Effect of tebipenem pivoxil hydrobromide on the normal gut microbiota of a healthy adult population in Sweden: a randomised controlled trial

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Summary

Background Antimicrobials cause perturbations in the composition and diversity of the host microbiome. We aimed to compare gut microbiome perturbations caused by oral tebipenem pivoxil hydrobromide (a novel carbapenem) and by amoxicillin–clavulanic acid (an orally administered β-lactam–β-lactam inhibitor combination widely used in clinical practice).

Methods We did a phase 1, single-centre, randomised, parallel-group, active-control trial to evaluate the effect of tebipenem pivoxil hydrobromide on the human gut microbiota. Healthy participants aged 18 years or older with no documented illnesses during recruitment were enrolled at Karolinska University Hospital (Stockholm, Sweden). Study participants were stratified by sex and block-randomised in a 1:1 ratio to treatment with either tebipenem pivoxil hydrobromide (600 mg orally every 8 h) or amoxicillin–clavulanic acid (500 mg amoxicillin and 125 mg clavulanic acid orally every 8 h). The study included 10 days of treatment (days 1–10) and four follow-up visits (days 14, 21, 90, and 180). The trial was open-label for clinical investigators and patients, but masked for microbiology investigators. Faecal samples were collected at all visits. Sequencing of 16S rDNA was used to measure the diversity metrics, and quantitative culture to quantify selected taxa. The primary outcomes were changes in the α and β diversity and log count of colony-forming units for selected taxa between samples compared with baseline (day 1), and whether any changes reverted during the follow-up period. The analyses were done in the intention-to-treat population. This study was registered with ClinicalTrials.gov (NCT04376554).

Findings The study was conducted between Jan 23, 2020, and April 6, 2021. 49 volunteers were screened for eligibility, among whom 30 evaluable participants (14 men and 16 women) were assigned: 15 (50%) to the tebipenem pivoxil hydrobromide group and 15 (50%) to the amoxicillin–clavulanic acid group. Baseline characteristics were similar between groups. Complete follow-up was available for all participants, and all participants except one completed treatment as assigned. The diversity metrics showed significant changes from baseline during the treatment period. Significant decreases in richness were observed on days 4–10 ($p \leq 0{\cdot}0011$) in the amoxicillin–clavulanic acid group and on days 4–14 ($p \leq 0{\cdot}0019$) in the tebipenem pivoxil hydrobromide group. Similarly, evenness was significantly decreased during treatment in the amoxicillin–clavulanic acid group (day 4, $p=0{\cdot}030$) and the tebipenem pivoxil hydrobromide group (days 4–10, $p<0{\cdot}0001$) compared with baseline. Quantitative cultures showed significant decreases in Enterobacterales (days 4–7, $p \leq 0{\cdot}0030$), *Enterococcus* spp (days 4–14, $p=0{\cdot}025$ to $p<0{\cdot}0001$), *Bifidobacterium* spp (days 2–4, $p \leq 0{\cdot}026$), and *Bacteroides* spp (days 4–10, $p \leq 0{\cdot}030$) in the tebipenem pivoxil hydrobromide group. Similarly, in amoxicillin–clavulanic acid recipients, significant changes were observed in Enterobacterales (days 4–10, $p \leq 0{\cdot}048$), *Bifidobacterium* spp (days 2–4, $p \leq 0{\cdot}013$), and *Lactobacillus* spp (days 2–4, $p \leq 0{\cdot}020$). Samples from the follow-up period were not significantly different from those at baseline in β diversity analysis (PERMANOVA, $p>0{\cdot}99$). By the end of the study, no significant change was observed compared with baseline in either group. There were no deaths or severe adverse events.

Interpretation The impact of tebipenem pivoxil hydrobromide on the gut microbiome was similar to that of amoxicillin–clavulanic acid. The safety of antibiotic use with regard to the microbiome should be given attention, as dysbiosis is associated with health and disease.

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Introduction

In the past two decades, accumulating evidence has shown that microbiome dysbiosis is associated with several potentially life-threatening health conditions, including cancer, heart diseases, mental illness, diabetes, and infectious diseases.[1–3] The normal gut microbiota confers

Research in context

Evidence before this study

Carbapenems are a cornerstone in the management of multidrug-resistant (MDR) infections, such as complicated urinary tract infections, including acute pyelonephritis, caused by cephalosporin-resistant microorganisms. All carbapenems available in Europe and the USA have so far been for intravenous use. However, the oral carbapenem tebipenem pivoxil hydrobromide has recently shown efficacy in the treatment of MDR Gram-negative bacilli. We searched PubMed, Embase, and Web of Science in all fields of primary research articles between database inception and Dec 31, 2019, with no language restrictions. "Tebipenem OR tebipenem pivoxil OR L084 OR SPR994" AND "microbiome OR microbiota OR dysbiosis" were the terms used to generate search string: [(((((ALL=(tebipenem*)) OR ALL=(L084)) OR ALL=(SPR994)) AND ALL=(microbiome)) OR ALL=(microbiota)) OR ALL=(Dysbiosis)]. Our search results showed that there was no previous study conducted to evaluate the effects of tebipenem pivoxil hydrobromide on gut microbiota. Existing evidence before this study was limited to the safety and efficacy of tebipenem pivoxil hydrobromide for the treatment of complicated urinary tract infections and acute pyelonephritis. The drug is efficacious against MDR strains, but evidence regarding possible damage to the healthy microbiome and potential selection of cephalosporin resistance is insufficient, although these factors could compromise patient safety and affect the use of this antibiotic.

Added value of this study

This study showed that the effect of tebipenem pivoxil hydrobromide on the microbiome is similar to that of amoxicillin–clavulanic acid. Moreover, a long-term impact was not observed, suggesting that the antibiotic might not confer sustained microbiome damage. Because perturbation of the microbiome has been associated with several long-term complications, evidence regarding the extent of microbiome damage and recovery is important for clinicians and prescribers. The current study fills the knowledge gap regarding the impact of tebipenem pivoxil hydrobromide on the gut microbiome.

Implications of all the available evidence

The currently available evidence suggests that oral tebipenem pivoxil hydrobromide can be used for treatment of urinary tract infections caused by cephalosporin-resistant strains, thus reducing the need for hospitalisation for administration of intravenous carbapenems. It could also be used to reduce the duration of hospital stay by switching intravenous to oral therapy. In addition to the current oral formulation, the findings herein could be used to guide future potential formulations of tebipenem pivoxil hydrobromide for other infections. Furthermore, this study could serve as a baseline for future studies. This evidence on the impact of tebipenem pivoxil hydrobromide on the gut microbiome together with previous studies on safety and efficacy provides comprehensive information to inform clinical practice.

protection (referred to as colonisation resistance) to the host against invasive pathogenic species.[1,4] This protection occurs through several mechanisms, including direct killing, competition for limited nutrients, and enhancement of immune responses.[5] The consensus is that a healthy gut microbiome is a complex, diverse community that is resistant to colonisation and proliferation by pathogens.[6,7]

Antimicrobials have been shown to cause disruptions to the gut microbiome by lowering bacterial diversity and thereby allowing pathogens to invade.[8] Once diversity has been compromised, it can be difficult to ameliorate. However, the increasing frequency of infections caused by multidrug-resistant (MDR) microorganisms demands the development of new antimicrobials, or modification of known drugs, with enhanced efficacy and reduced adverse effects.[9] Development of new antimicrobials has been quite challenging, particularly in the discovery of a new scaffold, while modification of known drugs has been more successful.[10]

Changing the route of antibiotic administration is an improvement that could influence therapeutic options.[11] For instance, at present, patients infected with MDR strains that need to be treated with carbapenems must be admitted to hospital to receive the drug intravenously. Hospital-based antibiotic administration imposes a risk of health-care-associated infections with MDR organisms, and can worsen the outcome of the patient compared with oral therapy (where possible) given at home or in other non-hospital-based settings.[12,13] Antimicrobials that can reduce hospital admissions contribute to decreasing hospital-acquired infections, lowering antibiotic use, reducing the development of antibiotic resistance, and minimising the overall cost of patient care.[14] Although various pump systems for at-home intravenous administration of antimicrobials have become available, there are still practical challenges with their use by patients outside of hospitals.[15] Hence, there is an unmet need for alternative oral carbapenems that can be used to treat infections caused by MDR pathogens in clinics and the community.[16]

Tebipenem pivoxil hydrobromide (initially known as SPR994) is a fast-track oral carbapenem that, if made clinically available, could potentially address an unmet medical need for oral treatment of serious MDR Gram-negative bacterial infections in adults[17] and children.[18,19] However, an assessment of the impact of oral antimicrobial agents on the composition of the gut microbiota is crucial. Generally, studies regarding potential collateral damage to the microbiome following antibiotic treatment are essential for guiding the rational use of antibiotics and ensuring patient safety. In this study, we aimed to characterise the effect of tebipenem pivoxil hydrobromide compared with oral amoxicillin–clavulanic acid on the gut microbiome of healthy individuals, with both phenotypic and genotypic microbiota analyses.

Methods

Study design

This study was a phase 1, single-centre, open-label, randomised, parallel-group, active-control trial to evaluate the impact of tebipenem pivoxil hydrobromide on the gut microbiota of healthy participants. The study was conducted at the Clinical Pharmacology Trial Unit at the Karolinska University Hospital (Stockholm, Sweden). All participants provided written informed consent. The protocol, informed consent form, investigator brochure, and other relevant documents (eg, advertisements) were reviewed and approved by the Swedish Ethics Review Authority and the Swedish Medical Products Agency (EudraCT number 2019-003059-12).

Participants

The study recruited men and women aged 18 years or older during screening. Consecutive individuals fulfilling the inclusion and exclusion criteria were enrolled. The main inclusion criterion was that participants should be medically healthy adults without clinically significant abnormal findings on haematological tests, clinical chemistry, urinalysis, physical examination, assessment of vital signs, or electrocardiography (ECG), as determined by the investigator during the screening period. Exclusion criteria included history or a significant presence of systemic diseases. A complete list of inclusion and exclusion criteria is presented in appendix 1 (pp 2–4).

Randomisation and masking

Participants were stratified by sex and block-randomised, followed by assignment to one of two antimicrobial treatment groups (tebipenem pivoxil hydrobromide or amoxicillin–clavulanic acid) in a 1:1 ratio using a computer-generated randomisation code (SAS version 9.4). Unique ten-digit codes (eg, 103-XXX-ZZZZ) were generated, comprising three parts: the first three digits indicated the study identifier (eg, 103), the second three digits indicated the site number, and the final four digits indicated a unique random number assigned to the participant. Randomisation numbers were not reassigned to other individuals in case of study dropouts. The study was open-label for clinical investigators and participants, but was blinded for microbiological investigators.

Procedures

Tebipenem pivoxil hydrobromide was administered orally at a dosage of 600 mg (as two 300 mg film-coated tablets) every 8 h (±1 h). Amoxicillin–clavulanic acid was given orally at a dosage of 500 mg amoxicillin and 125 mg clavulanic acid (as a single tablet) every 8 h (±1 h). Tebipenem pivoxil hydrobromide and amoxicillin–clavulanic acid were provided by the sponsor (Spero Therapeutics, Cambridge, MA, USA). Initial doses were administered at the clinic on day 1, and participants were given written instructions on how and when to take subsequent doses of the drugs, and how to record in their diary that they had taken the drugs. During each visit to the clinic, one of the doses for that day was taken there. Participants were asked about adverse events at each clinic visit and an investigator assessed the severity and relatedness to the investigational product.

The estimated study duration per participant was 7 months, including a 28-day screening period for health indicators for eligibility and re-screening for abnormal laboratory safety values, a 10-day treatment period, and four follow-up visits (days 14, 21, 90, and 180). Faecal samples were collected from study participants at nine different sampling points: day 1 (baseline), days 2–10 (treatment), and days 14–180 (follow-up). Changes in colony count, α and β diversity, and abundance of taxa (appendix 1 pp 10–12) were measured for all faecal samples collected at each visit including baseline, during treatment, and follow-up.

Selected clinically important taxa were targeted with use of selective culture media with and without antibiotics. The culture media were manufactured and supplied by the accredited substrate unit at Karolinska University Hospital (Stockholm, Sweden; appendix 1 p 5). Emergence of cephalosporin-resistant isolates at any timepoint, above a putative cutoff value in both groups of the study, was tested. Minimum inhibitory concentration (MIC) values were measured by broth microdilution (appendix 1 p 6) and, in the case of anaerobic bacteria, by agar dilution (appendix 1 p 7). MICs were interpreted using breakpoints specified by the European Committee on Antimicrobial Susceptibility Testing (2021 version). Broth microdilution plates were provided by ThermoFisher (Basingstoke, UK), while plates for agar dilution were prepared by the substrate unit at Karolinska University Hospital.

See Online for appendix 1

Total genomic DNA was extracted from 200 mg faecal samples using a Stool DNA isolation kit (Norgen Biotek, Thorold, ON, Canada) and sequenced at the BGI sequencing centre (Hong Kong, China). Library construction and sequencing were done with a dual-index paired-end sequencing approach, using standard fusion primers (Beijing Genomic Institute, Hong Kong, China), 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). To recover 16S rDNA from the metagenomic samples, the V3–V4 regions were targeted for amplification. The quality of the library was assessed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Subsequently, the qualified libraries were subjected to paired-end sequencing on the HiSeq 2500 System (Illumina, San Diego, CA, USA), using the MiSeq PE300 sequencing strategy (MiSeq-PE301+8+8+301) with a MiSeq reagent kit (appendix 1 pp 8–9).

Outcomes

The primary outcome of the study was perturbation of the gut microbiota during and after oral administration of tebipenem pivoxil hydrobromide and amoxicillin–clavulanic acid. Perturbation was assessed by quantitative culture (temporal changes in colony-forming units [CFU] per gram of stool for clinically relevant microorganisms [ie, species of Enterobacterales, *Enterococcus*, *Bifidobacterium*,

Lactobacillus, *Clostridioides*, *Bacteroides*, and *Candida*]) and by molecular characterisation of the microbiome diversity. Microbiome diversity was evaluated by 16S rDNA metagenomics in terms of α and β diversity of microbial communities and of operational taxonomic unit (OTU) composition and abundance. The perturbation of microbiome at each timepoint was compared with baseline within each group. The primary outcome was assessed in the intention-to-treat population.

A secondary outcome was the emergence of cephalosporin-resistant strains with MIC values above the putative epidemiological cutoff value for tebipenem pivoxil hydrobromide and the clinical breakpoint of amoxicillin–clavulanic acid following exposure to treatment in the two groups. Another secondary outcome was the correlation between the concentration of tebipenem in faeces and plasma after 10 days of oral administration of tebipenem pivoxil hydrobromide. A further secondary outcome was the safety and tolerability of treatment, assessed by the frequency of adverse events (by severity, seriousness, system organ class, preferred term, and antimicrobial group) and clinically significant changes from baseline in clinical laboratory values, physical examination findings, vital signs, and ECG findings. All secondary outcomes were assessed in the intention-to-treat population.

The associations between intestinal microbiota and faecal tebipenem concentrations compared with baseline were exploratory outcomes in the tebipenem pivoxil hydrobromide group.

Statistical and bioinformatic analyses

We recruited participants until the predefined number (n=30) was reached. The hypothesis was that the effect of tebipenem pivoxil hydrobromide on the gut microbiota of the healthy adult population would be similar to that of amoxicillin–clavulanic acid. The absence of a pilot study suggesting the extent of the antimicrobial impact of tebipenem pivoxil hydrobromide on the microbiome made it challenging to determine the sample size. Instead, the sizes of the two antimicrobial groups were based on previous experiences from trials with phenotypic methodology.[20]

We defined three populations for analysis: the intention-to-treat population (all randomly allocated participants), the microbiome population (all randomly allocated participants who took at least one dose of the study drug and provided one baseline and one post-treatment stool specimen for analysis), and the safety population (all randomly allocated participants who took at least one dose of the study drug).

The Illumina paired-end reads were processed using the QIIME 2 framework (released in April, 2020). After merging, denoising, and truncating the reads, a phylogenetic tree was constructed to identify amplicon sequencing variants and estimate α and β diversity metrics. The dissimilarities between all samples were transformed into a set of principal coordinates, and the two coordinates capturing the maximum variability were used to generate the principal component analysis plot. The changes in α diversity metrics within the antimicrobial groups were evaluated by aligned rank transform ANOVA using ARTool library (version 0.11.1) in R (version 4.3.0), where sampling days and sex were fixed effects and participants were random effects. In addition, a permutational multivariate analysis of variance (PERMANOVA) test with 1000 permutations was used to evaluate significant differences in β diversity among samples.

For taxonomic classification, we used VSEARCH (version 2021.4.0), primarily relying on the Greengenes database (version 13.8) to cluster reads with a 97% threshold. This led to identification of OTUs and their corresponding taxonomic ranks. The abundance of OTUs in a sample was defined as the number of sequences that were assigned to that OTU. The significance of the differential abundance of OTUs was assessed with the metagenomeSeq package (version 1.41.0), which uses a zero-inflated Gaussian model.

The statistical analysis of phenotypic quantitative microbiome data involved use of an aligned rank transform ANOVA in the ARTool library. Further details on statistical and bioinformatic analyses can be found in appendix 1 (pp 10–12). Spearman's and Pearson's correlation analyses were done to assess whether correlations existed between the faecal concentration of tebipenem and the richness during the treatment period.

This study was registered with ClinicalTrials.gov (NCT04376554).

Role of the funding source

The study was designed jointly by the principal investigator (SR), the principal microbiology investigator (CGG), and the sponsor and funder (Spero Therapeutics). The funder had no role in data collection, data analysis, data interpretation, or writing of the report, except generating the label sequence for study participants and samples, and sponsor oversight according to Good Clinical Practice guidelines.

Results

The study was conducted from Jan 23, 2020, to April 6, 2021 (when the last participant completed the study). We recruited and screened 49 participants, among whom 19 (39%) did not meet the eligibility criteria and the remaining 30 (61%) were enrolled and allocated to either the amoxicillin–clavulanic acid group (15 [50%] participants) or the tebipenem pivoxil hydrobromide group (15 [50%] participants). 29 (97%) participants in the intention-to-treat population completed treatment (figure 1). One participant allocated to the amoxicillin–clavulanic acid group prematurely discontinued from treatment on day 9 (the penultimate day of treatment) because of an adverse reaction of mild skin rash; the participant completed all follow-up visits. Baseline characteristics were similar across all participants in both groups (table).

From the phenotypic counts of the selectively studied microorganisms, decreased mean logCFUs were found for Enterobacterales and *Enterococcus* spp in the tebipenem pivoxil hydrobromide group during the treatment period

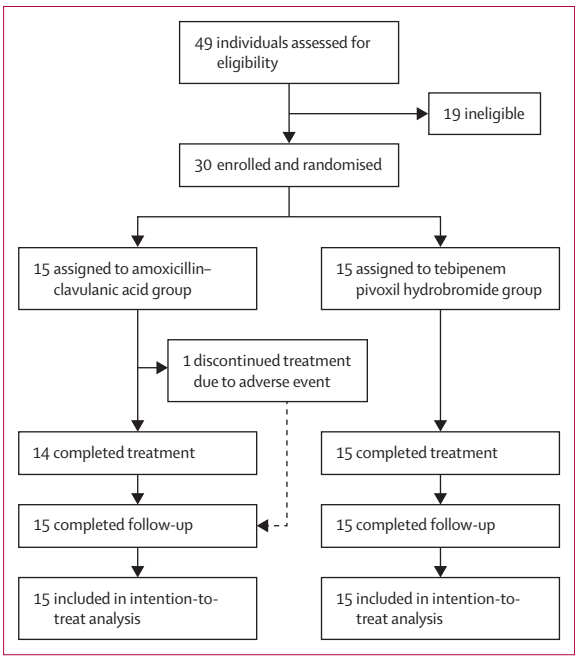

Figure 1: **Trial profile**

	Amoxicillin–clavulanic acid group (n=15)	Tebipenem pivoxil hydrobromide group (n=15)
Age, years		
Mean (SD)	31·7 (13·0)	28·6 (9·5)
Median (IQR)	25·0 (23·0–40·0)	25·0 (20·0–33·0)
Range	20–61	18–49
BMI, kg/m^2		
Mean (SD)	24·57 (5·15)	24·49 (3·84)
Median (IQR)	23·8 (21·4–28·3)	23·4 (20·9–27·4)
Range	16·7–34·5	20·0–32·0
Sex, n (%)		
Male	7 (47%)	7 (47%)
Female	8 (53%)	8 (53%)

Ethnicity data were not collected.

Table: **Demographic characteristics of the study participants**

(days 2–10) compared with baseline (day 1; appendix 1 pp 13–14). In the amoxicillin–clavulanic acid group, mean logCFU counts for *Enterococcus* spp were slightly decreased (without statistical significance) compared with baseline, whereas Enterobacterales were significantly increased during the treatment period compared with baseline (appendix 1 pp 15–16). *Candida* spp were significantly increased from baseline in the tebipenem pivoxil hydrobromide group, but not in the amoxicillin–clavulanic acid group, during the treatment period; however, CFU counts for *Candida* spp did not significantly differ from baseline in either group during the follow-up period (days 14–180).

Figure 2 and appendix 2 (p 2) provide an overview of the logCFU counts for specific taxa and their variations throughout the study period. With the exception of *Lactobacillus* spp, the logCFU counts of all selected taxa showed significant decreases during tebipenem pivoxil hydrobromide treatment compared with their baseline levels. The effect size, indicating the magnitude of changes, was smaller in the amoxicillin–clavulanic acid group than in the tebipenem pivoxil hydrobromide group. Nevertheless, the differences observed in the selected taxa between both groups diminished by the end of the study. Furthermore, stratification by sex (appendix 1 p 17) showed a similar pattern of results to that of the overall analysis (figure 2). However, fewer taxa showed significant differences in the sex-stratified analysis, which might be attributable to the smaller sample size in the strata as compared with the whole cohorts.

Metagenomic sequencing was done on all faecal samples collected from the microbiome population. However, in the amoxicillin–clavulanic acid group, two participants (S0032 and S0027) did not have a complete set of metagenomic sequencing data for all sampling days due to not meeting the DNA library quality control criteria. The number of reads after merging and denoising steps for each sequencing dataset are presented in appendix 2 (p 3).

Although some participants had different β diversity from the beginning, as observed in participant S0036, a cluster of samples obtained at baseline and the end of the study was formed in each of the antibiotic groups, suggesting recovery of microbiome as participants had similar microbial community compositions at both timepoints (figure 3). This observation was supported by the PERMANOVA test (appendix 1 p 18), which indicated that the β diversity of samples from day 1 of the tebipenem pivoxil hydrobromide group was not significantly different from day 90 ($p>0·99$) and day 180 ($p>0·99$), with day 21 ($p=0·072$) showing the start of return to baseline β diversity. However, for the amoxicillin–clavulanic acid group, the difference in β diversity diminished earlier, from day 10 ($p=0·18$), indicating a faster recovery.

The α diversity metrics indicated recovery of the microbiome following the initial impact of antibiotic treatment (figure 4; appendix 2 p 4). In both groups, significant effects on richness and evenness (both $p<0·0001$) were observed with sampling day, while the interaction between sampling day ($p=0·88$ for richness and $p=0·13$ for evenness) and sex ($p=0·12$ for richness and $p=0·18$ for evenness) was not significant, indicating that the effect of sampling day on diversity metrics was similar for males and females. Nevertheless, the distribution of α diversity metrics and the significant changes over time for each sex are presented in appendix 1 (p 19). Notably, significant differences compared with baseline in diversity metrics were primarily observed during the treatment phase (appendix 2 p 5), with the tebipenem pivoxil hydrobromide group taking longer to recover from the effect than the amoxicillin–clavulanic acid group. A longitudinal analysis of participants (appendix 1 p 20) revealed that α diversity metrics were not consistently lower compared with day 1; instead, they recovered higher

See Online for appendix 2

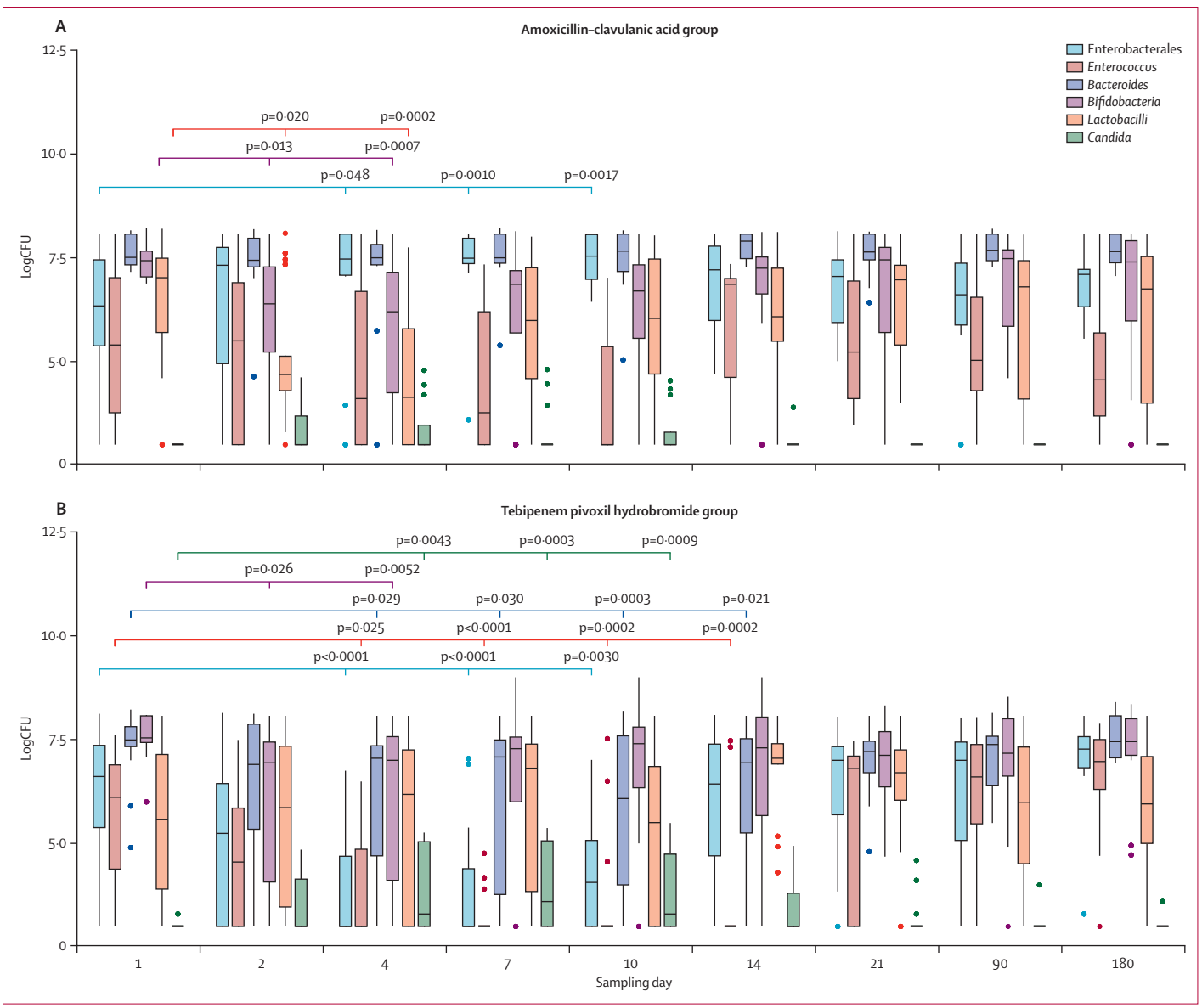

Figure 2: **Changes in the phenotypic count of selected taxa**

Graphs show logCFU counts per gram of faeces for Enterobacterales, *Enterococcus*, *Bifidobacterium*, *Bacteroides*, *Lactobacillus*, and *Candida* spp in the amoxicillin–clavulanic acid group (A) and tebipenem pivoxil hydrobromide group (B). Boxes in the plot indicate the IQR, with the horizontal line within each box representing the median. Whiskers extend to points up to 1·5 times the IQR from the quartiles, with any datapoints beyond this range being classified as outliers. p values are shown only for values that were significantly different from the baseline (day 1) value for the respective organism; detailed data are provided in appendix 2 (p 1). CFU=colony-forming units.

levels of richness and evenness following antimicrobial administration.

During the treatment period, several genera showed more pronounced reductions in abundance in the tebipenem pivoxil hydrobromide group than in the amoxicillin–clavulanic acid group. By contrast, some genera, such as *Enterococcus* and *Veillonella* in the tebipenem pivoxil hydrobromide group and *Parabacteriodes* in the amoxicillin–clavulanic acid group, had noticeable increases during treatment. However, towards the end of the study period (days 21–180), the abundances of these three genera returned to levels similar to those at baseline in both antimicrobial groups (figure 5). The identified OTUs and their abundance in each sample are presented in appendix 2 (p 6).

A zero-inflated Gaussian model was used to assess the abundance of OTUs across various taxonomic levels, with the aim of elucidating the effects of sampling day and sex, and their interactions. The coefficient matrices for this analysis are presented in appendix 2 (pp 7–8). Pairwise comparisons between sampling day and baseline values within each group are shown in appendix 2 (p 9). Although

the appendices contain unfiltered results, to maintain conciseness, we focused on the OTUs that exhibited more than a 2-fold change in response to antimicrobial use between days 2 and 14, with statistical significance at 0·05. In appendix 1 (p 21), OTUs are categorised by taxonomic order, showing their fold-change distributions. Most orders exhibit mixed increases and decreases in fold-changes of their OTUs. However, by the end of the study, changes were generally less than 2-fold and often minor compared with day 1 in each antimicrobial group. Notable exceptions are Enterobacterales and *Verrucomicrobiales* spp in the tebipenem pivoxil hydrobromide group, for which an OTU linked to *Escherichia fergusonii* and another related to probiotic *Akkermansia muciniphila*, respectively, were responsible. Within the amoxicillin–clavulanic acid group, there was a notable surge in OTUs associated with the genus *Klebsiella*, coupled with a decline in log fold-changes of OTUs connected to *Escherichia fergusonii*, uncultured Enterobacterales and *Enterobacter hormaechei*, which led to a widely spread distribution of Enterobacterales by day 180 (appendix 2 p 7). Moreover, OTUs of the genus *Klebsiella* and species *Escherichia fergusonii* were responsible for the increase in log fold-change of Enterobacterales on days 4 and 7. Furthermore, the abundances of specific taxa were assessed with 16S rDNA metagenomics and quantitative culture (appendix 1 pp 22–27).

A number of clinically relevant Enterobacterales were isolated on selective plates designed to culture them. The MIC values of *Enterobacterales* spp selected at a clinical breakpoint concentration of cefotaxime are presented in appendix 1 (p 28). Similarly, considerable numbers of meropenem-resistant *Bacteroides* spp were observed in both groups (appendix 1 p 29).

The selection of cephalosporin resistance was low for *Escherichia coli* and *Klebsiella pneumoniae* (2 [7%] of 30), but higher for all Enterobacterales across all study participants (9 [30%] of 30; appendix 1 p 7). The resistance was mainly observed among *Enterobacter* spp, with four cases in the tebipenem pivoxil hydrobromide group and five in the amoxicillin–clavulanic acid group. Typically, such resistance represents chromosomally derepressed bla_{AmpC}. In the tebipenem pivoxil hydrobromide group, isolates included *K pneumoniae* (n=2), *E coli* (n=1), and *Citrobacter freundii* (n=1). One of the *K pneumoniae* isolates encoded bla_{NDM-1}, and $bla_{OXA-181}$ at baseline. All isolates in the amoxicillin–clavulanic acid group were *Enterobacter* spp (appendix 1 p 7). Resistance to meropenem (MIC >16 mg/L) was observed in one *K pneumoniae* isolate in the tebipenem pivoxil hydrobromide group, as well as in *Enterobacter cloacae* and *Enterobacter bugandensis* isolates in the amoxicillin–clavulanic acid group. *Candida* spp significantly increased during antimicrobial use in the tebipenem pivoxil hydrobromide group, but reverted to baseline at the end of the follow-up period. Vancomycin-resistant *Enterococcus* spp were not observed, and *Clostridioides difficile* was found in one study participant from the tebipenem pivoxil hydrobromide group.

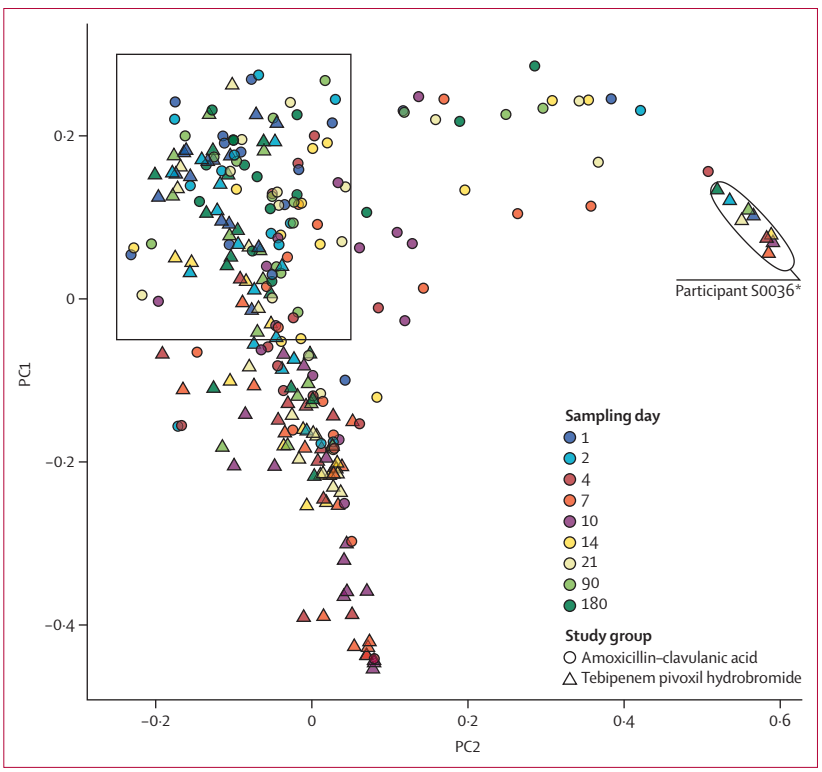

Figure 3: **Differences in β diversity of samples as distances in a Cartesian space**
The β diversity of samples are plotted based on the first two principal coordinates, PC1 and PC2, which capture the maximal variability in the dataset. At the beginning of the study, most participants had a microbial community composition located within the black box on the plot. As participants underwent treatment, compositions shifted outside of the box, before eventually re-entering it. *Participant S0036 displayed distinctive β diversity from the beginning, and their final microbiome composition closely resembled that at baseline, forming a distinct cluster separated from the microbiomes of other participants.

The highest mean faecal tebipenem concentration was observed on day 2 and the lowest on day 14, while the highest mean plasma concentration of tebipenem was seen 1 h post administration on day 1 (not shown). Faecal tebipenem concentration (total concentration) showed no clear correlation with richness (appendix 1 p 30) or dominance of extended-spectrum β-lactamase (ESBL)-producing strains (Pearson's r=−0·118; appendix 1 p 31). Only the free fraction of the antimicrobial is pharmacologically active, and the level of protein binding in different individuals was not assessed.

A total of 66 adverse events were reported across 14 of 15 participants in the tebipenem pivoxil hydrobromide group, and 51 adverse events were reported across 15 participants in the amoxicillin–clavulanic acid group. One participant in the amoxicillin–clavulanic acid group had a treatment-emergent adverse effect that led to study discontinuation (maculopapular rash; moderate severity and probably treatment related). One severe treatment-emergent adverse event was reported by a participant receiving tebipenem pivoxil hydrobromide (suspected COVID-19, unrelated). The most common adverse events (at the preferred term level) reported by two or more participants in the tebipenem pivoxil hydrobromide group were

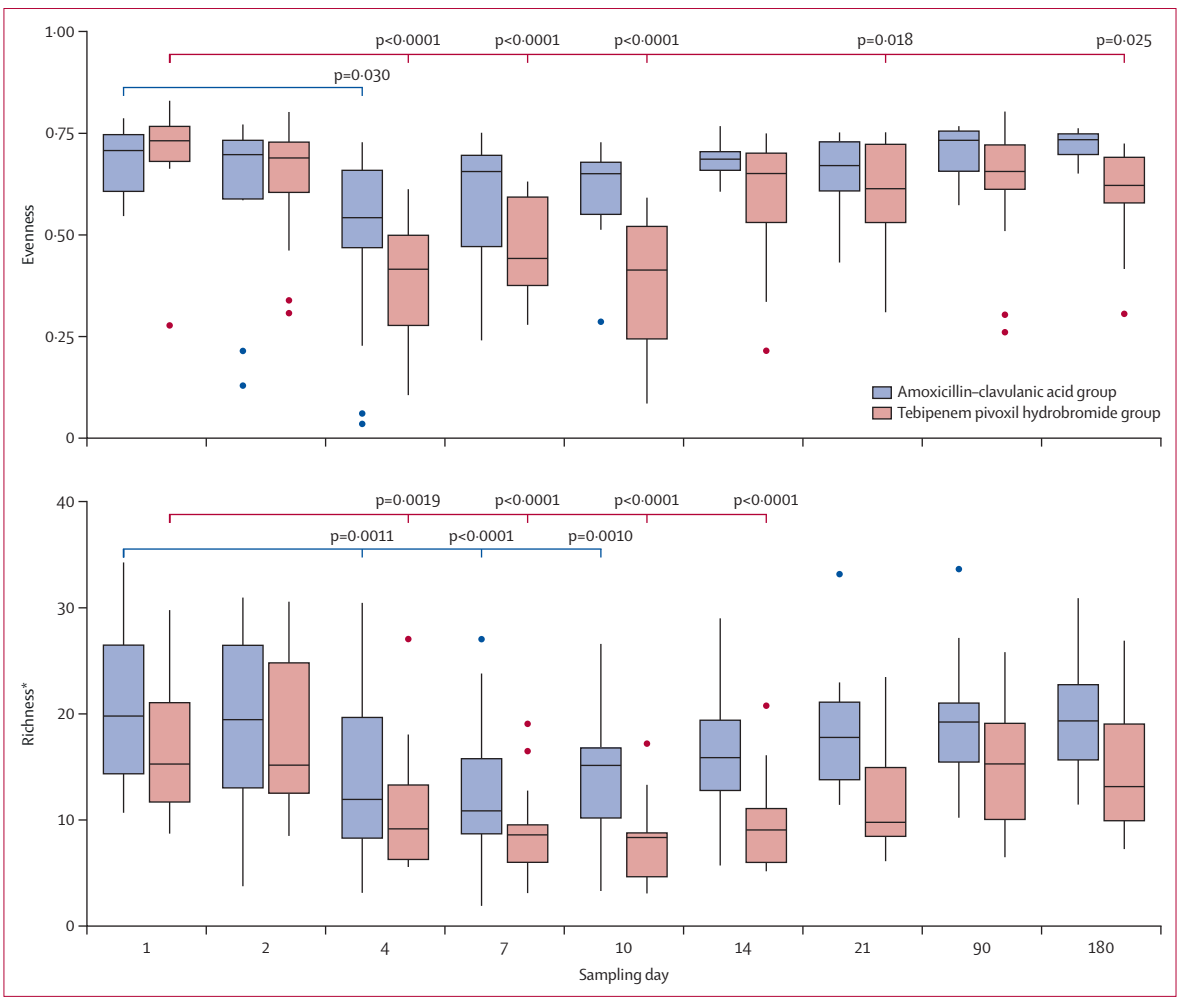

Figure 4: **α diversity metrics over the course of the sampling period**
p values are shown only for values that were significantly different from the baseline (day 1) value for the respective group; detailed data are provided in appendix 2 (p 5). *Faith's phylogenetic diversity.[21]

headache (11 [73%] of 15 participants), diarrhoea (nine [60%]), nausea (five [33%]), upper abdominal pain (three [20%]), and suspected COVID-19 (two [13%]). Adverse events reported by at least two participants in the amoxicillin–clavulanic acid group were headache (seven [47%] of 15 participants), diarrhoea (five [33%]), nasopharyngitis (four [27%]), nausea (three [20%]), back pain (two [13%]), myalgia (two [13%]), neck pain (two [13%]), and fatigue (two [13%]).

The most common treatment-related adverse events reported by at least two participants in the amoxicillin–clavulanic acid group were diarrhoea (five [33%]), headache (three [20%]), and fatigue (two [13%]). Similarly, in the tebipenem pivoxil hydrobromide group, treatment-related adverse events were diarrhoea (nine [60%]), upper abdominal pain (two [13%]), and headache (two [13%]). Alanine aminotransferase and aspartate aminotransferase were transiently increased in one participant in the amoxicillin–clavulanic acid group, and were identified and recorded as adverse events; no other clinically significant deviations were noted in the safety laboratory results in either group. Changes in systolic and diastolic blood pressure, temperature, and heart rate were generally small and not considered to be clinically meaningful. Detailed information, including on the coding of adverse effects, is provided in appendix 1 (pp 32–35).

Discussion

We observed that the effects of tebipenem pivoxil hydrobromide on the abundance of Enterobacterales and *Enterococcus* spp were more pronounced than those of amoxicillin–clavulanic acid. The abundance of *Bacteroides* spp was significantly affected in the tebipenem pivoxil hydrobromide group in contrast to the amoxicillin–clavulanic acid group, but pronounced effects were seen for *Bifidobacteria* and *Lactobacillus* spp in the amoxicillin–clavulanic acid group during the treatment period. However, during the follow-up period, the observed values were not significantly different from those at baseline in either

antimicrobial group. These findings show that after cessation of antimicrobial exposure, the changes either reverted to baseline or the residual differences did not reach statistical significance.

In the analysis of secondary outcomes, the emergence of resistance to cephalosporins among the most clinically relevant Enterobacterales (*E coli* and *K pneumoniae*; 7%) and selection of *C difficile* were low. Increased growth of *Candida* spp during tebipenem pivoxil hydrobromide treatment relative to baseline might have consequences in immunosuppressed individuals, but the observed changes did not persist. Furthermore, there were no serious adverse reactions observed in either the tebipenem pivoxil hydrobromide or the amoxicillin–clavulanic acid group, and both were well tolerated by participants. Microbiome analysis with quantitative culture and 16S rDNA metagenomic methods showed similar findings in most cases; however, quantitative culture had some advantages in the analysis of selected low-abundance but clinically relevant cultivable taxa. Generally, the changes in the anaerobic taxa were not significant in either group, and the significant changes observed in Enterobacterales and *Enterococcus* spp reverted to baseline values after treatment.

Given that antibiotics are one of the major disruptors of gut microbiota,[22] the impact of microbiome perturbation and recovery is important clinical information to consider before an inevitable antibiotic therapy.[23] The low concentration of faecal tebipenem in most samples makes it challenging to draw conclusions on effect of faecal drug concentrations on richness or dominance of ESBL-producing strains. The low faecal tebipenem concentration might be favourable in terms of the risk for selection of resistance; however, since the MICs were generally low, it is possible that low concentrations could be sufficient to confer selection of resistant isolates. The strategy of studying selective pressure at MIC levels is debatable, and future studies could also include determination of the minimum selective concentration.

No previous data were available on the impact of tebipenem pivoxil hydrobromide on the gut microbiome. The findings of this study provide valuable additional insight into the clinical use of tebipenem pivoxil hydrobromide, which could be used for the treatment of infections with MDR Enterobacterales, including *E coli*, *K pneumoniae*, and *Proteus mirabilis*.[24] In-vitro and in-vivo animal studies (using a murine ascending *E coli* urinary tract infection model and mouse thigh and lung infection models) have shown equivalence of tebipenem pivoxil hydrobromide with intravenous meropenem (300 mg/kg every 8 h in the lung infection model).[25] Similarly, orally administered tebipenem pivoxil hydrobromide given at 600 mg every 8 h has been shown to be non-inferior to intravenous ertapenem (1 g over 30 min every 24 h) for complicated urinary tract infections.[26,27] Even though reversal of changes was observed, oral carbapenems should be used cautiously, and antimicrobial stewardship teams should monitor use to ensure it is limited to treatment of cephalosporin-resistant infections.

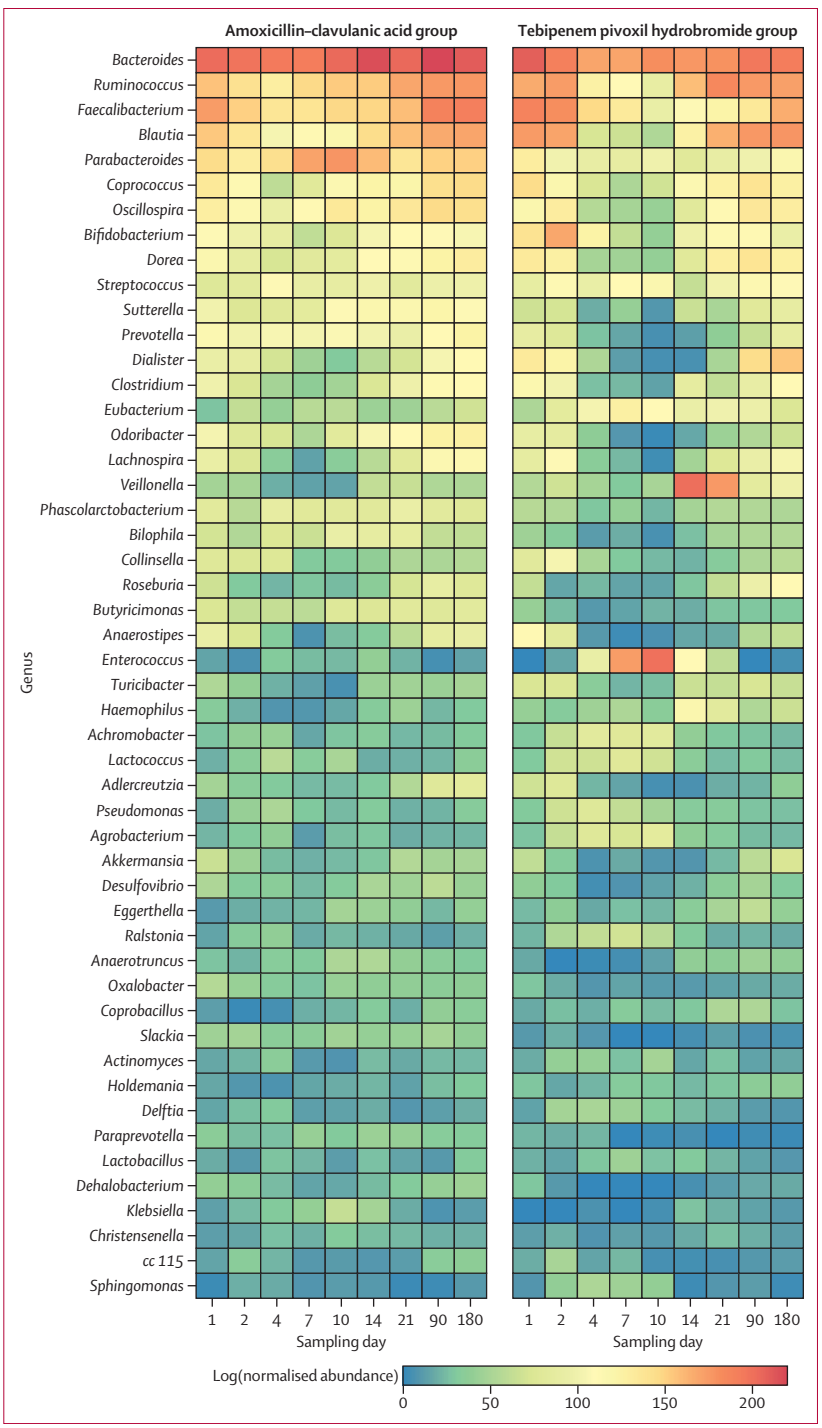

Figure 5: **50 most abundant genera observed in the studied microbiome population**
Data were aggregated across all participants, normalised using the cumulative sum scaling method, and log-transformed to enhance visibility.

The significant differences in perturbations of Enterobacterales can be explained by the differential activity of antibiotics on different phyla. Tebipenem and other

carbapenems are highly active against Enterobacterales. By contrast, amoxicillin–clavulanic acid exposure has been associated with increased Enterobacterales abundance while decreasing the counts of anaerobes.[28,29] In addition, in this study, increased proportions of amoxicillin–clavulanic acid-resistant Enterobacterales were isolated from the amoxicillin–clavulanic acid group compared with the tebipenem group and also relative to baseline. Moreover, significant perturbations from baseline of *Enterococcus* and *Bacteroides* spp in the tebipenem pivoxil hydrobromide group and of *Bifidobacterium* and *Lactobacillus* spp in the amoxicillin–clavulanic acid group were observed. Several other factors might have contributed to the differential effect of each antibiotic on different taxa, including the spectrum of the antibiotic, host factors, proportion of resistant isolates, and pharmcodynamic and pharmacokinetic properties of the antibiotic. Further mechanistic studies could be of interest to identify mechanisms of selection. However, we found that the perturbations were recovered back to baseline following the withdrawal of antibiotic exposure in both study groups.

A strength of our study is that it was longitudinal, enabling us to measure temporal changes in the microbiome within groups. Additionally, all study participants completed the study with adherence to the study protocol, including completion of diaries and full attendance at clinic visits during treatment and follow-up periods. Moreover, we used both quantitative culture and 16S rDNA metagenomics for microbiome analysis, and the findings with these respective methods showed that they are complementary; the use of quantitative selective culture compensated for the inherent limitation of 16S rDNA that a taxa with small fraction in a sample for microbiome analysis can be under-represented. Furthermore, data regarding phenotypically resistant fractions of the microbiota can only be analysed with quantitative culture.

A limitation of the study is that the study design did not include a sample size calculation. Some advances have been made regarding how sample size can be calculated for similar studies.[30] Such calculations depend on data from pilot studies, which were unavailable at the time we conducted the trial. Additionally, the low level of selection of resistance to cephalosporins might not be generalisable to other geographical regions where resistance to cephalosporins in the population is higher than at the present study site. Future studies should consider evaluating the effect of tebipenem pivoxil hydrobromide on the gut microbiome in areas with a high prevalence of antimicrobial resistance. In addition, studies considering the effects on the microbiome among patient populations will be important to consider. For example, the impact of tebipenem pivoxil hydrobromide on the microbiome of immunosuppressed patients or patients with underlying gastrointestinal diseases needs to be studied further.

In conclusion, tebipenem pivoxil hydrobromide was generally safe and well tolerated in this patient population, and there were no deaths or severe adverse effects observed during treatment or follow-up. The effect of tebipenem pivoxil hydrobromide on the gut microbiome was similar to that of amoxicillin–clavulanic acid. These results suggest that although amoxicillin–clavulanic acid and tebipenem pivoxil hydrobromide substantially affect the gut microbiome, there is still a relatively rapid recovery, which supports the safety of both compounds for clinical use. However, tebipenem pivoxil hydrobromide should be used restrictively to preserve its activity against MDR strains.

Contributors

TS, SR, CGG, and IAC contributed to study conceptualisation, design, and methods. CGG and SR conducted administrative elements of the project. SR was the principal investigator and acquired clinical data. CGG was the principal microbiological investigator. AC contributed to microbiological data acquisition. TS and MR curated the data, and MR conducted the bioinformatic analyses. TS, MR, and CGG were responsible for the data interpretation, drafted the manuscript, and validated the data with assistance from IAC, DM, MN, LBG, PBE, and CEN. All authors were involved in revising the work for intellectual content and approved the final manuscript. CGG, PBE, IAC, and SR accessed and verified all the data in the study. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

MN, DM, PBE, and IAC were employees of Spero Therapeutics and LBG was a consultant at Spero Therapeutics during the study. No personal honoraria were paid out to any of the academic investigators. All other authors declare no competing interests.

Data sharing

De-identified study data and the study protocol will be made available at the time of publication of this Article, on request to Karolinska Institutet. Study data requests should be made to Christian G Giske (christian.giske@ki.se) and protocol requests should be made to Staffan Rosenborg (staffan.rosenborg@regionstockholm.se). The statistical analysis plan is included in appendix 1 (p 28).

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