. Ethical approval was obtained by the Ethikkommission Nordwest- und Zentralschweiz (EKNZ 239/12).

2.2. Assessment of Gut Colonization due to ESC-R-Ent

As previously described, selective culture-screening and microbiological methods were implemented to assess intestinal colonization due to ESC-R-Ent and characterize strains (12). Briefly, a small portion of native stools was enriched overnight in Luria-Bertani broth containing cefuroxime [3 mg/L] and plated on BLSE, ChromID ESBL (bioMérieux) and Supercarba selective plates.

Positive ESC-R-Ent colonization was defined when *Enterobacteriaceae* species expressed an ESBL and/or a plasmid-mediated AmpC (pAmpC) based on phenotypic (e.g., MIC values for different antibiotics) and molecular methods (e.g., PCR/sequencing and microarray analyses) (12).

2.3. Microbiota analysis

Whole DNA was extracted from native stools with QIAamp DNA Stool Mini Kit (QIAGEN). Amplification of the V4 region of the 16S ribosomal RNA (rRNA) gene was performed as

previously described using the following primers with an Illumina adaptor sequence at the 5' end: V4_fw: TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG CCA GCM GCC GCG GTA A, and V4_rv: GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACH VGG GTW TCT AAT (13, 14). In brief, high throughput sequencing of obtained 16S rRNA amplicons was performed using Illumina MiSeq 2x250bp v2 chemistry. PCR products were subsequently purified with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) using QIACUBE (QIAGEN) and eluted in purified nuclease-free water with a final volume of 30 µL. DNA quantification was performed using the DNA 7500 kit with an Agilent 2100 Bioanalyzer (Agilent). Nextera XT libraries were prepared according to the Illumina MiSeq Protocol. Library preparation and high throughput sequencing steps were performed at the Next Generation Sequencing Platform, Institute for Genetics, University of Bern.

2.4. Analysis of sequence reads and definition of OTU

Obtained sequences were processed and analyzed using mothur version 1.39.0 (15), following the MiSeq standard operation procedure (16). Paired-end reads were aligned and all reads containing ambiguous bases, stretches of homopolymers longer than eight nucleotides, sequences longer than 260 base pairs, and sequences that did not align to the target region. Chimeras were identified and removed using UCHIME software (17) and sequences aligning to chloroplasts, mitochondria, *Archaea* and *Eukaryotes* were detected and removed as well. Operational taxonomic units (OTUs) were determined with average neighbor algorithm, using a 3% dissimilarity threshold and the taxonomy was assigned using SILVA alignment as a template (18). The data was normalized by random subsampling of sequences (rarefying). The SILVA database was used for taxonomical assignment (19). A phylogenetic tree based on the obtained OTUs was created with Clearcut (version 1.0.9.) and rooted with Figtree (version 1.4.2).

2.5. Alpha diversity calculations

Microbial community analysis was carried out using the packages *vegan* (version 2.4-3) and *phyloseq* (version 1.19.1) (20, 21) in R. Alpha-diversity was calculated using richness and the SDI. To correct for the dependency nature of the longitudinal study, a linear mixed model adjusted for colonization and time point was used to determine the influence of these factors on the richness and SDI using the package *lme4* (22). Fixed effects were the time point and the colonization status, whereas association to the individual was included as random effect. Richness and SDI values were transformed to their natural exponents ($\exp(\text{SDI})$) to achieve normally distributed residuals.

2.6. Beta diversity and hierarchical clustering calculations

Beta-diversity was calculated using variance adjusted weighted UniFrac (VAW-UniFrac) and Jaccard (unweighted) Indexes and were visualized in Non-metric multidimensional scaling (NMDS) plots. Differences between colonized and non-colonized individuals were assessed using permutational MANOVA (Multivariate analysis of variance). Stability of the microbiota was assessed by cluster analysis on the previously obtained matrix, containing Jaccard distance matrices. For this purpose, the Jaccard and VAW-UniFrac distance matrices were used and grouped using the Ward's hierarchical agglomerative clustering (Ward 2). Clusters were observed using Principal Coordinate Analysis (PCoA) for the Jaccard distances and NMDS for the VAW-UniFrac. Bacterial families statistically influencing cluster allocation were assessed using *vegan*'s function *envfit*. Relative abundance of bacterial families between the different time points, colonization status and clusters were calculated.

Differential abundance of bacterial taxa at different levels was assessed using a negative binomial mixed model effects using the *BhGLM* package (23). Fixed effects were the time point and the colonization status, whereas random effects were the identification of the

individual. P-values were corrected with a false discovery rate (FDR) and log₁₀-fold changes (log₁₀p) were calculated.

All resulting plots were generated using ggplot2 (24). P-values were considered significant when P or adjusted P (P_{adj}) based on false discovery rate < 0.05.

2.7. Accession number(s). The sequencing reads for this study were deposited at the European Nucleotide Archive (ENA) under accession no. PRJEB23775.

3. RESULTS

3.1. Prevalence of ESC-R-Ent Colonization Over time and strains characteristics

For the 40 volunteers, the prevalence of gut colonization due to ESC-R-Ent before traveling (T_0) was 10%, whereas after traveling (T_{1W}) was 76%. Colonization decreased over time, as rates of 33%, 26% and 18% were reported during the follow-ups at three (M3), six (M6) and twelve (M12) months, respectively (Figure 1). A summary of the demographic and clinical information of the volunteers including antibiotic use and further travel is depicted in Supplementary Table 1.

Phenotypic and molecular features of ESC-R-Ent found in the stools of volunteers were previously published (12, 25). In brief, most of the ESC-R-Ent were *Escherichia coli* that belonged to phylogenetic groups A and B1 and to highly diverse non-epidemic sequence types (STs); however, 15% of them belonged to clonal complex 10 (indicating a possible environmental origin) and mainly produced the worldwide spreading CTX-M-15 extended-spectrum β -lactamase (ESBL) (12, 25)

3.2. Decreased alpha-diversity in colonized individuals

We performed 16S rRNA genes sequencing for all samples receiving an overall number of 15,754,616 reads (median 81,904; range 9,385-348,321). Alpha-diversity values including

Shannon Diversity Index (SDI) and richness suffered slight fluctuations over time reaching the highest values three months (M3) after travelling (Figure 2A and 2B). However, these fluctuations were not statistically significant.

To better understand the influence of specific factors on SDI and richness, we designed a linear mixed model to correct for repetitive measurements of individuals (Figures 2C-2F). Fitted values indicated that SDI was lower in colonized versus non-colonized individuals (natural exponents SDI, expSDI: 29.87 vs 35.85, $p < 0.001$; Figure 2E and Supplementary Figure 1A) and after as compared to before travelling to India (expSDI 30.91 vs 34.81, $p = 0.02$ Supplementary Figure 1B). Individuals with abdominal complaints also had a lower SDI compared to those without such symptoms (expSDI 29.52 vs 34.35 vs, $p = 0.03$), whereas those that took antibiotics during the journey did not present significant differences in SDI (expSDI 36.75 vs 33.72, $p = 0.4$).

Richness values were also lower in colonized than non-colonized individuals (richness 293.98 vs 326.32, $p = 0.004$ Figures 2F and Supplementary Figure 1C), after than before traveling to India (richness 289.08 vs 324.14, $p = 0.004$ Supplementary Figure 1D), and in individuals with abdominal complaints (richness 281.43 vs 319.51, $p = 0.04$). In contrast, this phenomenon was not observed for people reporting antibiotic (richness 318.37 vs 315.35, $p = 0.8$).

3.3. Overall bacterial community of individuals affected by ESC-R-Ent colonization

Beta-diversity analyses were done using unweighted (Jaccard) distance matrices followed by non-metric multidimensional scaling (NMDS) plots (Figure 3A); subsequently, intra individual distances over time were created (Figure 3B). Unweighted distance matrices were used to up-weight the importance of rare taxa. However, weighted based analyses were also performed (VAW-UniFrac; Supplementary Figure 2A and 2B).

Overall microbial communities significantly differed using both weighted and unweighted matrices for the colonization status, though the degrees of differences (R^2) were very small

(Jaccard, $p=0.007$, $R^2=0.008$; VAW-UniFrac, $p=0.006$, $R^2=0.01$). Differences were not observed considering time points of sampling (Jaccard, $p=0.2$, $R^2=0.02$; VAW-UniFrac, $p=0.2$, $R^2=0.03$), abdominal complaints (Jaccard, $p=0.4$, $R^2=0.006$; VAW-UniFrac, $p=0.39$, $R^2=0.006$), and antibiotic intake (Jaccard, $p=0.59$, $R^2=0.006$; VAW-UniFrac, $p=0.87$, $R^2=0.004$).

3.4. Differential abundances of bacterial families and OTUs at different time points and colonization status

The relative abundances of the bacterial families at different time points (T_0 , T_{1w} , M3, M6 and M12) and in colonized versus non-colonized volunteers are illustrated in the supplementary Figures 3A and 3B. We found that the relative abundances of particular bacterial families were affected at T_{1w} compared to T_0 and M3 follow-up; in particular *Enterobacteriaceae*, *Prevotellaceae* and *Verrucomicrobiaceae* seemed to be increased, whereas *Lachnospiraceae* appeared to be decreased (Supplementary Figure 3A). However, these differences were not statistically significant.

Subsequently, we were focusing on the operational taxonomic units (OTU) rather than families. This means that every OTU was giving a number, depending on the overall abundance in the dataset (with one being the most abundant OTU). An overview of the taxonomy for all OTUs is depicted in Supplementary Table 2. As expected, the majority of OTUs belonged to the phyla *Bacteroidetes* and *Firmicutes* (26).

To verify whether specific OTUs were significantly correlated with colonization and the time points after traveling, a negative binomial mixed regression was then performed (and p -values were corrected with false discovery rate). The results for the model are depicted in Supplementary Table 3 for which the intercept is subjects colonized and T_{1w} . All statistically significant OTUs in the model were screened for OTUs that were significantly different at T_0 and M3, M6 and M12 follow-ups compared to T_{1w} , irrespective of the colonization status. In

particular, OTUs 83 and 587 were increased after traveling, whereas OTUs (also belonging to *Ruminococcaceae*) 269, 289, 475 and 587 were decreased at T_{1w} , irrespective of the colonization status. From the same family, OTUs 402, 467 and 508 were increased at T_{1w} compared to all other follow-ups and decreased in colonized individuals compared to non-colonized individuals, whereas OTU 778 had the opposite behavior (Supplementary Table 3). Other OTUs from *Veillonellaceae* (OTUs 28 and 49) and *Lachnospiraceae* (OTU 368 and 408) were also enlarged after travelling and decreased in colonized individuals. Only OTU 88 (unclassified from the order *Clostridiales*) and 236 (unclassified at all levels) were found reduced after travel, but increased in colonized individuals (Supplementary Table 3). Among OTUs of the *Enterobacteriaceae*, only *Hafnia* spp. (OTU 244) was found consistently decreased at all time points, compared to T_{1w} .

3.5. Stability of the microbiota composition and no association of particular compositions with subsequent ESC-R-Ent colonization

The hierarchical approach on the OTU abundances indicated that three main microbiota clusters could be identified among the 40 subjects by using either Jaccard or VAW-UniFrac distance matrices. Clustering was influenced by the presence of particular OTUs (Figure 4, Supplementary Figure 4, and Supplementary Tables 4 and 5).

Most samples of the 40 individuals were grouped into cluster 1 ($n=68$), followed by cluster 3 ($n=50$), and cluster 2 ($n=37$). Analyzing the cluster affiliation at different time points (T_0 , T_{1w} , M3, M6 and M12), the majority of individuals remained in their specific initial cluster observed in the T_0 sample. Very few individuals were found to transiently move to a different cluster at specific time points, but this was not related to travel ($p=0.5$), colonization status ($p=0.8$), or time ($p=0.06$) (Figure 5A and B).

When using VAW-UniFrac, individuals were also mainly grouped into cluster 1 ($n=72$), followed by cluster 3 ($n=62$), and cluster 2 ($n=26$). Compared to Jaccard, clustering with

VAW-UniFrac showed more shifts among clusters, but these were also not associated with travel ($p=0.3$), colonization status ($p=0.2$), or time point ($p=0.2$) (Supplementary Figures 5A and 5B).

Concerning Jaccard distances, the proportion of colonized individuals was the greatest in cluster 2 (45.6%, $n=26$), whereas cluster 1 and 3 were similar (26.3%, $n=15$ and 28.1%, $n=16$, respectively), though the difference was not statistically significant (Figure 5B, $p=0.5$). Moreover, none of the three clusters was significantly associated with subsequent ESC-R-Ent colonization.

Interestingly, using VAW-UniFrac cluster 2 also had the highest proportion of samples of colonized individuals (53.8%), followed by cluster 3 and 1 (32.2% and 31.9, respectively), but again this was not statistically significant (Supplemental Figure 5B, $p=0.6$) and no specific cluster was associated with subsequent ESC-R-Ent colonization.

4. DISCUSSION

In this work, we monitored the gut microbiota dynamics of healthy people traveling to India up to 12 months after their return in Switzerland in order to correlate it with the colonization due to ESC-R-Ent. To our knowledge, this is the first study to address microbiota dynamic changes due to traveling over a 12-month period follow-up.

The point-prevalence of ESC-R-Ent carriage recorded in our study was consistent to previous reports in which high carriage rates were observed when healthy volunteers returned from South Asia. Nevertheless, this ESC-R-Ent colonization is usually transient as a great amount of the travelers return decolonized within the first 3 months post-trip (27). However, we observed that even at 6 and 12 months after travel, colonization rates have not yet completely reverted. Similar findings have been described in other non-Swiss studies (27-29). This might be due to additional trips of the included volunteers or that there is a persistent ESC-R-Ent colonization possibly favored by a particular microbiota composition.

The above observations stimulated us to understand what happened to the overall gut microbiota throughout this time frame and perceive whether this colonization status was associated to a particular bacterial population pattern.

We initially observed that there were no major differences within the gut microbiota between colonized and non-colonized individuals, as indicated by both alpha- and beta-diversity analyses. After a more in depth modelling of our data, the SDI indicated a very small, but significant, difference between colonized and non-colonized subjects and this was further confirmed within the beta-diversity analyses using the Jaccard index and the VAW-UniFrac.

We also observed a lower SDI when considering traveling to South Asia as compared to before the travel (T_0). However, this could be mostly due to the colonization with ESC-R-Ent, rather than to the travel itself. In accordance to the literature, the SDI in people with abdominal complaints was also decreased in our study (8). It may come as a surprise, that we did not observe differences for SDI in individuals that used antibiotics. This may be explained

by the fact that only few travelers used antibiotics and/or the sampling at specific time points did not coincide with the antibiotic intake period and thus microbiota composition had the time to revert to a normal status (30). Interestingly, the beta-diversity was unaffected by travel, abdominal complaints, and antibiotic intake and thus, we further focused on the found differences based on the colonization status.

In order to assess the stability of the gut microbiota, we investigated whether individuals could be grouped into particular microbiota patterns with a hierarchical clustering approach. This was based on the idea of the enterotypes concept for population stratification which has been previously described (31). Within our study, individuals fell into three clusters depending on the composition of particular OTUs. Analyzing the follow-up samples, we noted few cluster shifts and no particular association was found in between the travel time points or when colonization status also shifted, regardless of the distance metric used. This may sound surprising, but high stability of the faecal microbiota has even been noted during antibiotic treatment with imipenem (32).

Our results indicate that there is no particular microbiota composition associated with increased risk for colonization with ESC-R-Ent. Essentially, we did not find clusters which were significantly associated with subsequent ESC-R-Ent colonization and, therefore, we conclude that all subjects had the same risk for ESC-R-Ent colonization. Overall, the individuals remained in their cluster and in the rare cases where we noted cluster shifts, this was observed temporarily. This stability of the microbiota explains why no major variations were identified immediately after traveling and in the follow-up period and this is also reflected in the diversity metrics. A stable microbiota was already observed in previous studies and it was only disturbed upon onset of travelers' diarrhea or antibiotic intake (8, 26, 33).

Finally, the negative binomial mixed effect models revealed the differentially abundant taxa that were found among the different time points, travel and colonization status. Taking into

account that there is great overlap of differentially abundant OTUs in between colonized individuals and the time points, it is difficult to understand if these are particular shifts that facilitate ESC-R-Ent colonization. In many instances, different OTUs of the same genera and/or families can be found increased or decreased after travel (e.g., OTUs belonging to *Ruminococcaceae*, *Lachnospiraceae* and *Veillonellaceae*). These observations are likely due to strain replacement in the gut, as people may encounter these bacteria during their journey. Species from the *Ruminococcaceae*, *Lachnospiraceae* and *Veillonellaceae* families are associated with the normal gut of humans and other mammals, but they are also involved in the production of short-chain fatty acids which are known to inhibit growth of several species belonging to the *Enterobacteriaceae* (34-36). Unfortunately, giving the well-known limitations of the 16S rRNA gene-based sequencing methodology, we cannot annotate exact species names to the OTUs.

A previous study assessing colonization in travelers identified that returning subjects and persistently colonized individuals have increased proportion of different antibiotic-resistant *Enterobacteriaceae* (27). The same study also observed that travelers returning from Asia had higher proportions of resistant *Enterobacteriaceae* in comparison to people coming back from other regions (27).

We speculate that people traveling to Asia are highly exposed to ESC-R-Ent and thus are at a greater risk of being colonized by these difficult to treat pathogens. This exposure probably leads to strain replacement in the gut, which is consistent with the fact that some strains in the gut are transient (37). Previous studies assessing the risk factors associated with colonization in returning travelers have identified the consumption of certain food products as significant for colonization by these MDR organisms (38). Studies assessing the colonization rates within the community in India are lacking and thus it is unknown if the relative abundance of *Enterobacteriaceae* is increased in Indian residents. However, it has been well documented that ESC-R-Ent have been found in high numbers in Indian environmental sources and food

chain (39-41). Moreover, well-known factors associated with increased expansion of antibiotic-resistant bacteria are lack of strict antibiotic policies, poor hygiene, and population density, all of which are problematic within this region (1).

Our study has several drawbacks. Firstly, the overall study population is small and factors that have small effects in the gut microbiota can only be properly studied when very large cohorts are analyzed (42). Secondly, several volunteers have traveled to other destinations during the follow-up period which might influence the microbiota composition (Supplementary table 1). However, as the travel destinations and durations varied a lot, we decided to not include this information in our statistical models. Finally, model diagnostics for the dataset are unfeasible given the number of OTUs retrieved from fecal samples and also given that all taxa are influenced by different factors that cannot be considered for every single taxon.

5. CONCLUSION

The prevalence of ESC-R-Ent in people, animals, and environment on the Indian subcontinent is high and, therefore, travelling to India might be associated with an increased risk for subsequent ESC-R-Ent gut colonization. This risk may depend on an individual's microbiota. We have therefore characterized the gut microbiota in a longitudinal study of 40 volunteers before and after travelling to the Indian sub-continent. We recorded high rates of ESC-R-Ent carriage after traveling, but only minimal changes in the gut microbiota of the volunteers. Microbiota composition seems not playing a key role in subsequent ESC-R-Ent colonization.

AUTHORS AND CONTRIBUTORS

Conception and design (AE, MH); acquisition of data (JP, EK, SK, RT); analysis and interpretation of data (JP, JK, EK, MH, CH, AE); drafting the work (JP, AE, MH); revising it critically for important intellectual content (all authors); final approval of the version to be published (all authors); agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (all authors).

CONFLICT OF INTEREST STATEMENT

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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LEGEND TO THE FIGURES AND TABLES

Figure 1. Alluvial plot with the colonization status over time. Each line corresponds to one individual. "+" indicates positive colonization with ESC-R-Ent; "-" indicates negative colonization for ESC-R-Ent; "NA" indicates sample not available. M3 - 3 months; M6 - 6 months; M12 - 12 months.

Figure 2. Shannon Diversity Index (SDI) and richness values. SDI (**2A**) and richness (**2B**) values obtained at each time point. M3 - 3 months; M6 - 6 months, M12 - 12 months. Raw values of the exponential value of the SDI (expSDI) (**2C**) and richness (**2D**) for colonized and non-colonized individuals are longitudinally plotted. Fitted values of the expSDI (**2E**) and richness (**2F**) obtained from the linear mixed effects model using the lme4 R package. Fixed effects were the time point and the colonization status, whereas association to the individual was included as random effect. expSDI values were used to obtain normally distributed residuals. Plots were generated using ggplot2.

Figure 3. Jaccard beta-diversity plots. **A** - Non-metric Multidimensional Scaling plot using the distance matrix generated with R package vegan with 1000 iterations in a two dimensional space. Centers of the two groups colonized and non-colonized are enlarged and segments link the different samples to the center. **B** - Scatter plot using the intra-colonization status distances over time.

Figure 4. PCoA plot showing the results of hierarchical clustering using the Jaccard distance matrix. The center of the cluster is enlarged and samples from the same cluster are linked to clusters' center. Only the first 20 most abundant OTUs influencing the clustering are shown. These were obtained with the envfit function from vegan.

Figure 5. Alluvial plots obtained from the hierarchical clustering with Jaccard. Each line corresponds to one individual. The color assigned indicates the first cluster in which each individual was allocated to. Clusters are identified with the respective number. "NA" indicates sample not available. **A** - Alluvial plot showing the clustering at the different time points. **B** - Alluvial plot showing the clustering overtime split by the different colonization status. All plots were obtained with the R package alluvial.

Supplementary Figure 1. Boxplots comparing fitted values obtained from the models; **A** – illustrated are the fitted values of expSDI at different colonization status and **B** - fitted expSDI at travel; **C** – illustrated are the fitted values of richness at different colonization status and **D** - fitted richness at travel.

Supplementary Figure 2. Variance Adjusted Weighted UniFrac (VAW-UniFrac) beta-diversity plots. **A** - Non-metric Multidimensional Scaling plot using the distance matrix generated with R package vegan with 1000 iterations in a two dimensional space. Centers of the two groups colonized and non-colonized are enlarged and segments link the different samples to the center. **B** - Scatter plot using the intra-colonization status distances over time.

Supplementary Figure 3. Relative abundance plots for **A** - at the different time points and; **B** - at the different colonization status. Only the first 16 most abundant families are shown and the remaining collapsed into "Others".

Supplementary Figure 4. NMDS plot showing the results of hierarchical clustering using the VAW-UniFrac distance matrix. The center of the cluster is enlarged and samples from the

same cluster are linked to clusters' center. Only the first 20 most abundant OTUs influencing the clustering are shown. These were obtained with the envfit function from vegan.

Supplementary Figure 5. Alluvial plots obtained from the hierarchical clustering with VAW-UniFrac. Each line corresponds to one individual. The color assigned indicates the first cluster in which each individual was allocated to. Clusters are identified with the respective number. "NA" indicates sample not available. **A** - Alluvial plot showing the clustering at the different time points. **B** - Alluvial plot showing the clustering overtime split by the different colonization status. All plots were obtained with the R package alluvial.

Supplementary Table 1. Demographic and clinical data for the participants.

Supplementary Table 2. Taxonomy for the obtained OTUs.

Supplementary Table 3. Results for the Negative Binomial Mixed Effects Model for differential OTU identification

Supplementary Table 4. OTUs influencing the hierarchical clustering with the Jaccard Distance Matrix. OTU names can be found within Supplementary Table 8.

Supplementary Table 5. OTUs influencing the hierarchical clustering with the VAW-UniFrac Distance Matrix. OTU names can be found within Supplementary Table 8.

Figure 1

Figure 3A

Figure 3B

Figure 4

A Figure 5

B