



# Effects of Ampicillin on the Gut Microbiome of an Adult Male as Determined by 16S rRNA V4 Metagenomics Sequencing and Greengenes Bioinformatics Suite

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## Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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## ABSTRACT

**Aims:** To determine the effects of ampicillin on the relative abundance of the gut microbiota using Next Generation Sequencing (NGS) metagenomics platform and to predict metabolic functions with bioinformatics suite.

**Methods:** The subject who has taken no antibiotics for the past six months provided fecal sample on day 0 (pre-antibiotics), day 10 (antibiotics) to treat sour throat infection and after 90 days (post-antibiotics). DNA was extracted, metagenomics library prepared and 16S rRNA V4 region was amplified using custom bar-coded primers before sequencing with IlluminaMiseq program. Sequence reads were analyzed with IlluminaBasespace algorithm and greengenes bioinformatics suites. Metabolic functional prediction was accomplished using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) algorithm.

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**Results:** Pre-antibiotics sequence reads were classified into 20 phyla, 35 Class, 71 Order, 147 Family, 275 Genera, and 401 Species-level categories. Ampicillin significantly influenced the gut microbiota as the sequence reads were reduced to 8 phyla, 15 Class, 24 Order, 41 Family, 72 Genera, and 98 Species-level categories. Post-antibiotics sample (90 days after antibiotic treatment) generated sequence reads classified into 17 phyla, 28 Class, 60 Order, 120 Family, 221 Genera, and 313 Species-level categories.

**Conclusion:** Ampicillin reduced the diversity of the core bacterial phylogenetic taxa with a corresponding increase in *Firmicutes-Bacteroidetes* ratio from 2.4:1 (pre-antibiotics) to 6.5:1 (antibiotics). A high proportion of *Veillonella* species were observed during ampicillin intake. Some bacterial metabolic functions such as carbohydrate (Ascorbate and aldarate), amino acid (D-Arginine and D-ornithine) and vitamin (pantothenate and CoA biosynthesis) metabolisms were stimulated by ampicillin in the subject.

**Keywords:** Ampicillin; gut; microbiome; metabolic functions; metagenomics; greengenes.

## 1. INTRODUCTION

Introducing antibiotics in medical practice in the 1930s advanced survival of humans from infectious diseases. However, it has also led to extinct of copious numbers of “beneficial” microbes from the environment. The human gut microbial community is in a continuous metabolic state from *in-utero* life to even after the end of life. Microbial kinetics are involved in the production of enzymes, vitamins, and various compounds that play significant roles in food metabolism and regulation of the immune system [1]. For more than half a century, scientists have estimated that microbes in the human gut are up to  $10^{14}$  with over 1000 different species encoding over 3 million genes and an approximate biomass of 2 kg [2]. The previous assumption that the human microbiota out-number the human cells in the ratio of 10:1 has been challenged, by proposing that the proportion of microbial cells to human cells might be 1.3:1 [3]. The relative abundance and diversity of the gut microbiota is influenced by multiple factors such as nutrition, environmental exposures, geographic location, and host genetics. It results in microbial community variations among individuals and niches [4].

Unwarranted and inappropriate use of antibiotics for treatment of infections is one of the leading processes that lead to perturbations of the human microbiome [5] and emergence of antibiotic-resistant microbes [6]. In the so-called developed countries of the Western hemisphere, antibiotics are prescribed before and after surgery to prevent infections [7]. In countries undergoing developmental transition, antibiotics are available over-the-counter often due to lack of governmental legislative control, thus leading to self-medication and irrational use [8,9].

Several authors have investigated the impact of other antibiotics on the gastrointestinal tract microbiota [10,11]. Ampicillin is a beta-lactam antibiotic used as a broad-spectrum. The effects on the relative abundance of the gut microbiota and bacterial metabolic functions are not studied with the NGS metagenomics platform in Africa, nay Nigeria. In this study, the first objective is to determine the impact of ampicillin on the relative abundance and diversity of faecal microbiota of an adult Nigerian male. Second objective is to use metagenomic bioinformatics to predict the influence of ampicillin on the microbiome metabolic functionality in the gut of the subject.

## 2. MATERIALS AND METHODS

### 2.1 Ethics declaration

A written consent was obtained from the subject with absolute adherence to the Helsinki protocol. The subject enrolled in the study to know the effect of consuming a broad-spectrum antibiotic on the gut microbiome.

### 2.2 Gut Sampling

Gut microbiota were sampled non-invasively using the gut sample collection kit provided by uBiome<sup>®</sup>. The subject who has taken no antibiotics for the past six months provided fecal sample on day 0 (Pre-antibiotics). Second sample was collected on the last (10<sup>th</sup>) day (Antibiotics) after consumption of 10-days course of antibiotics (Ampicillin-500 mg x2 daily) to treat sour throat infection. Third sample was collected after 90 days (Post-antibiotics). The subject took none other antibiotics before the 90 days duration. The three samples were collected at the targeted time points following the instructions provided by uBiome<sup>®</sup>.

## 2.3 DNA Extraction and Sequencing

Metagenomic DNA was extracted using the PowerSoil-htp DNA isolation kit (MoBio Laboratories Ltd, Carlsbad, CA), following uBiome extraction protocols, which involves both chemical and physical lysis of the cells. Extracted DNA was amplified using custom bar-coded primers (515F and 806R primers as previously described [12] prior to sequencing and sequenced with paired-end 150 bp reads on an IlluminaMiSeq program.

The primers have attached to them barcodes, indexes and other sequences needed for paired-end sequencing in the Illumina protocol. The paired-end sequencing was performed in an IlluminaMiSeq sequencer that has a flow cell with four lanes. This means that each sample was read in four different lanes (L001 to L004), and each produced forward (R1) and reverse (R2) reads.

## 2.4 Data Analysis

The 8 reads were imported into BaseSpace sequence bioinformatics hub, which is a cloud-based genomics environment for next-generation sequencing (NGS) data management and analysis.

## 2.5 Fast QC Check

The paired end sequence raw reads were checked for quality using the FastQC version 0.11.3. [13]. Four analytical quality parameters of the reads were selected which includes per base sequence quality, per sequence quality scores, per sequence GC content, and sequence length distribution. Microbial diversity in all the samples points were determined by using the inverse Simpson's Diversity Index. Simpson's diversity index (D) is a mathematical measure that characterizes species diversity in a community. The proportion of species relative to the total number of species is calculated and squared. The squared proportions for all the species are summed, and the reciprocal is taken. Scores range from 0 to 10, with 10 being the most diverse.

Quantitative Insights into Microbial Ecology (QIIME) pipelines [14] was used for 16S rRNA recognition. Operational Taxonomy Unit (OTU) clustering and Microbial taxonomy to species level was generated using the Greengene database (gg\_13\_5\_species\_32bp.database).

## 2.6 Functional Prediction

Analysis of the 16S rRNA amplicons was accomplished using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) tool version 0.9.1 [15]. This is based on the data derived from the KEGG pathway database, which predicts functional capabilities by multiplying each normalized OTU abundance by the predicted functional characteristic.

## 3. RESULTS

The eight reads for each sample produced on average 13,489,184 base pair count per read containing 89,587 sequences ranging from 32 bp to 151 bp and averaging 150bp in length (std. deviation from average length 5.580). All of the sequence reads have unique identities. Table 1 shows the classification statistics presenting the total sequence reads for the three sample points and the taxonomic level categories. The 16S rRNA metagenomics report showed that in general the sequence reads generated for pre-antibiotics, antibiotics and post-antibiotics samples as represented in Table 1 and Fig. 1. are classified by taxonomic level categories. Specifically, in pre-antibiotics sample, 99.56% of the total sequence reads were assigned to bacterial domain, while 0.44% (1,514 reads) remained unclassified at kingdom level and only 1 sequence read was assigned to archaea domain. Pre-antibiotics resulting sequence reads were classified into 20 phyla, 35 Class, 71 Order, 147 Family, 275 Genera, and 401 Species-level categories (Table 1).

Sample collected after 10 days consumption of ampicillin antibiotics generated sequence reads of which 78.01% were unclassified at Kingdom level, and only 21.99% were assigned to bacterial domain. The sequence reads were classified into 8 phyla, 15 Class, 24 Order, 41 Family, 72 Genera, and 98 Species-level categories.

Post-antibiotics sample (90 days after antibiotic treatment) generated 184,445 sequence reads representing 85.38% classified as bacteria, while 31,572 sequence reads (14.62%) were unclassified at Kingdom level. The sequence reads were classified into 17 phyla, 28 Class, 60 Order, 120 Family, 221 Genera, and 313 Species-level categories.

**Table 1. Classification statistics showing the total sequence reads and categories of taxonomic levels identified**

<b>Classification statistics</b>	<b>Pre-antibiotics</b>		<b>Antibiotics</b>		<b>Post-antibiotics</b>	
Upload base pair counts	13,489,184 bp		1,378,892 bp		6,863,315 bp	
Post QC base pair count	547,713 bp		89,590 bp		329,162 bp	
Total sequence reads	343,860		75,002		216,017	
<b>Classification results by taxonomic level</b>						
Taxonomic Level	Reads Classified to Taxonomic level	% Total Reads Classified to Taxonomic level	Reads Classified to Taxonomic level	% Total Reads Classified to Taxonomic level	Reads Classified to Taxonomic level	% Total Reads Classified to Taxonomic level
Kingdom -Classified (unclassified)	342,346 (1,514)	99.56 (0.44)	16,494 (58,508)	21.99 (78.01)	184,445 (31,572)	85.38 (14.62)
Phylum -Classified (unclassified)	340,777 (3,083)	99.1 (0.90)	16347 (58,655)	21.8 (78.20)	183,427 (32,590)	84.91 (15.09)
Class -Classified (unclassified)	338,108 (5,752)	98.33 (1.67)	16,165 (58,837)	21.55 (78.45)	182,348 (33,669)	84.41 (15.59)
Order -Classified (unclassified)	336,751 (7,109)	97.93 (2.07)	16,116 (58,886)	21.49 (78.51)	181,783 (34,234)	84.15 (15.85)
Family -Classified (unclassified)	333,679 (10,181)	97.04 (2.96)	16,067 (58,935)	21.42 (78.58)	180,742 (35,275)	83.67 (16.33)
Genus -Classified (unclassified)	329,304 (14,556)	95.77 (4.23)	16,003 (58,999)	21.34 (78.66)	178,117 (37,900)	82.46 (17.54)
Species -Classified (unclassified)	190,091 (153,769)	55.28 (44.72)	10,595 (64,407)	14.13 (85.87)	107,139 (108,878)	49.60 (50.40)
Total phylum level categories identified	20		8		17	
Total Class level categories identified	35		15		28	
Total Order level categories identified	71		24		60	
Total Family level categories identified	147		41		120	
Total Genus level categories identified	275		72		221	
Total Species level categories identified	401		98		313	

In terms of relative abundance dominance at phylum level, pre-antibiotic sample was dominated by Firmicutes (55.48%), followed by Bacteroidetes (26.15%), Actinobacteria (14.05%), Proteobacteria (3.23%), Unclassified at phylum level (0.90%), Spirochaetes (0.07%), Fusobacteria (0.06%), Tenericutes (0.03%), Chloroflexi (0.022%), Chlorobi (0.004%), Cyanobacteria (0.004%), Thermi (0.002%), Acidobacteria (0.001%), Chrysiogenetes (0.001%), Deferribacteres (0.001%), and Verrucomicrobia (0.001%). The following phyla; Crenarchaeota, Nitrospirae, Thermodesulfobacteria and Thermotogae had only one sequence read respectively (Fig. 1).

Antibiotic sample was dominated by reads that are unclassified at phylum level (78.20%),

*Firmicutes* (16.95%), *Bacteroidetes* (2.48%), *Actinobacteria* (2.15%), *Proteobacteria* (0.15%), *Spirochaetes* (0.07%), *Tenericutes* and *Thermi* had only one sequence read individually.

Post-antibiotics sample had relative abundance in the following order; *Firmicutes* (49.39%), *Bacteroidetes* (25.21%), Unclassified at phylum level (15.09%), *Proteobacteria* (8.81%), *Actinobacteria* (1.38%), *Spirochaetes* (0.08%), *Fusobacteria* (0.01%), *Cyanobacteria* (0.01%), *Chloroflexi* (0.004%), *Chlorobi* (0.004%), *Tenericutes* (0.004%), *Verrucomicrobia* (0.004%), *Acidobacteria* (0.001%), *Chrysiogenetes* (0.001%), *Synergistetes* (0.001%), *Deferribacteres* and *Chlamydiae* are represented by one sequence read separately.

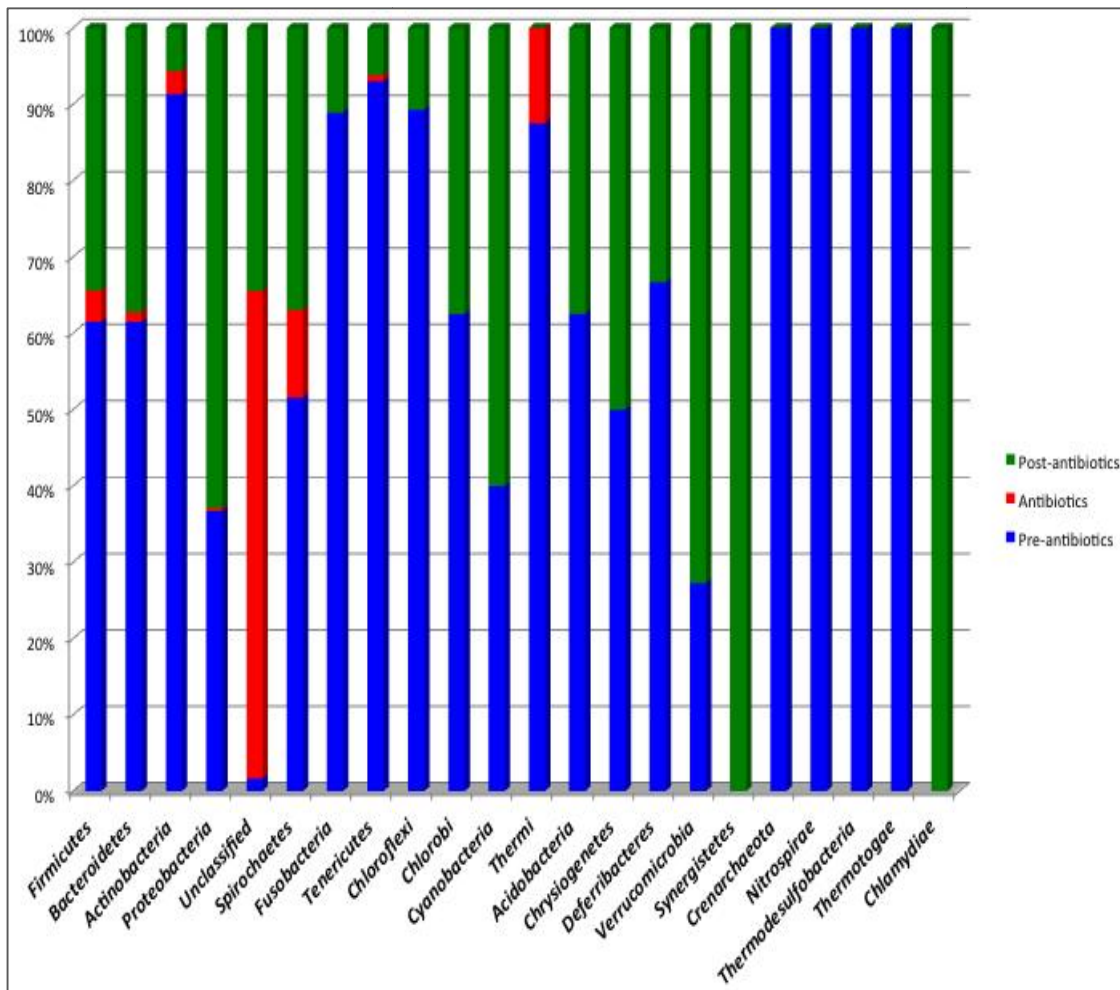


Fig. 1. Relative abundance of the phyla taxa represented as 100% stacked bar

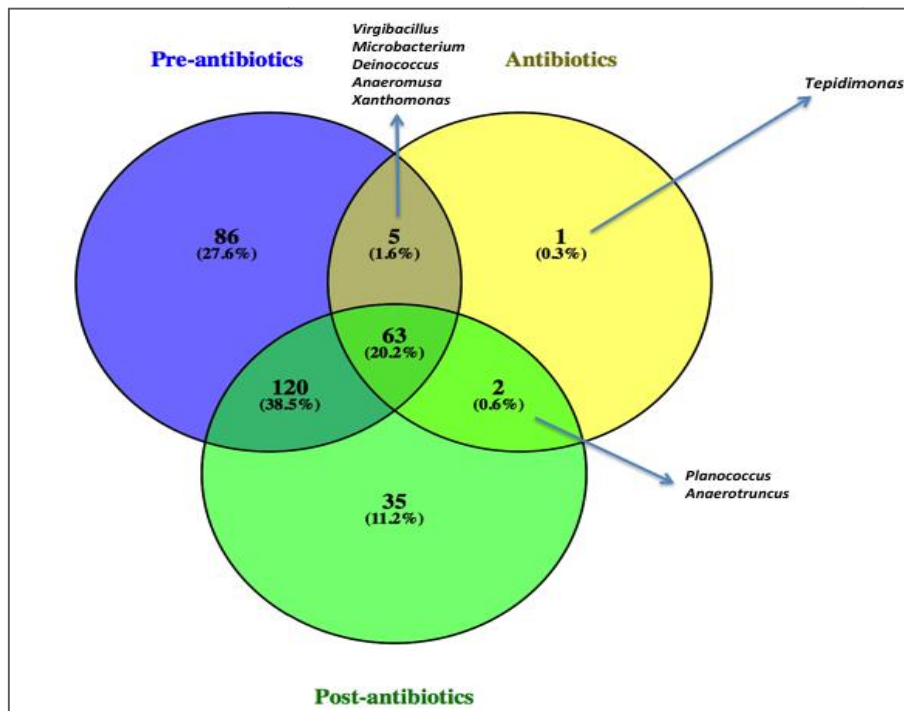


Fig. 2. Venn diagram showing the genera that are common and shared at the sampled time points

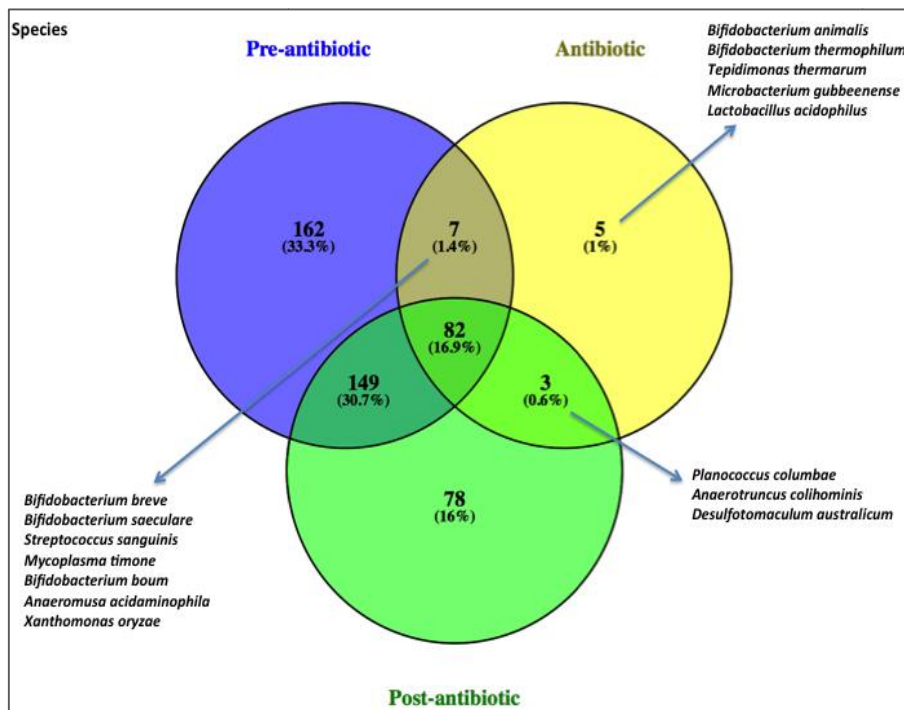


Fig. 3. Venn diagram representing the species that are shared and common during the pre-ampicillin, ampicillin and post-ampicillin administration

At the genera taxonomic level, 86 (27.6%) are exclusively present in pre-antibiotics, 1 (0.3%) in antibiotics and 35 (11.2%) in post-antibiotics. Interestingly, 63 (20.2%) genera were common in all the sample points (Fig. 2).

Among the common genera are *Faecalibacterium*, *Blautia*, *Bifidobacterium*, *Bacteroides*, *Dorea*, *Coprococcus*, *Lachnospira*, *Oscillospira*, *Ruminococcus*, *Johnsonella*, *Anaerofilum*, *Clostridium*, *Sutterella*, *Odoribacter*, *Serratia*, *Roseburia*, *Collinsella*, *Coprobacillus*, *Pedobacter*, *Phascolarctobacterium*, *Peptostreptococcus*, *Mogibacterium*, *Streptococcus*, *Dysgonomonas*, *Slackia*, *Oribacterium*, *Porphyromonas*, *Turicibacter*, *Actinomyces*, *Lactobacillus*, *Alkalibacterium*, *Adlercreutzia*, *Pseudobutyrvibrio*, *Leptospira*, *Legionella*, *Peptococcus*, *Selenomonas*, *Veillonella*, *Pediococcus*, *Anaerostipes*, *Peptoniphilus*, *Acidaminococcus*, *Lachnobacterium*, *Macrococcus*, *Alkaliphilus*, *Sedimentibacter*, *Caloramator*, *Mycoplasma*, *Bacillus*, *Streptomyces*, *Roseomonas*, *Desulfotomaculum*, *Moorella*, *Sporotomaculum*, *Cohnella*, *Actinobacillus*, *Negativicoccus*, *Propionispora*, *Helicobacter*, *Methylnatronum*, *Stenotrophomonas*, *Brachyspira*, and *Thiorhodococcus*. At the species taxonomic level, 82 (16.9%) were common in all the sample points as shown in Fig. 3.

*Bifidobacterium gallicum* (7.55%), *Bacteroides stercoris* (6.41%) and *Faecalibacterium prausnitzii* (3.51%), *Blautia coccoides* (2.86%), *Bacteroides vulgatus* (2.81%), *Bacteroides thetaiotaomicron* (2.76%) and *Bacteroides fragilis* (2.70%) predominated pre-antibiotics (Fig. 4), while antibiotics event was dominated by *Veillonella atypica* (4.32%), *Bacteroides vulgatus* (1.04%), *Phascolarctobacterium faecium* (1.04%), *Bifidobacterium gallicum* (0.76%), *Bacteroides stercoris* (0.75%), *Coprococcus eutactus* (0.53%), and *Lachnospira pectinoschiza* (0.51%).

The species that dominated in post-antibiotics include *Bacteroides stercoris* (8.46%), *Faecalibacterium prausnitzii* (6.69%), *Bacteroides vulgatus* (6.35%), *Haemophilus parainfluenzae* (3.41%), *Lachnospira pectinoschiza* (2.71%), *Bacteroides dorei* (2.13%), and *Roseburia faecis* (1.46%). In post-antibiotics, *Akkermansia muciniphila* (0.003%) belonging to the phylum Verrucomicrobia, reported to have clinical relevance was identified. Fig. 5 shows the taxonomic level highlighting the impact of the ampicillin on *Bifidobacterium* species. For example twenty-three *Bifidobacterium* species were identified pre-antibiotics, showing *Bifidobacterium gallicum* as the most abundant.

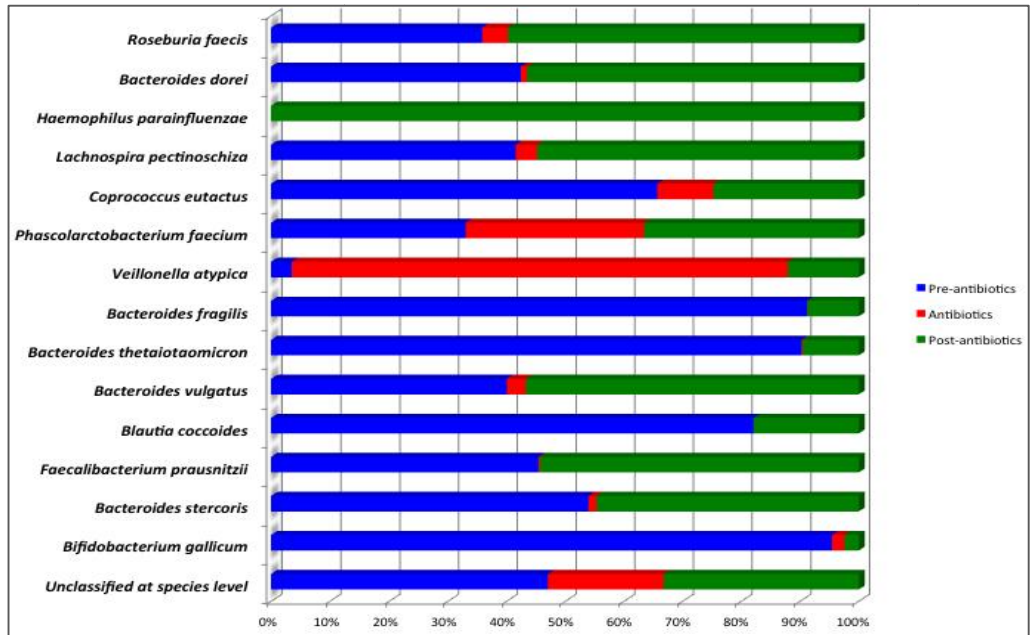


Fig. 4. Stacked bar chart (100%) showing core species that occurred with high relative abundance



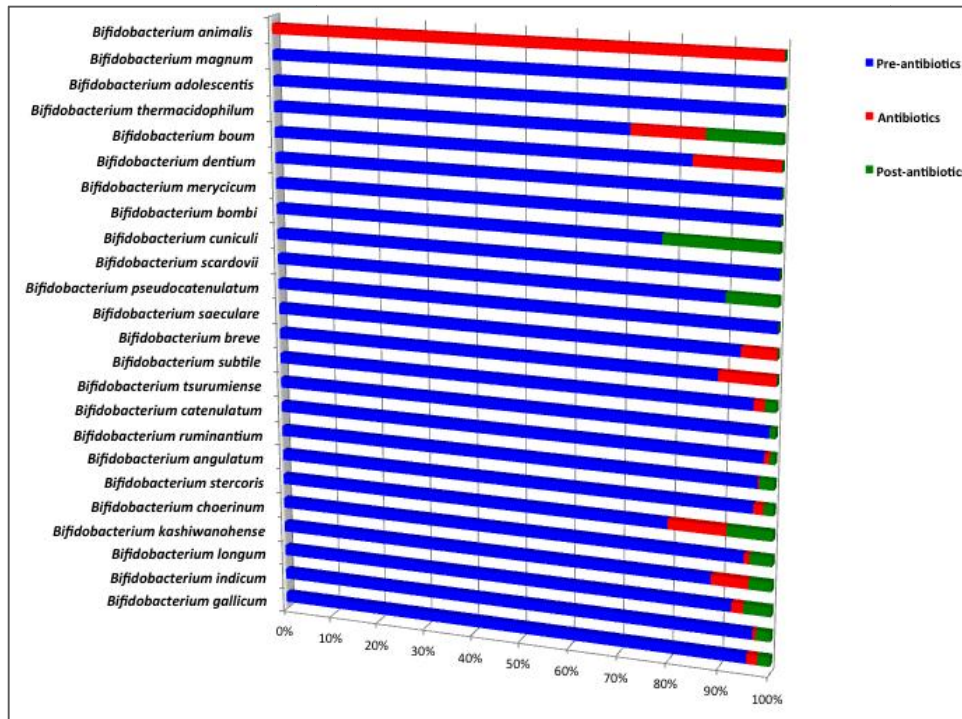


Fig. 5. Stacked bar chart (100%) at the species taxonomic level highlighting the impact of the ampicillin on *Bifidobacterium* species

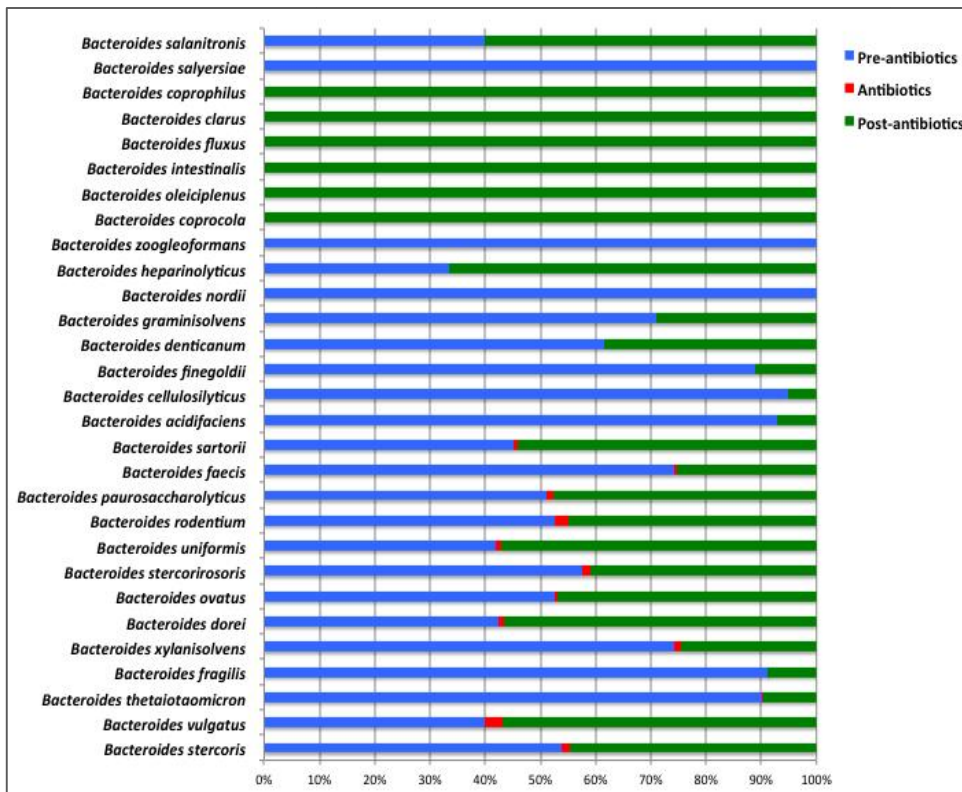


Fig. 6. Stacked bar chart (100%) presenting effects of ampicillin on *Bacteroides* species



The antibiotic episode reduced the *Bifidobacterium* species to 15 with 97.09% decrease in total abundance. Post-antibiotics (90 days after antibiotics) show a marginal recovery with 96.59% decrease from pre-antibiotics period with a corresponding over abundance of *Bifidobacterium animalis* during antibiotic event.

In the same vein, Fig. 6. presents effects of ampicillin on *Bacteroides* species showing *Bacteroides stercoris* as the most abundant with 97.5% decrease of the total abundance from the pre-antibiotics to 31.15% after 90 days post-antibiotics.

Interestingly, 6 species (*Bacteroides coprophilus*, *Bacteroides clarus*, *Bacteroides fluxus*, *Bacteroides intestinalis*, *Bacteroidesoleiciplenus* and *Bacteroides coprocola*) that were not identified pre-antibiotics and antibiotics period were found 90 days post-antibiotics administration.

Fig. 7. shows the effect of ampicillin on *Clostridium* species. A total of 23 species were identified pre-antibiotics with *Clostridium alkallicellulosi* as the most abundant species.

*Clostridium* species were drastically reduced (99.75% decrease) to only two species (*Clostridiumalkalicellulosi* and *Clostridium thermosuccinogenes*) during the course of the antibiotic. After 90 days post-antibiotics, fourteen species re-emerged, still with a decrease of 90.12% from pre-antibiotics.

Fig. 8. presents the impact of ampicillin on *Streptococcus* species. A total of 19 species were identified pre-antibiotics with *Streptococcus vestibularis* as the most abundant species. *Streptococcus* species were severely reduced (97.29% decrease) to only four species (*Streptococcus vestibularis*, *Streptococcus tigurinus*, *Streptococcus australis* and *Streptococcus sanguinis*) during the course of the antibiotic.

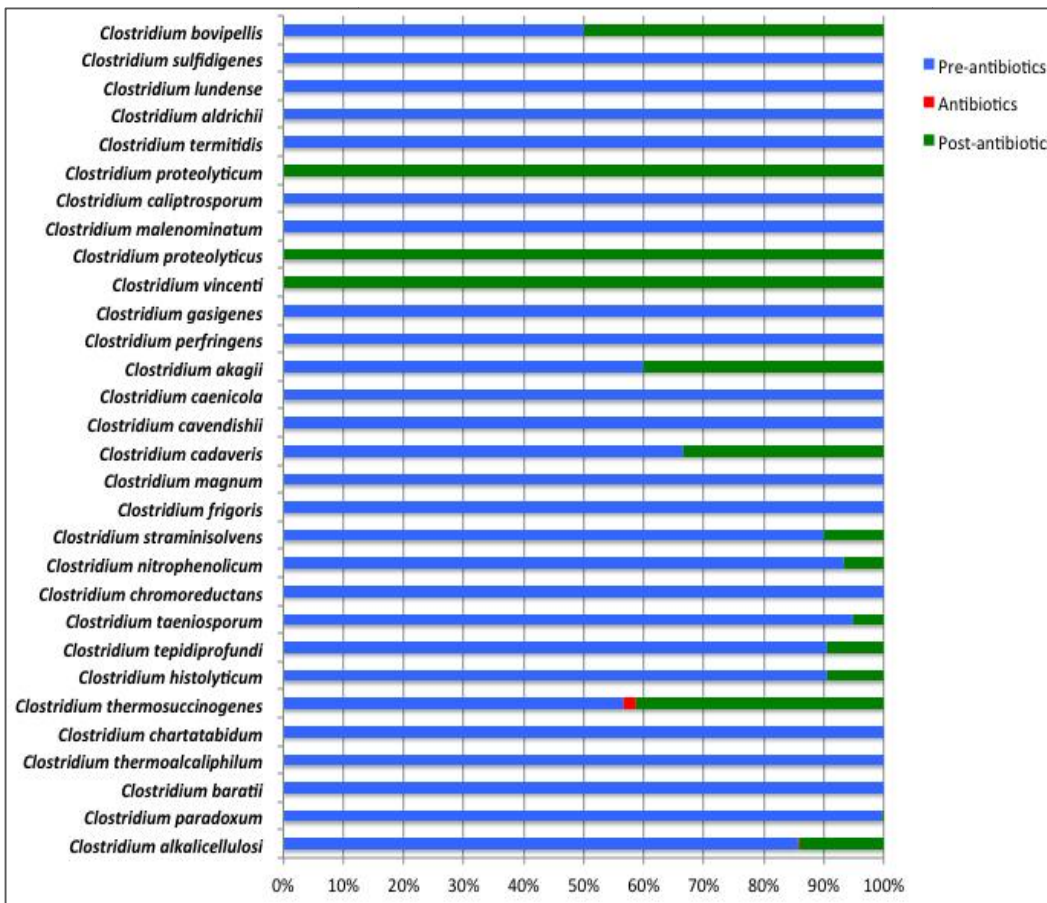
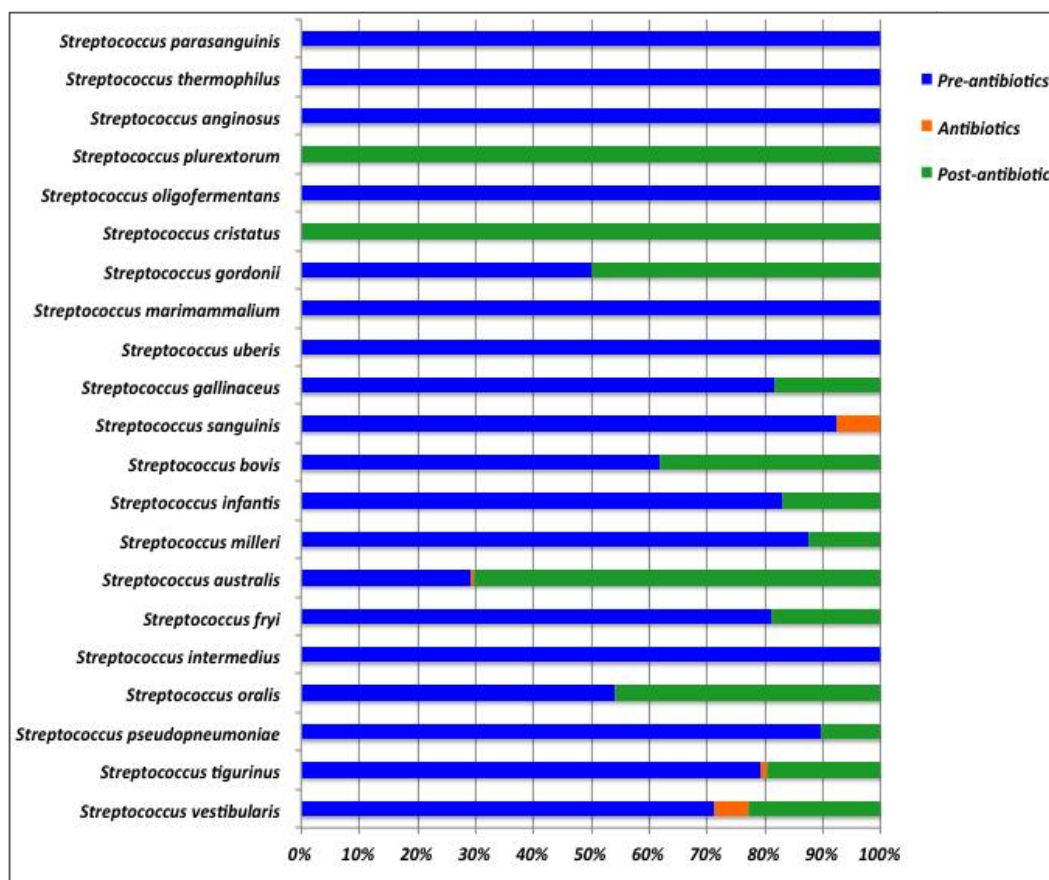


Fig. 7. Stacked bar chart (100%) showing the effect of ampicillin on *Clostridium* species



**Fig. 8. Stacked bar chart (100%) presenting the impact of ampicillin on *Streptococcus* species**

After 90 days post-antibiotics, thirteen species re-emerged (with *Streptococcus sanguinis* as the most abundant) still with a decrease of 59.14% from pre-antibiotics.

**InverseSimpson's Diversity Index (SDI):** Pre-antibiotics had Simpson's diversity index of 8.05, while antibiotics period produced an index of 7.76 and post-antibiotics had 7.53.

### 3.1 Functional Predictions

PICRUSt was used to predict the functional capabilities of the microbial community's metagenome from the 16S rRNA libraries by associating the OTU to a given function present in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Carbohydrates metabolisms in the gut microbiome were affected by ampicillin intake showing 14.14% increase.

High proportions of ascorbate and aldarate metabolism, inositol phosphate, butanoate, and propionate metabolisms were prominent. Post

antibiotics showed a marginal reduction of carbohydrate metabolism (11.42%) as shown in Fig. 9. There was a 13.64% increase in the amino acid metabolism from pre-antibiotics to antibiotics period with high proportion occurring in D-Arginine and D-ornithine, Lysine degradation, valine/leucine/isoleucine and beta-Alanine metabolism. In contrast, Cyanoaminoacid metabolism, Glycine/serine/threonine decreased marginally during antibiotics episode (Fig. 10.).

Ampicillin affected secondary metabolite degradation, by increasing caprolactam, benzoate, and styrene degradation, while it decreased atrazine and bisphenol degradation (Fig. 11). On average, there was a marked decrease of Lipid metabolisms mostly affecting steroid biosynthesis, alpha-linolenic acid metabolism, with a corresponding increase in arachidonic acid metabolism (Fig. 12.). Ampicillin increased vitamin metabolism particularly pantothenate and CoA metabolism as shown in Fig. 13.

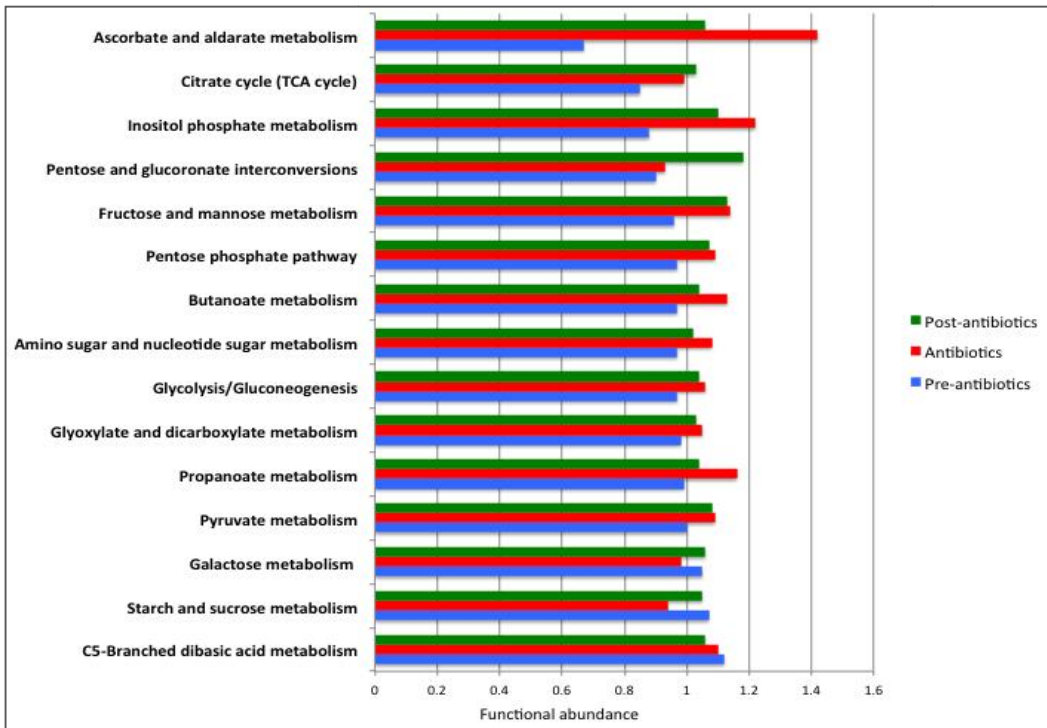


Fig. 9. Clustered bar chart showing the effect on carbohydrate metabolisms

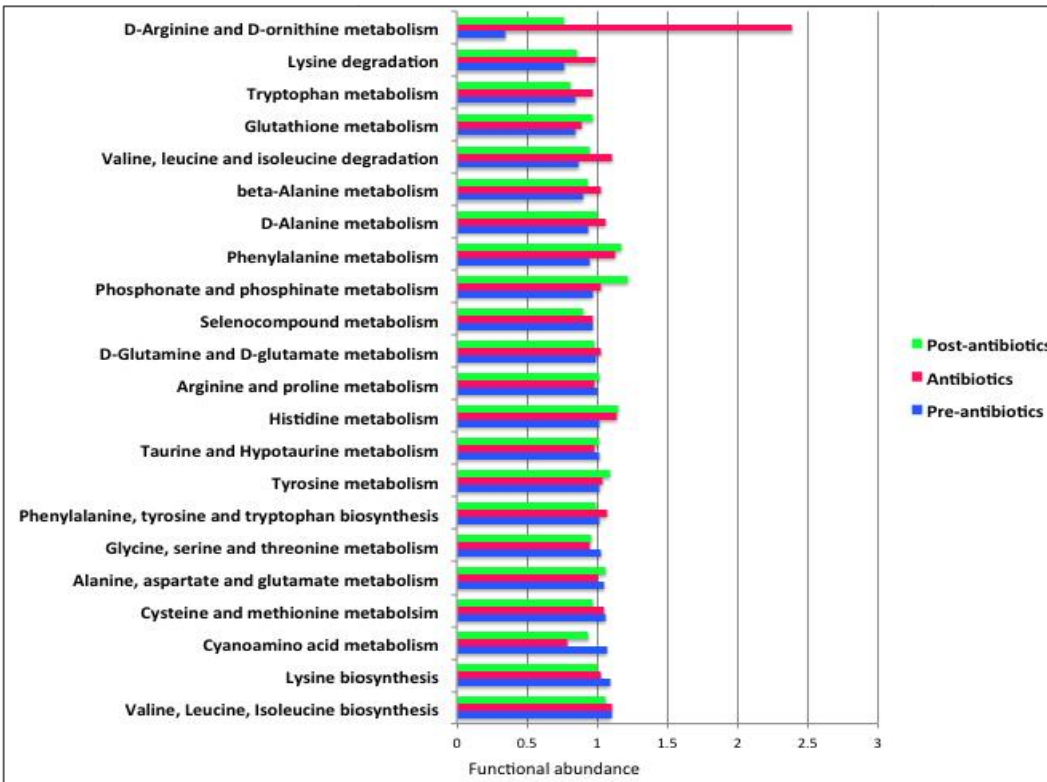
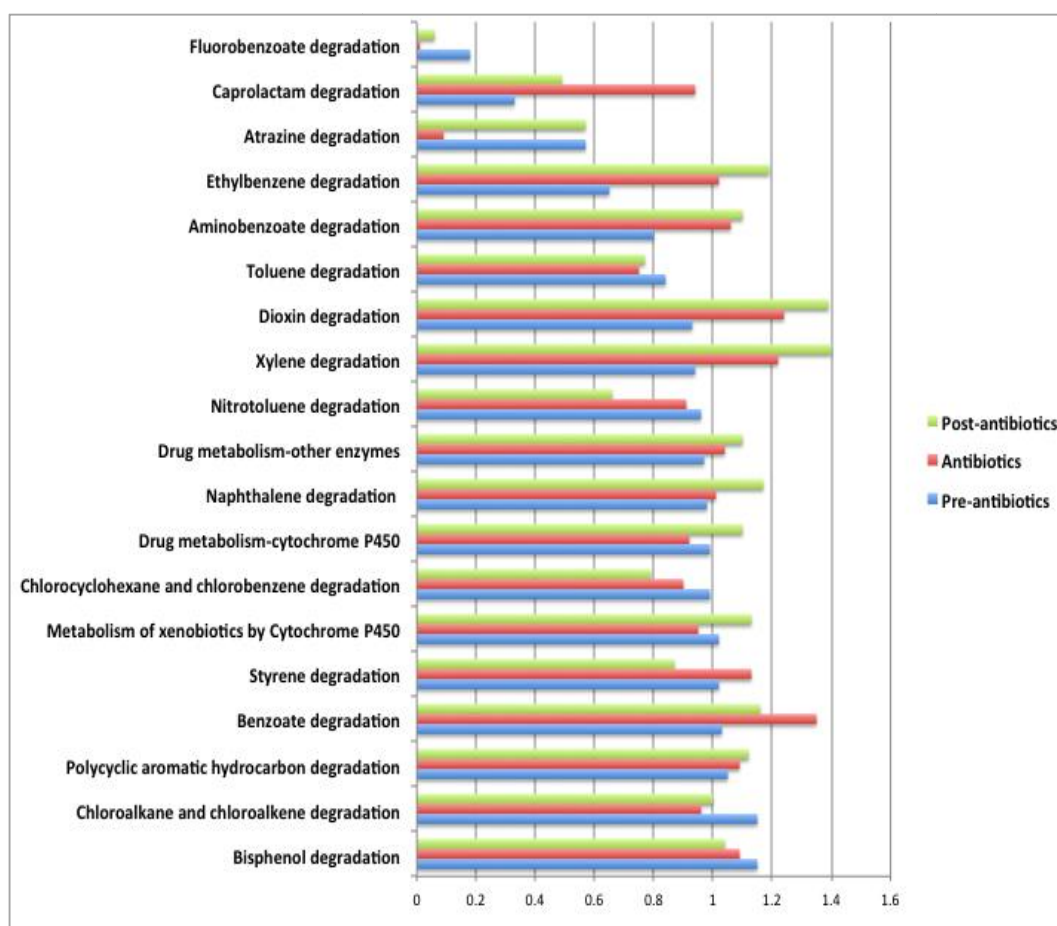


Fig. 10. Clustered bar chart showing the effect on amino acid metabolisms



**Fig. 11. Clustered bar chart on secondary metabolite degradation**

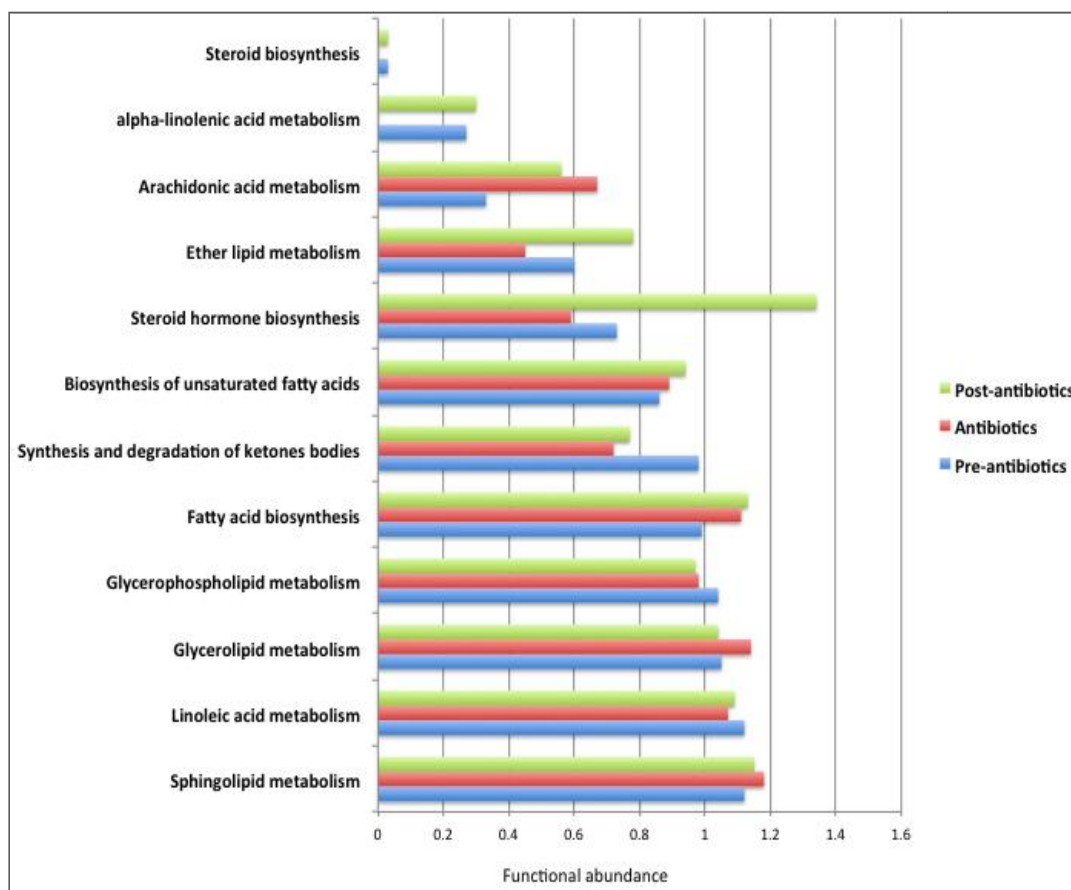
In the secondary metabolite biosynthesis, Flavone and flavonol were markedly decreased (Fig. 14.), while bacterial abilities such as Lysozyme decreased marginally.

#### 4. DISCUSSION

The contributions of antibiotics in changing the landscape of modern humans to survive various bacterial diseases are germane. However, the control of infectious diseases with antibiotics has caused significant damage to the human body and to the environment. This study has shown that the broad-spectrum antibiotics, ampicillin caused a remarkable decrease in the relative abundance and diversity of the microbiome with a substantial decrease in the sequence reads. Ampicillin is a beta-lactam antibiotics that targets the enzyme transpeptidase. Other studies have reported that ampicillin led to a decrease in bacterial diversity and a greater prevalence of *Enterobacter spp.* [16]. The microbiome of this

case study before antibiotics administration had higher diversity with *Firmicutes-Bacteroidetes* ratio of 2.4:1. Ampicillin reduced the diversity of the core bacterial phylogenetic taxa from 20 to 8 and reduced species from 401 to 98 as shown in Table 1. with a corresponding increase in *Firmicutes-Bacteroidetes* ratio of 6.5:1. Previous studies have reported that antibiotics reduced the number of different microbes with corresponding pathological consequences of decreased ecological stability and increased susceptibility to infections [17]. At the phylum taxonomic level, ampicillin caused *Firmicutes* to increase by 45% while *Bacteroidetes* decreased by 46% and *Actinobacteria* by 88%. This study revealed that at the genera and species taxonomical level, *Veillonella atypica* increased by over a 1000-fold. *Veillonella denticariosi*, *Veillonella dispar* and *Veillonella montpellierensis*, increased by over a 100-fold during antibiotic administration, thus contrasting universal view on the effects of broad spectrum antibiotics on the gut microbiome.



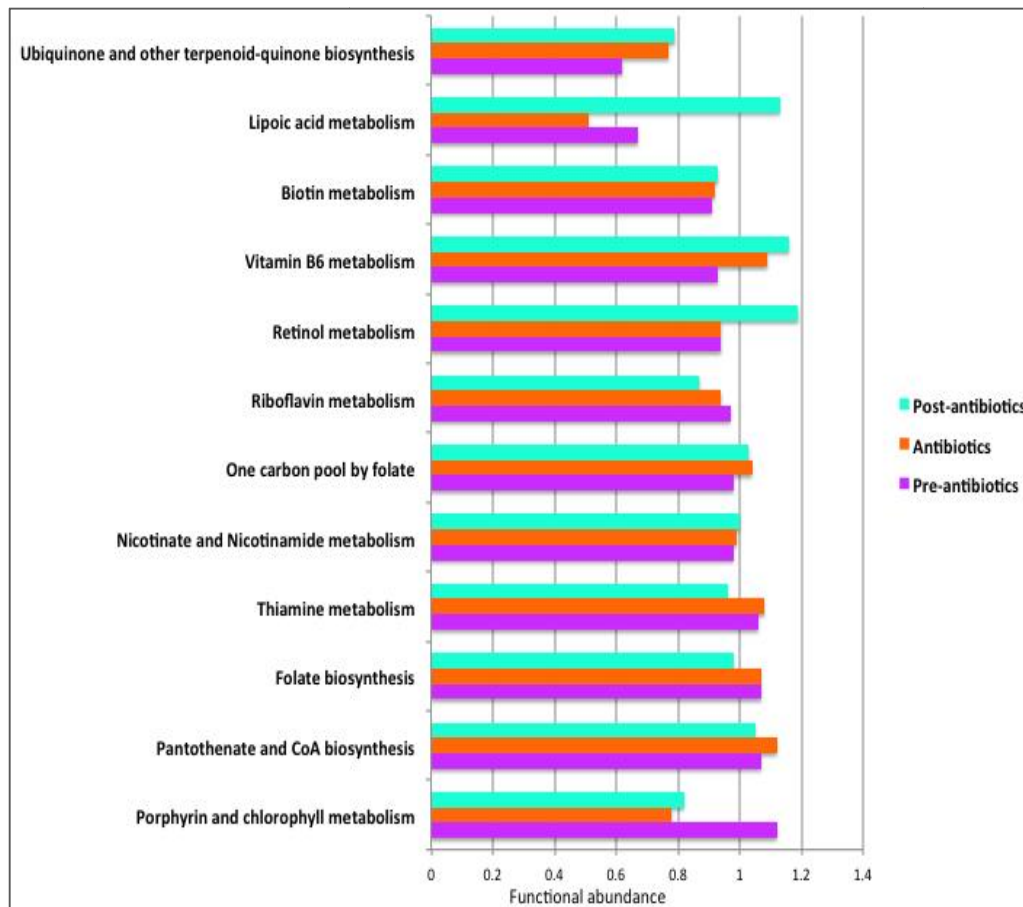


**Fig. 12. Clustered bar chart on lipid metabolisms**

Although not surprising, previous studies have shown that beta-lactams antibiotics increased microbial load by a two-fold [18] and high-calorie diet shifts the microbial ecology toward *Firmicutes* at the expense of *Bacteroidetes*, thus increasing the energy harvesting capacity of the microbiota [19]. Another study showed that antibiotic exposure during infancy was found to increase the risk of overweight in preadolescence for boys [20]. *Proteobacteria*, *Fusobacteria*, *Acidobacteria*, and *Verrucomicrobia* were wiped out by ampicillin leading to decrease in the microbial diversities. However, oral exposure of ampicillin to the adult male caused an obvious alteration even after 90 days, which led to only 85% recovery of the bacteriome at the phylum taxonomic level. In another study, oral amoxicillin exposure caused marked shifts in microbiome composition that lasted 30 days on average and were observed for over 60 days [21]. In comparison, Raymond et al., [22] reported a decrease in the

abundance of several bacterial families after the antibiotic course, but the microbiota recovered to its initial state after 90 days. Another study showed a reduction in bacterial diversity for up to 4 and 12 months after exposure [23].

Besides reducing the diversity of the gut microbiome, ampicillin led to the selection of some species particularly *Bifidobacterium animalis*, *Bifidobacterium thermophilum*, *Tepidimonasthermarum*, *Microbacterium gubbeenense* and *Lactobacillus acidophilus*, that were absent in the pre-antibiotics compositions. Other studies have shown that some antibiotics can increase microbial resistance genes through selection of strains with drug resistance reservoirs [24]. In terms of relative abundance, *Veillonella atypica* was highly selected in the antibiotic episode typified by over 85% increase from the pre-antibiotic exposure as shown in Fig. 4.



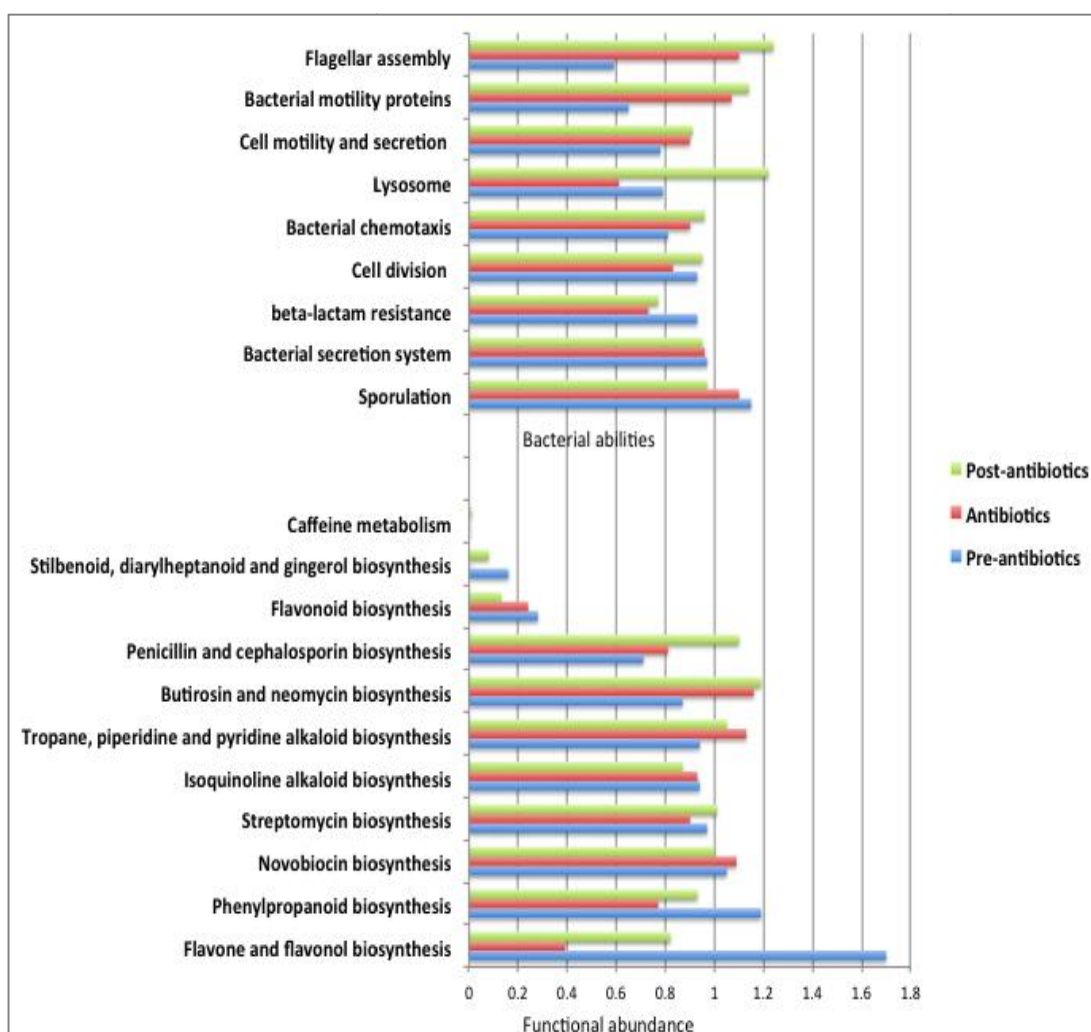
**Fig. 13. Clustered bar chart on vitamin metabolisms**

Reduction of *Bifidobacterium* species taxa from 23 to 15 is in concordance with the study of Newton et al. [25], showing the bacteriostatic effect of ampicillin on *Bifidobacterium* species. In the study, growth rate was dependent on several metabolic activities that were affected by antibiotic addition, including fermentation product formation and enzyme synthesis. The ecological and physiological roles of *Bifidobacteria* in the gut [26], especially in the biosynthesis of vitamin K, biotin, folate and thiamine are documented [27]. This study has revealed that even after 90 days of oral ampicillin administration, less than half of the species were recovered. The study reflects the general impact of irrational use of antibiotics and the corresponding potential consequences of increasing colonization by pathogenic species.

The effect of ampicillin on *Bacteroides* in this case study corroborated previous study whereby

ampicillin resulted in substantial reductions in *Bacteroides* [25]. The role of *Bacteroides* in breaking down complex polysaccharides that would otherwise be inaccessible to most other gut-adapted bacteria has been elucidated in several studies [28]. A recent study found species of *Bacteroides* to be connected in the ecological network of the gastrointestinal (GI) track microbiota [29]. Reduction of *Bacteroidetes* taxa abundances in previous studies has been associated with obesity, showing a fundamental role for these bacteria in maintaining a healthy gut microbiota [30,31]. Similar effects were observed on *Clostridium* species with extensive reduction of most species and their absolute counts. Earlier study found that ampicillin caused counts of *C. perfringens* to drop 10-fold [25]. This study showed a 100% decrease and did not rebound after 90 days post antibiotics administration.





**Fig. 14. Shows clustered bar chart on secondary metabolite biosynthesis and bacterial abilities**

It is widely believed that antibiotics are meant to obliterate pathogenic bacteria in a particular niche and possibly reduce metabolic functions to the barest minimum. However, this study has shown that some bacterial metabolic functions such as carbohydrate (Ascorbate and aldarate), amino acid (D-Arginine and D-ornithine) and vitamin (pantothenate and CoA biosynthesis) metabolisms are stimulated by ampicillin administration as observed in Fig. 9, Fig. 10 and Fig. 11). Previous studies have demonstrated that ampicillin increased bacterial expression of genes involved in tRNA biosynthesis, translation, vitamin biosynthesis, stress response and antibiotic resistance [32]. Even as ampicillin stimulated biosynthesis of some vitamins, it depleted vitamin-producing bacteria such as *Bifidobacteria* associated with vitamin K

biosynthesis. A previous study showed that some broad-spectrum beta-lactams with an N-methylthiotetrazole moiety could lead to depletion of vitamin-producing bacteria and cause vitamin K deficiency [33].

## 5. CONCLUSIONS

It is not in doubt that antibiotics contributed to the advances of modern medicine, however, the effects on the micro-ecology of the gut microbiome is now becoming more relevant than ever before. The impact of ampicillin on the gut microbiota of the subject and bacterial metabolic functions reduced the diversity of *Bifidobacteria*, *Bacteroides* and *Clostridium* species. In addition, ampicillin led to proliferation of *Veillonella* species in the subject. With the variability

observed in individual responses to antibiotic administration, the time is now ripe to ponder on the potential introduction of “smart” monitoring of bacterial responses to therapeutics with NGS and “smart” probiotics, to cushion the effects of ampicillin on the gut microbiome.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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