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RESEARCH ARTICLE

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Alterations of the oral-gut microbiome axis of Egyptian teenagers after *Helicobacter pylori* eradication therapy

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Abstract

Helicobacter pylori is a gram-negative carcinogenic intestinal pathogen that infects about 50% of humans worldwide. The impact of *H. pylori* eradication therapy on the oral and gut microbiota of teenagers is yet unknown. Thus, this study evaluated alterations in the oral and gut microbiome after triple therapy eradication in teenagers. A total of 20 salivary and 20 fecal samples were collected pre and 8 weeks post eradication therapy. The orointestinal microbiota axis was analyzed with 16S rRNA next-generation sequencing of the V3-V4 hypervariable region. The composition and diversity of oral and gut microbiota were compared before and after *H. pylori* eradication therapy. For oral samples, there was a decrease in the abundance of the families *Streptococcaceae, Saccharimonadaceae, Actinomycetaceae, Fusobacteriaceae, Lachnospiraceae, Staphylococcaceae* and *Micrococcaceae* after eradication treatment versus an increase in the relative abundance of *Prevotellaceae, Neisseriaceae, Veillonellaceae, Leptotrichiaceae, Pasteurellaceae, Porphyromonadaceae, Family_XI* and *Carnobacteriaceae*. For stool samples, the relative abundance of *Clostridiaceae_1, Peptostreptococcaceae, Coriobacteriaceae, Streptococcaceae, Pseudomonadaceae, Enterobacteriaceae, Eggerthellaceae, Prevotellaceae* decreased after eradication treatment, whereas the relative abundance of *Ruminococcaceae, Lachnospiraceae, Bifidobacteriaceae, Bacteroidaceae, Methanobacteriaceae, Veillonellaceae,* and *Erysipelotrichaceae* increased. There was significant difference in alpha diversity of oral and fecal samples pre and post eradication treatment. Triple therapy reduced the microbial diversity after eradication treatment in both oral and fecal samples.

Keywords: *Helicobacter pylori;* eradication; treatment; teenagers; oral-gut axis; orointestinal; oral-gut microbiome axis; Egypt.

1. Introduction

The human microbiome balance and dysbiosis are related to health and disease **(Hou et al., 2022).** The oral cavity and the gut are regarded as the most complex microbial inhabitants found in humans. The interactions between the oral and gut microbiota are interconnected, unstable, and complicated **(Acharya et al., 2017).** The transmission of microorganisms between oral cavity and gut can reshape the microbial community ecosystem in these two niches and regulate pathogenicity of various diseases **(Park et al., 2021).**

Anatomically, the oral cavity and gut belong to the digestive tract, and they are chemically and physically linked together by the stomach **(Park et al., 2021)**. The oral and gut microbiota are separated under healthy state but are interacted under pathological state **(Seedorf et al., 2014)**. Gastric diseases are associated to alterations of the oral-gut microbiota axis **(Olsen and Yamazaki, 2019)**.

H. pylori is a gram-negative intestinal pathogen that infects about 50% of humans worldwide **(Lee et al., 2022).** It can survive under drastic acidic conditions with the ability to alter the acidity of the human's gastric environment **(Kang and Blaser, 2006)**. *H. pylori* infection could be related to various diseases such as peptic ulcer, chronic gastritis, gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma **(Chen et al., 2022)**. The World Health Organization's International Agency for Research on Cancer classified *H. pylori* as carcinogenic to humans **(Camilo et al., 2017)**.

H. pylori can be transmitted between the mouth and stomach in the gastrointestinal tract through both oral-oral and fecal-oral routes **(Chen et al., 2022)**. Due to this mode of transmission, *H. pylori* was found to alter the microbiome of the oral-gut axis **(Mladenova and Durazzo, 2018)**. The abundance of *H. pylori* in the oral cavity is very low compared to the stomach where it constitutes about 42% to 97% of the total gastric microbial community **(Schulz et al., 2018)**. Despite its low abundance, *H. pylori* could have a significant impact on the oral community **(Vasapolli et al., 2019)**. Moreover, gut diseases such as colorectal cancer and inflammatory bowel disease are closely associated to the disturbance of the oral-gut microbiome axis **(Park et al., 2021)**. Furthermore, *H. pylori* infection may remodel the gut microbiome **(Baj et al., 2021)** causing various systematic diseases including hypertension, inflammation, hyperglycemia, dyslipidemia, and arteriosclerosis **(Beydoun et al., 2018)**.

The current *H. pylori* eradication treatment using antibiotics change the diversity of the gut microbiome **(Gotoda et al., 2018)**. Dysbiosis and alterations in the gut microbiome composition may induce different pathogenic disorders. The impact of *H. pylori* eradication therapy on the oral and gut microbiota in teenagers is still unclear. Thus, the aim of the current study was to investigate the alterations of the oral and gut microbiome through 16S rRNA next-generation sequencing of salivary and stool samples collected from infected teenagers pre and post eradication therapy.

2. Methods

2.1 Ethical statement

All study procedures involving human subjects were reviewed and approved by the Research Ethics Committee at the Faculty of Pharmacy, Suez Canal University, Egypt (Reference number 202009PHDH1). The

study was conducted in accordance with all applicable ethical regulations. All participants provided their consent with knowledge.

2.2 Study participants

Salivary and fecal samples were obtained from 20 teenagers attending the "Ismailia laboratory" in Ismailia, Egypt. Samples were collected in the period from January 2021 to August 2021. The teenagers were in the age range from 14 to 19 years old. All teenagers participated in the study and their parents approved with a written informed consent for providing the samples.

2.3 Detection of *H. pylori* positive subjects

As a primary test, anti-*H. pylori* antibody test was performed for fecal samples and urea breath test was done as a secondary test.

2.4 Sample collection

All positive subjects with a body weight ≥ 40 kg administrated eradication therapy according to the physician prescription. Patients with a history of drug allergy to the prescribed treatment were excluded. The eradication therapy composed of 500 mg amoxicillin, 400 mg clarithromycin, and 20 mg proton pump inhibitor twice a day for one week **(Gotoda et al., 2018)**. Salivary (10 samples) and fecal (10 samples) samples were collected from the same subjects before and 8 weeks after the completion of the eradication therapy. Hence, 20 samples were collected pre and post treatment with a total of 40 samples. Eradication of *H. pylori* was confirmed using urea breath test before samples collection. Samples were immediately stored at −80°C until being processed.

2.5 DNA extraction and 16S rRNA next-generation sequencing

DNA was extracted from frozen oral and fecal samples using Qiagen DNeasy power soil kit (cat. No 12888-50). The V3-V4 hypervariable region of the 16S rRNA gene was amplified using the forward primer 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG -3` and the reverse primer 5`-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC -3` according to the standard Illumina MiSeq protocol (https://support.illumina.com/documentation.html)**.** Illumina MiSeq (Illumina, San Diego, CA) next-generation sequencing in 300 bp paired-end mode was done at IGA Technology Services (Udine, Italy).

2.6 Sequences processing and statistical analysis

Raw sequences were analyzed using the Quantitative Insights Into Microbial Ecology 2 platform (QIIME2) **(Estaki et al., 2020).** Denoising and dereplication was achieved through the DADA2 plugin **(Callahan et al., 2016)** (truncation length for forward reads = 270 bp and reverse reads = 210). The output is a feature table of unique amplicon sequence variants (ASVs). Taxonomy assignment against SILVA database (V138) **(Quast et al., 2013)** was done with a trained RDP's naive Bayesian classifier at 97% sequence similarity **(Wang et al., 2007).** Taxa abundance, alpha diversity, and beta diversity was performed through MicrobiomeAnalyst (https://www.microbiomeanalyst.ca). Alpha diversity was measured using Chao1 richness estimate, observed OTUs, Abundance based coverage (ACE), and Shannon alpha diversity indices. Beta diversity was estimated using Bray-Curtis index distance

matrix and visualized using two-dimensional principal coordinate analysis (PCoA) plots. Differences in bacterial community composition before and after eradication treatment were tested using Permutational Multivariate Analysis of Variance (PERMANOVA). Statistically significant differences were measured using Kruskal Wallis rank-sum test and Wilcoxon rank-sum test. The *p* value was adjusted by false discovery rate (FDR) for multiple comparisons. FDR adjusted p value < 0.05 was considered statistically significant.

3. Results

3.1 Data integrity check and data filtering

The samples revealed a total of 782626 read counts. The total number of ASVs was 7847 with 19565 average counts per sample and 28617 maximum counts per sample. Based on prevalence and inter-quantile range, a total of 1881 low abundance features and 230 low variance features were removed respectively. After data filtering, a total of 2062 features remained.

3.2 Relative abundance of most dominant bacterial families

For oral samples, the relative abundance of the 10 most predominant families was *Streptococcaceae* (33.32%), *Prevotellaceae* (13.14%), *Veillonellaceae* (8.42%), *Neisseriaceae* (8.12%), *Leptotrichiaceae* (5.27%), *Actinomycetaceae* (3.64%), *Pasteurellaceae* (3.63%), *Fusobacteriaceae* (3.31%), *Porphyromonadaceae* (2.77%) and *Saccharimonadaceae* (2.55%). For fecal samples, the relative abundance of the 10 most predominant families was *Ruminococcaceae* (19.43%), *Lachnospiraceae* (13.3%), *Clostridiaceae_1*(13%), *Prevotellaceae* (8.8%), *Peptostreptococcaceae* (7%), *Bifidobacteriaceae* (5.6%), *Enterobacteriaceae* (5.4%), *Enterococcaceae* (4.9%), *Coriobacteriaceae* (4%), and *Bacteroidaceae* (3.13%). The most abundant families of oral and gut microbiota in teenagers before and after *H. pylori* eradication treatment for individual samples are shown in Figure 1.

3.3 Alterations of the oral and gut families after *H. pylori* eradication treatment

For oral samples, there was a significant decrease in the relative abundance of the following families: *Streptococcaceae, Saccharimonadaceae, Actinomycetaceae, Fusobacteriaceae, Lachnospiraceae, Staphylococcaceae* and *Micrococcaceae* Their relative abundances before treatment were 38%,5%,4%,4% ,3%,3%, and 2.8% respectively. These changed to 29%, 0.68%, 3%, 2.9%, 0.5%, 0%, and 2% respectively after treatment. On the contrary, the relative abundance of the families *Prevotellaceae, Neisseriaceae, Veillonellaceae, Leptotrichiaceae, Pasteurellaceae, Porphyromonadaceae, Family_XI* and *Carnobacteriaceae* increased after treatment. Their relative abundances before treatment were 12%, 5%, 5%, 4%, 2.7%, 2.4%, 1.9%, and 1.8% respectively. These increased to 14%, 11%, 12%, 6%, 4%, 3%, 2.3%, and 2.14% respectively after treatment.

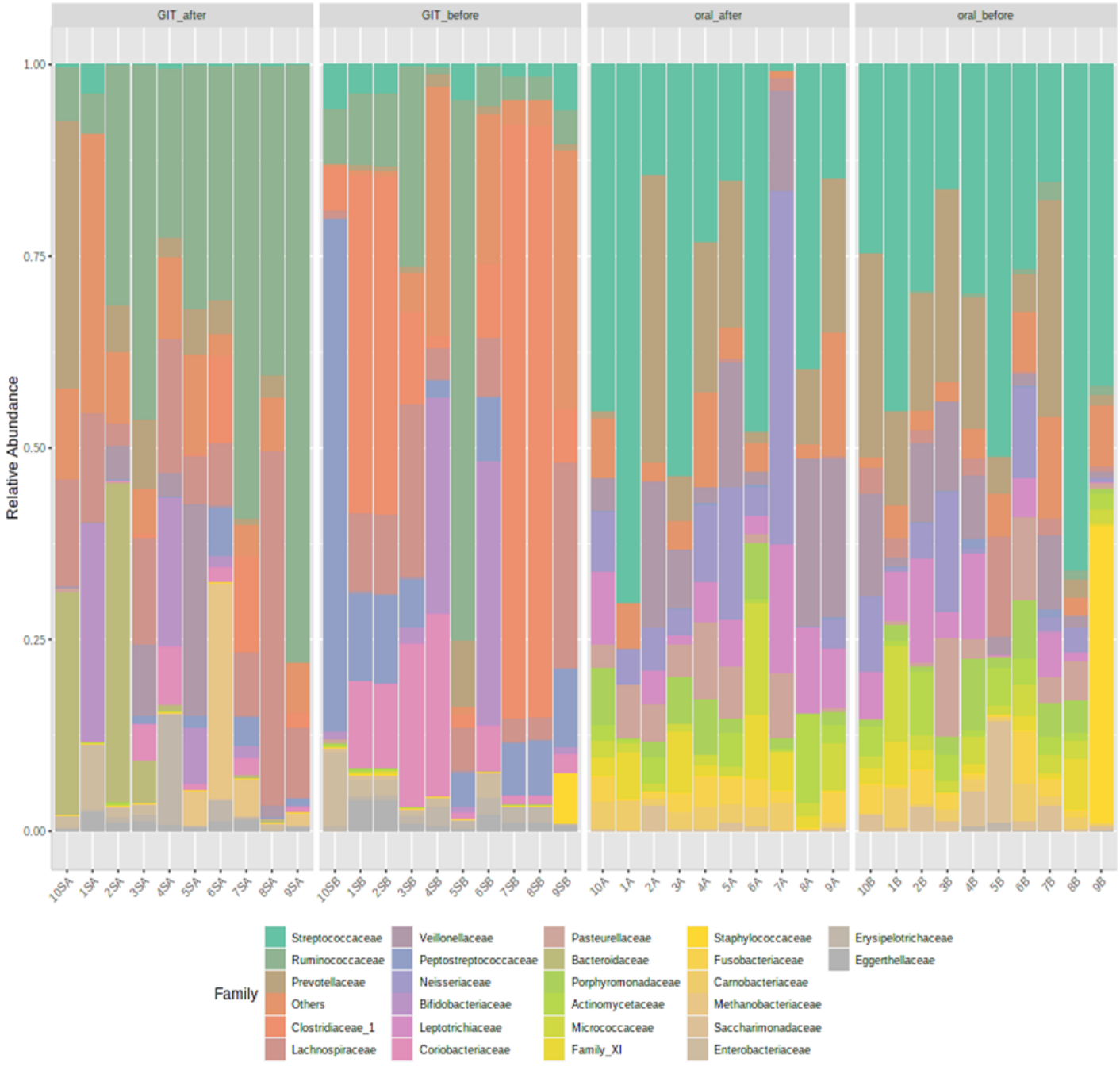

Figure 1: Stacked bar charts showing relative abundance (> 0.5%) of top oral and gut bacterial families before and after *H. pylori* eradication treatment. Each bar represents one sample.

For fecal samples, the relative abundance of *Clostridiaceae_1, Peptostreptococcaceae, Coriobacteriaceae, Streptococcaceae, Pseudomonadaceae, Enterobacteriaceae, Eggerthellaceae, Prevotellaceae* decreased after eradication treatment from 32.34%, 16.42%, 5.68%, 3.01%, 2.4%, 2.3%, 1.6%,4.43% to 2.65%, 1.32%, 2.06%, 0.9%, 0%, 1.35%, 1.03%, 7.01% respectively. However, there was an increase in the relative abundance of the families *Ruminococcaceae, Lachnospiraceae, Bifidobacteriaceae, Bacteroidaceae, Methanobacteriaceae, Veillonellaceae,* and *Erysipelotrichaceae*. Their relative abundances before eradication treatment were 14.8%, 8.83%, 4.35%, 0.02%, 0.01%, 0.07%, and 1.08% respectively. These increased after treatment to 32.5%, 14.98%, 6.05%, 9.03%,4.16%, 3.94%, and 2.43% respectively. Hierarchical clustering and heatmap visualization for oral and fecal samples before and after eradication treatment at family taxanomy level is decipated in Figure 2.

3.4 Alpha diversity

Alpha diversity revealed a significant change in the richness within the bacterial communities of oral and fecal samples after treatment (Figure 3). This change was revealed by the alpha indices Chao1 (p = 0.01785), observed OTUs (p = 0.0155), ACE (p = 0.0099), and Shannon (p = 0.0445). The alpha diversity indices in both oral and fecal samples were significantly decreased 2 months post treatment as illustrated in Table 1.

3.5 Beta diversity

PCoA plots based on Bray-Curtis index using PERMANOVA (Figure 4) indicated significant difference between oral and fecal samples pre and post eradication treatment (p = 0.001).

3.6 Classical Univariate Statistical Comparisons for oral and fecal samples before and after treatment

Using Mann-Whitney test and Kruskal-Wallis test, a total of 53 significant families (Table 2) were identified for oral and fecal samples before and after eradication treatment ($p < 0.05$).

4. Discussion

This is the first preliminary study to estimate alterations in the oral and gut microbiome before and after *H. pylori* eradication treatment in Egypt using Illumina Miseq next-generation sequencing. The aim of the current study was to investigate the short-term effects of standard triple regimen on both oral and gut microbiota. Oral and fecal samples were collected 2 months following eradication therapy. Oral and fecal samples were obtained from the same subjects before and after *H pylori* eradication to investigate changes in the oral-gut microbiome axis.

Previous literature suggested that dysbiosis of the gut microbiota was associated with a wide range of diseases **(Durack and Lynch, 2019).** However, antibiotics also change the gut microbiome taxonomically, genomically, and functionally. The use of broad-spectrum antibiotics decreases the diversity of bacterial communities by the expand and collapse of specific taxa **(Modi et al., 2014).** In the present study, alpha diversity decreased 2 months post-eradication therapy. There was a significant difference in the diversity of microbial communities within samples. They

Figure 2: Hierarchical clustering and heatmap visualization of salivary and fecal samples before and after eradication treatment in respect to family taxonomy level.

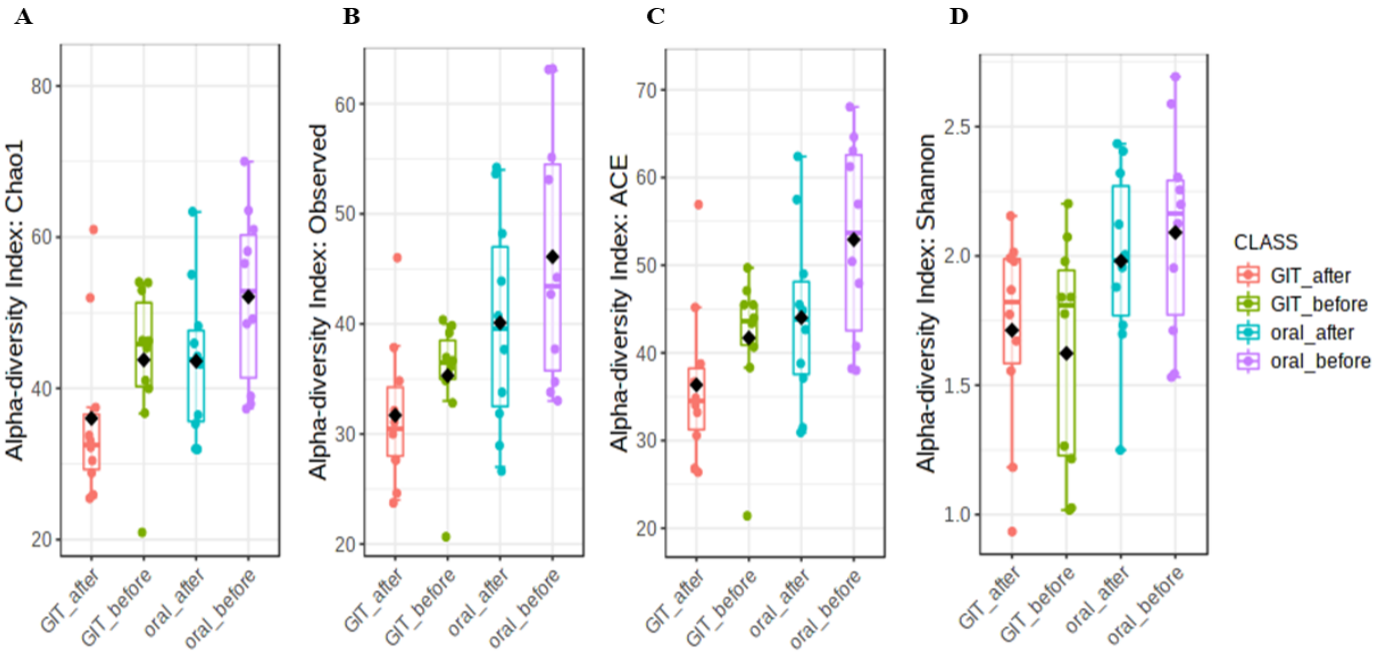

Figure 3: Alpa diversity indices of the oral and gut microbial communities in teenagers before and after *H. pylori* eradication treatment. **(A)** Box plot of Chao1 alpha diversity index. **(B)** Box plot of observed OTUs alpha diversity index. **(C)** Box plot of Abundance based coverage richness estimate (ACE) alpha diversity index. **(D)** Box plot of Shannon alpha diversity.

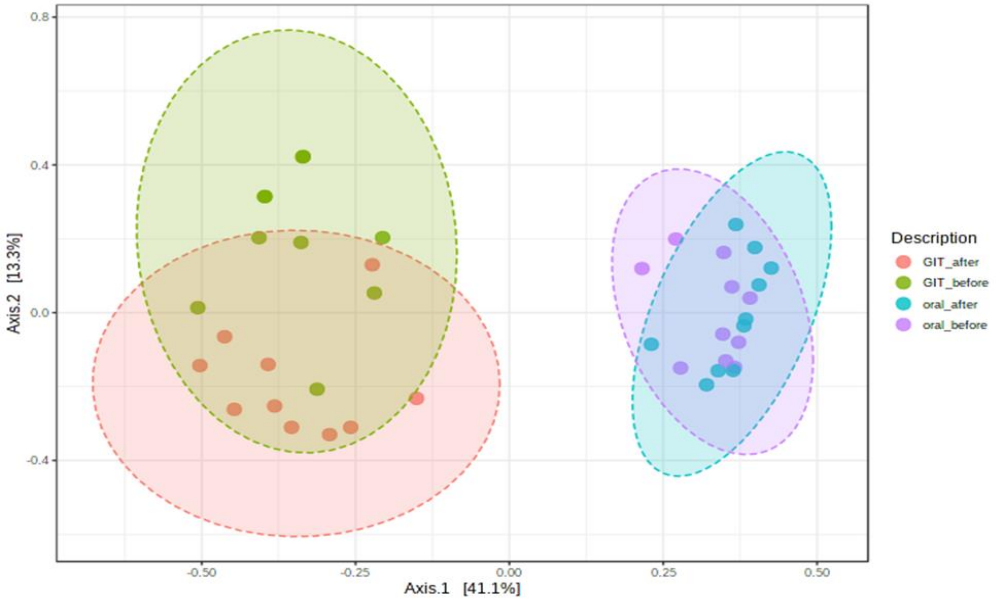

Figure 4: PCoA two-dimensional graph of oral and gut microbial communities in teenagers pre and post *H. pylori* eradication therapy. Purple and blue balls represent oral samples before and after treatment respectively. Green and pink balls represent fecal samples before and after treatment respectively.

Table 1: Alpha-diversity pre and post *H. pylori* eradication treatment of salivary and fecal samples.

Gender	Body Site	Time	Description	samples	Chao1	ACE	Observed OTUs	Shannon
Female	oral	before	oral_before	1B	48.6	50.44	215	1.95
Female	GIT	before	GIT_before	1SB	54	45.48	207	2.01
Female	oral	after	oral_after	1A	32	30.92	108	1.25
Female	GIT	after	GIT_after	1SA	26	26.4	145	1.84
Female	oral	before	oral_before	2B	49.14	56.96	186	2.26
Female	GIT	before	GIT_before	2SB	54	45.48	207	1.84
Female	oral	after	oral_after	2A	43.25	42.67	148	2
Female	GIT	after	GIT_after	2SA	37.5	38.75	206	1.67
Male	oral	before	oral_before	3B	61	57.93	217	2.2
Male	GIT	before	GIT_before	3SB	46.33	49.7	225	1.98
Male	oral	after	oral_after	3A	55.11	47.5	169	1.88
Male	GIT	after	GIT_after	3SA	32.2	34.84	206	1.87
Female	oral	before	oral_before	4B	48	49.75	216	2.41
Female	GIT	before	GIT_before	4SB	52	45.42	259	2.15
Female	oral	after	oral_after	4A	39.2	40	170	2.3
Female	GIT	after	GIT_after	4SA	21	21.19	95	1.78
Male	oral	before	oral_before	5B	37.33	38.21	144	2.32
Male	GIT	before	GIT_before	5SB	40	40.67	204	1.99
Male	oral	after	oral_after	5A	35.33	37.8	138	1.71
Male	GIT	after	GIT_after	5SA	25.5	26.84	179	1.22
Female	oral	before	oral_before	6B	70	68.05	303	2.59
Female	GIT	before	GIT_before	6SB	61	56.59	278	2.2
Female	oral	after	oral_after	6A	44.25	44.86	192	1.96
Female	GIT	after	GIT_after	6SA	41	41.91	248	1.98
Male	oral	before	oral_before	7B	63.5	64.61	339	2.69
Male	GIT	before	GIT_before	7SB	45.33	43.37	207	1.56
Male	oral	after	oral_after	7A	36.5	37.14	128	1.73
Male	GIT	after	GIT_after	7SA	33	34.05	182	1.02
Male	oral	before	oral_before	8B	56.5	61.24	313	1.7
Male	GIT	before	GIT_before	8SB	46.33	44	184	1.18
Male	oral	after	oral_after	8A	32	31.48	116	1.53
Male	GIT	after	GIT_after	8SA	30.5	33.23	177	1.03
Male	oral	before	oral_before	9B	58.11	63.03	256	2.43
Male	GIT	before	GIT_before	9SB	36.67	38.31	238	2.07
Male	oral	after	oral_after	9A	63.33	62.4	255	1.54
Male	GIT	after	GIT_after	9SA	28.86	30.59	181	0.93
Female	oral	before	oral_before	10B	46	45.01	209	2.13
Female	GIT	before	GIT_before	10SB	53	47.09	199	1.77
Female	oral	after	oral_after	10A	38	38.51	147	2.12
Female	GIT	after	GIT_after	10SA	33.75	36.67	159	1.26

Table (2): Univariate Statistical analysis at family level for salivary and fecal samples before and after eradication treatment (p <0.05).

Family	*p* value	FDR adjusted *p* value
Flavobacteriaceae	2.22E-07	7.56E-06
Neisseriaceae	5.15E-07	7.56E-06
Campylobacteraceae	5.15E-07	7.56E-06
Leptotrichiaceae	5.36E-07	7.56E-06
Ruminococcaceae	6.30E-07	7.56E-06
Micrococcaceae	7.53E-07	7.74E-06
Eggerthellaceae	1.11E-06	9.75E-06
Carnobacteriaceae	1.22E-06	9.75E-06
Weeksellaceae	2.30E-06	1.32E-05
Streptococcaceae	2.35E-06	1.32E-05
Acidaminococcaceae	2.49E-06	1.32E-05
Actinomycetaceae	2.55E-06	1.32E-05
Family_XI	2.62E-06	1.32E-05
Coriobacteriaceae	2.75E-06	1.32E-05
Clostridiaceae_1	3.41E-06	1.52E-05
Fusobacteriaceae	3.60E-06	1.52E-05
Erysipelotrichaceae	8.22E-06	3.29E-05
Lachnospiraceae	1.36E-05	5.15E-05
Pasteurellaceae	1.49E-05	5.36E-05
Saccharimonadaceae	2.46E-05	8.12E-05
Bifidobacteriaceae	2.48E-05	8.12E-05
Porphyromonadaceae	3.03E-05	9.49E-05
Enterobacteriaceae	4.12E-05	0.000123
Spirochaetaceae	9.10E-05	0.000262
Christensenellaceae	0.000103	0.000276
Corynebacteriaceae	0.000104	0.000276
Peptostreptococcaceae	0.000107	0.000276
Veillonellaceae	0.000195	0.000484
Akkermansiaceae	0.000553	0.001327
Cardiobacteriaceae	0.000677	0.001573
Succinivibrionaceae	0.000734	0.001652
Paludibacteraceae	0.000951	0.002024
Aerococcaceae	0.000956	0.002024
Burkholderiaceae	0.001239	0.00255
Moraxellaceae	0.001387	0.002774
candidate_division_SR1_bacterium_taxon_345	0.002426	0.00472
Methanobacteriaceae	0.002818	0.00534
Prevotellaceae	0.005351	0.009712
candidate_division_SR1_bacterium_MGEHA	0.005396	0.009712
Bacteroidaceae	0.005544	0.009735
Geodermatophilaceae	0.006824	0.01168
TM7_phylum_sp__oral_clone_FR058	0.006976	0.01168

Family	*p* value	FDR adjusted *p* value
Rikenellaceae	0.007144	0.01169
Enterococcaceae	0.009579	0.015326
Synergistaceae	0.011056	0.017305
Coriobacteriales_Incertae_Sedis	0.012527	0.018967
Muribaculaceae	0.01282	0.018967
Lentimicrobiaceae	0.012908	0.018967
Lactobacillaceae	0.015974	0.023003
Marinifilaceae	0.017396	0.024559
Leuconostocaceae	0.021777	0.030153
Mycoplasmataceae	0.022279	0.030265
Clostridiales_vadinBB60_group	0.03095	0.040516

tended to be less diverse. There was also a significant change in the composition of the microbial communities of oral and fecal samples before and after eradication therapy as revealed by beta diversity. *H pylori* colonization in the stomach demonstrated a significant decrease in the alpha diversity of the microbial community under high relative abundance of *H. pylori* **(Noto and Peek, 2017).** On the contrary, *H. pylori* negative subjects tended to have more diverse microbial communities **(Noto and Peek, 2017)**. In concordance to our results, **(Hsu et al., 2018)** reported *H. pylori* eradication therapy resulted in a significant decrease in the diversity of the gut microbiota.

In the recent years, administration of *H. pylori* treatment to younger generations at early stages was recommended in order to prevent gastric atrophy and intestinal metaplasia **(Suzuki and Matsuzaki, 2018).** A prospective study indicated that patients at early stages of gastric carcinoma receiving *H. pylori* treatment, had reduced rates of gastric cancer **(Choi et al., 2018).** The administration of the triple therapy, which is composed of proton-pump inhibitors, amoxicillin, and either clarithromycin or metronidazole is recommended by the European and North American Societies for Gastroenterology, Pediatric, and Nutrition as a first-line treatment in children **(Koletzko et al., 2011).** It is also highly recommended to use probiotics along with the triple therapy to minimize toxicity **(Gotoda et al., 2018).**

5. Conclusion

In conclusion, *H. pylori* infection and eradication can alter the structure and composition of the oral-gut microbiome axis affecting the development and progression of oral and gastrointestinal tract diseases. Furthermore, *H. pylori* infection results in imbalance of oral and intestinal microbial community which may be related to series of systemic diseases.

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