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Analysis of the Associations Among Gut Microbiota, Intestinal Parasites, and Nutritional Conditions in Mothers and Children from Zanzibar, Tanzania

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To my grandmother, **Régine Efouba**, who passed away in 1998.

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CHAPTER I: GENERAL INTRODUCTION

1.1 GUT MICROBIOTA COMPOSITION AND FUNCTION.

The assemblage of bacteria, archaea, and eukaryotes inhabiting the gastrointestinal tract (GI) is known as the **'gut microbiota**,' which has evolved alongside the host for millennia, establishing a complex mutually beneficial relationship (1). The population of microorganisms residing in the GI tract is estimated to surpass 10^{14}, representing approximately ten times the number of human cells and over a hundred times the genomic content (**microbiome**) compared to the human genome (2). Although "**microbiota**" and "**microbiome**" are used interchangeably, there are certain differences between the two terms. **Microbiota** refers to the living microorganisms found in a defined environment, such as oral and gut microbiota while **microbiome** denotes the collection of genes of all the microorganisms found in a specific environment, which includes not only the community of the microorganisms, but the microbial structural elements, metabolites, and the environmental conditions (3).

Although there is debate about the starting point of infant gut colonization, it is widely accepted that the gut microbiome formation takes place over several years of life from the moment of birth (4). This colonization evolved throughout life to reach the adult like microbiota at around three years old (5). However, other studies argued that the maturation of the children microbiota may occur later than 3 years old (6). Characterizing the microbiota and its progression in the early years of life may help identify '**windows of opportunity**' when interventional strategies might be effective in promoting health and preventing diseases (7). Mode of delivery significantly influences the composition of children's microbiota, with naturally born infants acquiring their initial gut bacteria primarily from their mothers through the faecal-oral route (8). Emphasizing the crucial role of mothers as the primary source of infant microbiota, Frese and Mills humorously remarked that for the birth of the infant gut microbiome, "moms deliver twice"! (9) The study of Makino et al. which did not find evidence showing the occurrence of horizontal transmission for bifidobacteria confirmed the role of the mother as first source of children microbiota (10). Subsequent research indicated that not all maternal gut inhabitants are transmitted to the infant. Only specific maternal taxa, notably bifidobacteria and *Bacteroides*, establish a permanent and relatively dominant presence in the infant's gut (11). In the initial days after birth, aerobic and facultative bacteria, such as streptococci and enterobacteria prevail in the infant gut microbiota. The environmental conditions in maternal vagina and breast differ from those in the infant gut, suggesting that the

maternal gut serves as the main source of microbes colonising the infant gut. The lack of vertical transmission in infants born via caesarean section supports the hypothesis that microbial colonisation normally begins at birth rather than in utero (12).

Despite the widespread adoption of the term "**core microbiome**" and its increasing usage, there remains a lack of consensus regarding its practical quantification (13). Thursby and Juge conducted a review of numerous studies and found that that 93.5% of species belonged to Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes phyla (1). Similarly, King et al. (14), seeking to define the baseline gut microbiota of healthy human, found that members of the Firmicutes and Bacteroidetes phyla constitute the majority of the bacterial species that were present in the human intestinal microbiota. Their study also revealed that the healthy human gut microbiome consists of 8 phyla, 18 families, 23 classes, 38 orders, 59 genera and 109 species. More than half of Firmicutes are members of the Clostridia class, which is the most abundant class, followed by Bacteroidia, Actinomycetia (Bifidobacteria), Gammaproteobacteria (Enterobacteria) and Bacilli **(**Lactobacilli).

Although there are instances where the microbiota of both mothers and infants exhibit similarities (15), there are notable differences between the microbiota of children and adults, not only in terms of composition but also function (16). Analysis of predicted functional metagenomes revealed that children display an overrepresentation of pathways related to glycan degradation, riboflavin (vitamin B2), pyridoxine (vitamin B6), and folate (vitamin B9) biosynthesis. Conversely, the gut microbiome of adults exhibits higher abundances of pathways associated with carbohydrate metabolism, beta-lactam resistance, and the biosynthesis of thiamine (vitamin B1) and pantothenic acid (vitamin B5) (17).

The gut microbiota significantly influences human health, with growing evidence supporting its importance (3). Various levels of evidence, including studies on animals and humans, highlight the role of gut microbiota in human health. Despite its dynamic nature, the gut microbiota plays fundamental roles in immunological, metabolic, structural, and neurological aspects of the human body. Dietary fibers, such as lignin, non-starch polysaccharides, resistant starch (RS), and oligosaccharides, like fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), resist digestion by host enzymes. Gut microbes possess a variety of enzymes to effectively utilize these diverse carbohydrates (18). The digestion of these resistant carbohydrates leads to the production of short chain fatty acids as one of the main products.

Gut microbial species such as *Roseburia* spp., *Eubacterium rectale* and *Faecalibacterium prausnitzii* and *Clostridium* groups IV and XIVa are the main producers of SCFAs (19) . This important contribution of the gut microbiota to human metabolism stems from its possession of enzymes not encoded by the human genome. These enzymes, such as those involved in polysaccharide and polyphenol breakdown, as well as vitamin synthesis, enable this contribution. Studies conducted with germ-free and conventional rodents, along with human volunteers, have long revealed the gut microbiota's ability to synthesize certain vitamins. Notably, these include vitamin K and various B group vitamins like biotin, cobalamin, folates, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, and thiamine (20).

Furthermore, the gut microbiota assumes crucial roles in maintaining host health by modulating gut immunity and metabolizing xenobiotics and drugs. The immune-modulating process involves various components and cell types of the immune system, such as gut-associated lymphoid tissues (GALT), effector and regulatory T cells, IgA-producing B cells (plasma cells), Group 3 innate lymphoid cells, as well as resident macrophages and dendritic cells in the lamina propria. The gut microbiota's involvement in establishing a healthy GALT is indicated by its impact on the development of Peyer’s patches and isolated lymphoid follicles, which typically exhibit an abundance of IgA+ B cells but may show an altered presence of IgE+ B cells in cases of dysfunction (21). Likewise, the ability of the gut microbiome to metabolize xenobiotics and drugs was acknowledged more than 40 years ago. The influence of the gut microbiota on xenobiotic metabolism could have a significant impact on future therapies for various diseases (22).

1.2 HOW TO STUDY THE MICROBIOME?

The common methods for studying the microbiome include culturomics, metagenomics, meta-transcriptomics, and metabolomics. (23).

Culturomics are approaches in which large numbers of cells are isolated and cultured (24). However, around 85% of microbes in the human gut are anaerobic and cannot be cultured in an open Petri dish. They require research laboratory anaerobic chambers for growth. Despite advancements in culturing techniques, there's still bias in what can be cultured, as certain bacteria grow faster under specific conditions than others (23).

Metagenomics approaches encompass two main categories: **amplicon analyses** and **whole metagenomics analyses**. In amplicon analyses, a particular DNA segment is massively amplified, often through PCR. Among these approaches, sequencing of the 16S rRNA gene stands out as the most commonly employed method (25). Although 16S rRNA sequencing has enabled a great deal of scientific research on microbial communities, simply knowing the genera of bacteria and its relative abundance is not as useful for clinical analysis. This is because each genus can have a wide range of strains that are genomically distinct (26).

Shotgun metagenomics differs from the targeted approach of utilizing a single gene, like the 16S rRNA gene. Instead, it involves breaking down all the DNA present in a sample into smaller fragments, sequencing these fragments, and subsequently assembling them to construct a comprehensive picture of the microbiome (27). This approach offers the benefit of delving deeply into the composition of the microbiome, down to the species and even strain level. Additionally, it furnishes comprehensive insights into the entirety of the environmental genome proving information not only on bacteriome, but also virome, mycobiome, and archea.

Metatranscriptomics involves sequencing transcribed RNA, while **metaproteomics** utilizes mass spectrometry to identify the diverse proteins within a sample. These methods hold significant potential for understanding gene expression but pose notable challenges. It's worth noting that the transcripts of most bacterial genes have a short lifespan, typically lasting only a few minutes (23).

Metabolomics approaches facilitate the examination of nonprotein small molecules, which encompass metabolic byproducts. This burgeoning field is particularly thrilling as it directly correlates with the functionality of the microbial community. Typically, metabolites are separated through gas chromatography or liquid chromatography before being analysed via mass spectrometry to detect charged ions (23). Metabolomics analysis primarily employs two approaches: targeted metabolomics and untargeted mass spectrometry. In targeted metabolomics, a predefined list of molecules, often with available reference standards, is scrutinized. This method tends to be highly sensitive for detecting specific molecules of interest and offers superior quantification. However, it lacks the capacity for discovery since it focuses solely on predetermined compounds (28).

1.3 FACTORS THAT AFFECT THE GUT MICROBIOTA

1.3.1 Mode of delivery

Mode of delivery is generally accepted as a major factor determining initial colonization, although not all studies agree and some suggest that infant microbiota undergoes substantial reorganization during the first months of life, which is primarily driven by body site and not by mode of delivery (29).

Although the gut microbiota typically exhibits significant interindividual variability, its development during infancy generally follows a discernible and consistent pattern, influenced by the mode of birth. As depicted in Figure 1, Bifidobacterium typically dominate until weaning, after which there is a gradual increase in the relative abundance of taxa that metabolize plant-based carbohydrates, primarily members of the clostridial families Lachnospiraceae and Ruminococcaceae (30).

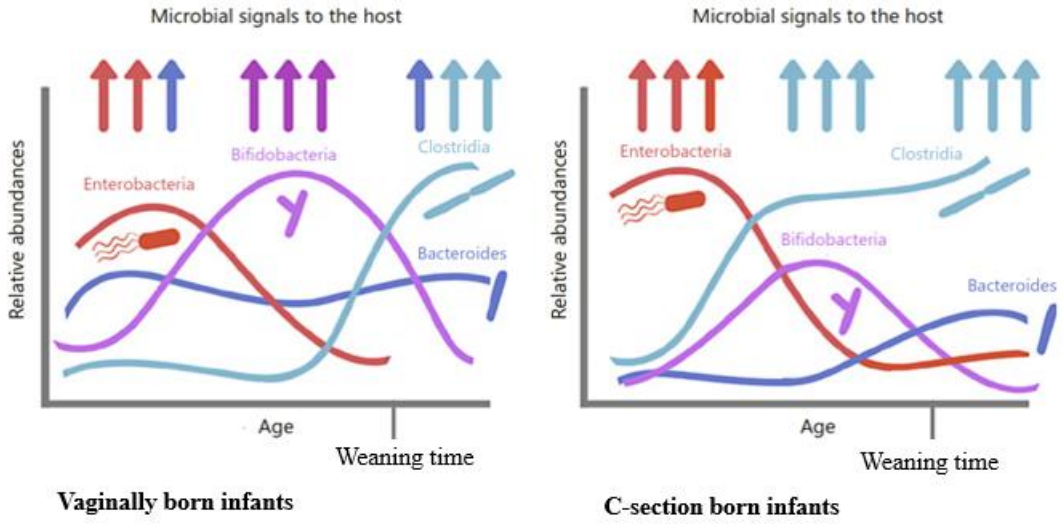


Figure 1. 1. Temporal development of the relative abundances of the dominant classes of bacteria in the infant gut. (Modified from Katri Korpela. Ann Nutr Metab 2021;77(suppl 3):11–19)

In a study contrasting the gut microbiota of infants based on delivery mode, Brief Mitchell et al. found that *Bacteroides* were present early on regardless of delivery method. However, C-section-delivered infants, regardless of exposure to vaginal labour, experienced a decline in *Bacteroides* by two weeks post-birth. Interestingly, the strains of *Bacteroides* found in infants closely resembled those from the maternal rectum rather than the vagina (31). The same study noticed a higher abundance of the genera *Streptococcus* and *Haemophilus* in C-section born

children in week 1 samples, before the disappearance of *Bacteroides*. Across all samples and all time points, the relative abundances of *Bacteroides* and either *Streptococcus* or *Haemophilus* were inversely correlated (31). This suggests that colonisation of the gut is characterized by competition among microorganisms. The most adapted will remain and the less will disappear or decrease.

Another significant aspect of early gut colonization is the establishment pattern of *Escherichia-Shigella*. Research on healthy Canadian infants revealed that *Escherichia-Shigella* and *Bacteroides* species were less prevalent in infants delivered via caesarean section. Particularly, those born through elective caesarean delivery exhibited notably diminished bacterial richness and diversity. Compared to vaginally delivered infants, those born via caesarean section displayed bacterial communities with significantly lower levels of *Escherichia-Shigella* (32). While a clear distinction exists in colonization patterns between caesarean section and vaginal delivery, the specific type of caesarean section also impacts gut colonization. Jakob Stokholm et al. discovered that elective versus emergency caesarean sections were associated with distinctly different colonization patterns (33). Muriel-Ohayon et al. proposed that the variation in microbiota development between elective and emergency caesarean sections might be linked to differing progesterone levels. Their studies in mice and humans revealed a significant alteration in gut microbial composition during late pregnancy, marked by increased *Bifidobacterium* levels. Through in vitro and in vivo experiments, they demonstrated that progesterone supplementation influences microbial communities, notably boosting *Bifidobacterium* abundance (34).

The link between early-life colonization and gut microbiota manifests in varying susceptibility to certain diseases later in children born vaginally versus through C-section. Martal Reyman et al. noted that, among others, *Bifidobacterium* showed no association with respiratory infection occurrences. Conversely, taxa like *Veillonella* and *Staphylococcus* were negatively linked to fewer respiratory infections. Notably, differences in *Klebsiella* abundance persisted for over four months beyond the initial neonatal period (35). An investigation comparing the microbial composition and diversity of 21 allergic and 18 healthy infants at 3 weeks, 3 months, and 6 months of age, and linking these findings to allergy development at 18 and 36 months, revealed that a heightened *Klebsiella*/*Bifidobacterium* ratio in early life was associated with the subsequent development of paediatric allergies (36).

1.3.2 Infant feeding mode.

The establishment of human gut microbiota in early life is closely associated with both short- and long-term infant health (37). Delivery mode and feeding pattern are two important determinants of infant gut microbiota (38). Coker et al. examined how delivery mode and infant feeding influence the taxonomic composition and functional capacity of the developing gut microbiota throughout the first year of life. They found that breastfeeding altered the longitudinal effects of delivery mode on the microbiota's taxonomic composition by age one. Among infants delivered via caesarean section, longer breastfeeding durations were associated with increased abundance of *Bacteroides fragilis* and *Lactobacillus*, while vaginally delivered infants showed an increase in *Faecalibacterium*. Their findings reinforced the idea that duration of breastfeeding plays a critical role in restoring a health-promoting microbiome (39). Xiong et al. noticed a compensatory effect of breastfeeding on C-section delivered infants. Their microbiota composition may eventually resemble that of vagina-delivered infants provided enough *Bifidobacteria* is supplied (40). Highlighting the significance of breastfeeding, Fehr et al. demonstrated that the composition, diversity, and maturity of infant gut microbiota are linked to the exclusivity and duration of breastfeeding, rather than occasional pumped milk feeding. Their research revealed that certain bacteria found in mothers' milk, like *Streptococcus* spp. and *Veillonella dispar*, are also present in their infants' stool, with reduced co-occurrence when infants receive pumped breastmilk. Moreover, they found positive correlations in the relative abundances of shared species between breastmilk and stool. The study concluded that the composition of the gut microbiota strongly correlates with the exclusivity and duration of breastfeeding, regardless of whether the milk is directly nursed or pumped. Additionally, breastmilk bacteria contributed significantly to the overall variation in the infant microbiome, akin to other factors like birth mode (41). Another cohort study revealed that modes of infant feeding are associated with asthma development. Direct breastfeeding is most protective compared with formula feeding; indirect breast milk confers intermediate protection (42). Hence, breast milk, particularly through breastfeeding, should be advocated as the optimal nutrition mode for infants. It supplies essential nutrients crucial for growth and development, along with passive immunity that safeguards against infectious diseases during infancy (43).

1.3.3 Host genetics.

Experiments aimed at establishing causality are now shedding light on the intricate interplay among host genetics, microbiome composition, and disease. This relationship can manifest in various ways. Firstly, a genetic variant might directly induce the disease phenotype, subsequently leading to changes in the microbiome. Secondly, a genetic variant could modify gene expression in the host, thereby influencing the microbiome and contributing to the development of the disease phenotype. Lastly, the genetic variant may directly impact the microbiome, consequently leading to the manifestation of the disease phenotype (44). Significant insights into this point have emerged from twin research. Microbiome studies involving twin cohorts, both monozygotic and dizygotic, suggest that host genetics play a crucial role in shaping the composition of the gut microbiome. Furthermore, these studies indicate that the colonization by specific taxa is strongly influenced by hereditary factors (45). Investigating on the genetic determinants of the gut microbiome on UK twins, Goodrich studies found that there were the association of a SNP (rs2164210) in the lactase gene (*LCT)* with the abundance of *Bifidobacterium* and a SNP (rs2276731) in the gene for an aldehyde dehydrogenase family member (*ALDH1L1)* with the abundance of unclassified SHA-98 bacteria (46). Moreover, another twin study revealed that a SNP (rs651821) in the apolipoprotein A5 (*APOA5*) gene was associated with the abundance of *Bifidobacterium* in patients with metabolic syndrome (47). Furthermore, Raivo Kolde et al.'s study revealed that the taxonomic composition and functional potential of the microbiome were notably correlated with genetic principal components in the gastrointestinal tract and oral communities. However, such correlations were not observed in the nares or vaginal microbiota. Additionally, the associations between microbial features and both high-level genetic attributes and single variants were found to be site-specific (48).

1.3.4 Geographical location or host environment

Ethnicity and geographical location are recognized as influential factors in shaping the composition and diversity of the gut microbiota (49). Numerous studies have highlighted differences not only across diverse international regions with varying socio-economic conditions but also among individuals sharing similar cultural and genetic backgrounds. For instance, research on Latinos in the USA revealed that sociodemographic factors and migration-related variables significantly influenced the host microbiome. Furthermore, relocating to the US mainland was found to be associated with changes in gut microbiota

composition, underscoring the impact of geographical location (50). Similarly, Shin et al. found notable alterations in the gut microbiota of elderly women residing in either island or inland regions of South Korea. However, these two groups differ from each other for lifestyle, dietary patterns and physical activities (51). Likewise, differences in microbiota composition between urban and rural populations in Russia were attributed to diverse diets, lifestyles, and environmental factors (52). While it could be argued that the population samples in a forementioned studies exhibited socio-economic disparities, an Italian study countered this notion. It explored whether the gut microbiota profiles of healthy Italian volunteers varied based on their geographical origins and discovered differences in microbiota composition among participants grouped by their region of origin. Additionally, species richness varied significantly across Italian regions such as Apulia, Lazio, and Lombardy. This shows that a variability exists even within small-scale geographical regions (53). Other studies in Egypt (54) and India (55) that included population with same ethnical backgrounds and similar socio-economic conditions also revealed that the geographical setting impacts on the gut microbiota composition and function.

1.3.5 Medication/ Antibiotics

The gut microbiota is known to plays an important role in host health and can be perturbed by several factors including antibiotics. As a result, alterations induced by antibiotics in microbial composition can adversely affect host health. These changes may include decreased microbial diversity, shifts in the functional characteristics of the microbiota, and the emergence and selection of antibiotic-resistant strains. Consequently, hosts become more vulnerable to infections by pathogens such as *Clostridioides difficile* (56). The influence of antibiotic can start even before birth as perinatal and peripartum antibiotic use can impact gut microbial colonization and the resistome profile in infants (57). Moreover growing evidences reveal that early childhood exposure to antibiotics can lead to several gastrointestinal, immunologic, and neurocognitive conditions (58). Research also reveals that the use of broad-spectrum antibiotics is associated to reduce gut microbiota diversity (59). By impacting the composition of the microbial community, antibiotics also alter microbiota functionality and thus the metabolites produced (60). In such condition, killing the pathogen of concern can be accompanied with the eradication of beneficial microbes (61). Antibiotic eradication of beneficial bacteria in the gut enables the development of opportunistic bacteria and pathogenic bacteria such as *Clostridium difficile* (62). One study also concluded that oral antibiotic use is associated with

an increased risk of colon cancer (63). Amoxicillin, a frequently prescribed antibiotic, has been demonstrated to completely eliminate the *Bifidobacterium adolescentis* species in children, resulting in a reduced diversity of the bifidobacteria population (64). This finding is worrisome because *Bifidobacterium* species are widely acknowledged for their crucial role in maintaining children's health. Furthermore, the adverse effects of antibiotic usage are exemplified by the administration of antibiotics to eradicate *Helicobacter pylori*. This treatment can disrupt the indigenous microbiota and promote the development of resistant strains, which may persist for years following the completion of the treatment (65). Azithromycin, another widely prescribed antibiotic, has been shown to reduce the diversity of the gut microbiome and significantly increase macrolide resistance within just five days post-treatment (66). Anthony et al. found that this antibiotic also delays the recovery of species richness, leading to greater compositional differences. Some individuals experienced a persistent reduction in microbiome diversity following antibiotic treatment and showed similarities in composition with patients hospitalized in intensive care units (67). Conversely, certain species present in the microbiota before treatment can influence personalized responses to antibiotics in humans (68).

Antibiotics are not the only group of drugs that affects the gut microbiota. Other commonly used drugs such as proton-pump inhibitors, metformin, and laxatives impact strongly the microbiome with changes in taxonomy, metabolic potential and resistome (69). Proton pump inhibitor-induced changes in the gut microbiome can lead to decreased colonisation resistance and the development of enteric infections, including *Clostridium difficile* infections (70).

1.3.6 Lifestyle: physical activity and smoking

The impact of lifestyle on the gut microbiota is well acknowledged (71).The interplay between physical activity and gut microbiota are recognised in literature. Moderate endurance exercise reduces inflammation, improves body composition, and leads to positive effects on gut microbial diversity and composition and its metabolic contribution to human health. On the other hand, endurance exercise exhibits positive effects on human health and on the gut microbial ecosystem, provided that the exercise intensity is controlled (72). A few years ago, Clarke et al. conducted a study indicating that professional rugby athletes exhibited higher alpha diversity and a greater abundance of the health-associated genus *Akkermansia* compared to sedentary controls with both high and low body mass indexes (73). Furthermore, other studies have shown that the gut microbiota influences sports performance. Microbes in the gut produce molecules that activate sensory neurons, thereby stimulating reward circuits in the

brain associated with exercise. This newly uncovered pathway in mice influences motivation for prolonged physical activity (74). The available evidence suggests that SCFA may have performance-enhancing effects through their influence on glycogen storage and skeletal muscle metabolism (75).

Tobacco smoking is a prevalent aspect of modern lifestyles. Shapiro et al.'s study revealed that the microbiome alterations induced by smoking can directly impact smoking-related diseases. Additionally, smoking-associated dysbiosis may influence the development of weight gain after smoking cessation. In individuals who smoke, distinct faecal microbiome compositions were observed compared to non-smokers. Smokers exhibited higher abundance of faecal *Prevotella*, *Veillonella*, *Bacteroides*, *Acidaminococcus*, and *Oscillospira* compared to non-smokers. Moreover, smokers showed reduced abundance of the Firmicutes phylum (76). Fan et al. corroborated the impact of smoking on three taxa (*Intestinimonas*, *Catenibacterium*, and Ruminococcaceae) with increased evidence strength. Furthermore, they identified an additional 13 taxa that might be directly influenced by tobacco smoking. Intriguingly, they discovered that reduced abundance of *Bifidobacterium* could lead to earlier initiation of smoking. This finding challenges conventional beliefs suggesting that early smoking initiation in children is solely due to exposure to third-hand smoke or imitation of paternal smoking behaviour (77). On the contrary, another study revealed the protective effect of the microbiota against nicotine induced damage with the gut bacterium *Bacteroides xylanisolvens* standing as an effective nicotine degrader. Colonization of *B. xylanisolvens* reduces intestinal nicotine concentrations in nicotine-exposed mice, and it improves nicotine-exacerbated Non-alcoholic fatty liver disease (NAFLD) progression (78). Recently, Shotgun metagenome sequencing revealed that the commensal *Parabacteroides distasonis* was enriched by ^{13}C-inulin. Integration of ^{13}C-inulin metagenomes and metabolomes suggested that *P. distasonis* used inulin to produce pentadecanoic acid, an odd-chain fatty acid, which was confirmed in vitro and in germ-free mice. *P. distasonis* or pentadecanoic acid was protective against Non-alcoholic steatohepatitis (NASH) in mice (79).

1.3.7 Diet

Various factors influence the composition of the human GI microbiota, including genetics, sex, ethnicity, age, medication, diseases/disorders, and notably, diet (80). Extensive research on the gut microbiota has shown that diet modulates the composition and function of this community of microbes in humans and other mammals. The human gut microbiota swiftly responds to

significant dietary changes and plays a crucial role in energy extraction. For instance, germ-free rats exhibit lower energy harvesting from a diet rich in polysaccharides, while germ-free mice have reduced adiposity despite consuming more food compared to their colonized counterparts (81). As an illustration, the *Prevotella* enterotype has been linked to high carbohydrate intake and is commonly found in individuals following vegetarian or vegan diets. Conversely, the *Bacteroides* enterotype is associated with high consumption of animal protein, amino acids, and saturated fats (80). Furthermore, the production of gut bacteria-derived metabolites from dietary substrates is constrained by the composition of the gut microbiota (82). Partula et al. discovered that foods typically considered healthy showed a positive correlation with α-diversity, whereas foods advised to be consumed in moderation were negatively associated with α-diversity. Among these, fruits, fried products, ready-cooked meals, and cheese were the primary drivers of dissimilarity in microbiota composition (83). Moreover, the analysis at the nutrient level indicated that individuals obtaining nutrients mainly from plant-food sources tended to have a more diverse gut microbiota than individuals obtaining them from animal-food sources (84). Another crucial aspect to consider is the interindividual variability in response to diet. Individuals may exhibit diverse metabolic responses to the same foods. For instance, those with a higher *Prevotella*-to-*Bacteroides* ratio (P/B) experienced greater weight loss after following a high-fibre diet for six months compared to those with a lower P/B ratio. Similarly, obese individuals with elevated levels of *Akkermansia muciniphila* demonstrated improved metabolic outcomes, such as lower insulin resistance and LDL cholesterol levels, when adhering to a hypocaloric diet rich in protein and fibre, in contrast to those with lower baseline concentrations of this microbe (85). This supports the notion that a meal may not be universally regarded as "good" for everyone.

Numerous dietary patterns have been identified for their positive impacts on health, operating through the intricate ecosystem of the intestinal microbiota. Among these, the Mediterranean-type diet (MD) stands out, long acknowledged for its protective health effects in scientific literature (86). An interesting hypothesis posits a mutually influential relationship between the MD and the gut microbiome: the diet shapes the assembly and functional capacity of the microbiota, while microbial activity in turn metabolizes dietary components into beneficial compounds, fostering a probiotic state (87). By favourably modulating the gut microbiota, the MD enhances diversity and alters the abundance of certain bacterial species. This modulation, characterized by increased production of short-chain fatty acids (particularly butyrate species), appears to correlate with reduced incidence of certain cancers (notably colorectal cancer) and

cardiometabolic pathologies among MD adherents (86). Research by Vázquez-Cuesta et al. revealed a correlation between adherence to the Mediterranean diet and alpha diversity, with better adherence associated with higher diversity. Moreover, good adherents exhibited increased abundance of *Paraprevotella* and *Bacteroides* (88).

The ketogenic diet, a low-carbohydrate protocol dating back to the 1920s, has gained attention for its efficacy in treating refractory epilepsy. Recent investigations have explored its impact on the gut microbiota, particularly in relation to its anti-seizure effects in mice. Within just four days of adopting the diet, significant alterations in gut bacterial taxonomy were observed. Notably, species such as *Akkermansia* and *Parabacteroides* showed marked increases in mice on ketogenic diets, and colonization experiments demonstrated their potential anti-seizure effects in germ-free or antibiotic-treated mice (89). Additionally, the Dietary Approaches to Stop Hypertension (DASH) diet, when combined with fasting, induces distinctive changes in both the microbiome and immunome, persisting even three months post-intervention (90).

1.4 GUT MICROBIOTA IN DISEASE CONDITIONS

Extensive research has focused on the human gut microbiome and its significance in both health and disease, revealing its influence on human metabolism, nutrition, physiology, and immune function. Disruption of the typical gut microbiota balance has been associated with various gastrointestinal conditions like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), as well as broader systemic disorders including obesity, type 2 diabetes, and atopy (91). The gut microbiome's involvement extends beyond gastrointestinal health to encompass neurological, cardiovascular, and respiratory ailments (92). Recent studies are revealing the implication of gut microbiota in infection diseases such as viral infections and mycobacteria infections (93). This overview highlights the implications of both common chronic conditions and infectious diseases.

1.4.1 Gut microbiota and obesity

Microbes in the human intestines impact the absorption, breakdown, and storage of nutrients, and have potential consequences on host physiology. Studies have found that obese subjects had significantly reduced numbers of *Bacteroidetes* and higher *Firmicutes* to *Bacteroidetes* ratios compared with non-obese subjects (94). Furthermore, research suggests that the composition and functions of gut microbiota not only correlate with obesity but may also contribute to its development. The gut microbiota not only provides metabolites and energy to

the host but also regulates nutrient absorption in the intestine, thereby influencing human energy metabolism (95). It was discovered that transferring the gut microbiota from obese subjects into germ-free mice partially replicated the increased body weight and fat mass gain, suggesting a potential causal relationship between the microbiota and obesity (96). Additionally, data collected thus far have revealed that several other phyla or specific bacteria are also influenced by obesity or leanness. This includes well-known Actinobacteria such as *Bifidobacterium* and the Verrucomicrobia representative *Akkermansia muciniphila* (97). One the practical use of those finding is the application of *Akkermansia muciniphila* for preventing or treating metabolic syndrome (98).

1.4.2 Gut microbiota and type 2 diabetes mellitus

Type 2 Diabetes mellitus (T2DM) is characterized by high blood sugar levels that occur because of decreased pancreatic insulin production or decreased insulin sensitivity in tissues that typically respond to insulin signalling. The extent of gut microbiota dysbiosis correlates with the severity of the disease, and restoring it through probiotic administration in both animal models and human patients has been linked to symptom improvement and disease progression (99). Lili Zhang et al. found that the gut microbiota plays an important role in T2DM. A decrease of butyrate-producing bacteria, such as *Faecalibacterium* and *Roseburia*, and reduction of butyrate are common in T2DM, which may be the principal causes of T2DM (100). Another study summarized evidence on microbial associations with the disease and identified supporting preclinical studies or clinical trials utilizing probiotic treatments. Among the frequently reported findings, genera such as *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, *Akkermansia*, and *Roseburia* were negatively associated with T2DM, whereas genera like *Ruminococcus* and *Fusobacterium* were positively associated with T2DM (101). Recently, it was found that the gut microbiome of T2DM patients had distinct gut microbiome functionality potential, characterized by enriched modules associated with the degradation of amino acids, and urea and notably an increased acetogenic potential of the microbiome that could be explained by the increase of acetogenic lineages like *Marvibryantia formatexigens* (102). There is increasing evidence of the gut microbiota in the unset of type 2 diabetes. However, there is also evidence that therapeutic approaches targeting the microbiota can be helpful to control this disease.

1.4.3 Gut microbiota and infection diseases

Despite the numerous benefits, harbouring a microbiota also entails significant biological costs. The abundant populations of microbes in our guts are beneficial until a viscus ruptures—as in

the case of appendicitis, diverticulitis, or a perforated ulcer (103). Bacterial infections are strongly associated with microbial dysbiosis (104–106) Recently, researchers have begun exploring the link between viral infections and intestinal microbial balance. Although less understood, investigations into how fungal infections shape the microbiota and reciprocally how the microbiota influences fungal infection development are also underway. Additionally, studies have uncovered a reduced diversity of intestinal microbiota in rats infected with *Aspergillus fumigatus* (*A. fumigatus*) in the lungs during the early stages of infection (107). A recent study reported that the gut a natural population of *Bifidobacterium* increased enhancing the hosts' resistance to influenza (108).

1.4.4 Gut-brain axis and neurodegenerative diseases

The gut microbiota is considered essential for brain physiological functions including myelination, neurogenesis, and microglial activation. It also plays a role in regulating human behaviour and influencing mental processes such as mood and cognition (109). Neurodegeneration, as the name implies, refers to the degeneration of neurons (110). While the exact mechanisms through which bacteria influence neurodegenerative diseases remain unclear, insights from well-researched host-microbe interactions suggest involvement in inflammatory responses and various aspects of host proteostasis, including protein synthesis, folding, trafficking, and degradation (111,112).

Alzheimer's Disease (AD) often referred to as dementia or cognitive impairment, is a common degenerative condition affecting the central nervous system in older individuals. In elderly individuals with AD, the microbiome typically exhibits a lower proportion of bacteria involved in synthesizing butyrate, which contributes to anti-inflammatory activity and immunity regulation. Conversely, there is a higher abundance of taxa known to induce proinflammatory states (112). The frailty observed in these individuals correlates with diminished diversity among core microbiota groups such as *Lactobacilli*, *Bacteroides*, and *Prevotella*, alongside an increased prevalence of *Ruminococcus*, *Atopobium*, and Enterobacteriaceae (113).

Similarly, the microbiome of **Parkinson's Disease** (PD) patients is characterized by a decreased abundance of butyrate-producing bacteria with an increased abundance of pro-inflammatory Proteobacteria, which may trigger inflammation-induced misfolding of Alpha-synuclein (a-syn) (114). It is increasingly evident that gut microbes may hasten the progression of neurodegenerative diseases by triggering autoimmunity and generating metabolites.

Conversely, modifying the composition of gut microbes holds promise for mitigating these diseases (112).

1.5 PUBLIC HEALTH PROBLEMS OF SOIL TRANSMITTED HELMINTH INFECTIONS, MALNUTRITION, AND THEIR RELATIONSHIPS WITH GUT MICROBIOTA

1.5.1 Soil transmitted helminth infections and gut microbiota.

Infections caused by soil-transmitted helminths (STHs) are widespread in Africa overall, particularly in sub-Saharan Africa (115), as well as in other developing regions. These infections predominantly affect the most economically disadvantaged populations, significantly affecting human health, nutrition, and workforce productivity, thereby worsening poverty (116).

Approximately 1.5 billion individuals worldwide are infected with soil-transmitted helminths (STHs), which are classified as neglected tropical diseases by the World Health Organization (WHO). Notably, roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), hookworms (*Necator americanus* and *Ancylostoma duodenale*), and *Strongyloides stercoralis* are of significant public health concern for certain populations. These infections lead to various health issues such as malnutrition, iron deficiency, and developmental deficits in children (117,118). In the case of the Zanzibar Archipelago in Tanzania, parasitic infections have been recognized as major public health challenges for decades (119).

As investigations continue with the aim to better understand the disease and to find efficient strategy to eliminate STH, research has come out with an established association between helminth infection and gut microbiome. Besides, the microbiota is necessary for the establishment of some helminth infections and can also influence the evolution of these diseases. Specific bacterial taxa can contribute to the resistance or susceptibility to certain helminths (120)**.** A recent meta-analysis conducted by Kupritz et al. revealed that intestinal helminth parasites have the capacity to produce a dynamic shift of the host gut microbiome. Among the helminths residing in the large intestine, *Enterobius vermicularis* and *Trichuris trichiura* exerted the most pronounced influence on the composition of the microbiome. This suggests that the impact on the microbiome appears to be specific to soil-transmitted helminths (STH) species and their anatomical niche within the host (121)**.** Another research group found that the effects of helminth infection status on the microbiome was dependant to village or origin of the host (122). Although several papers converge about an impact of the helminth

infection on the host microbiota, it is important to acknowledge that the mechanism by which helminths influence the microbiota remains to be fully elucidated.

In a cross-sectional study involving participants from various countries and continents, Rosa et al. discovered that seven out of twelve taxa, which increased with infection in both countries, belonged to the Firmicutes phylum, including four genera from the Clostridiales orders. They also observed that all seven individual taxa positively associated with soil-transmitted helminth (STH) infection in Ecuador were from the phylum Firmicutes, highlighting the association of this phylum with helminth infection. Notably, the family *Lachnospiraceae* was significantly lower in infected samples in Ecuador ($P = 0.037$), and this was also one of just two taxa significantly lower in infected individuals in both Liberia and Indonesia (123).

1.5.2 Malnutrition and gut Microbiota

Despite ongoing efforts to alleviate malnutrition globally, the task of completely eradicating it remains daunting. Projections indicate that nearly 600 million individuals will still suffer from chronic undernourishment by 2030, underscoring the significant challenge in meeting the Sustainable Development Goal (SDG) of eliminating hunger. The 2023 report revealed that millions of children under the age of five continue to endure stunting (148 million), wasting (45 million), and overweight (37 million), highlighting the persisting severity of the issue as of 2022 (FAO, IFAD, UNICEF, WFP and WHO. 2023. *The State of Food Security and Nutrition in the World 2023. Urbanization, agrifood systems transformation and healthy diets across the rural–urban continuum*. Rome, FAO. https://doi.org/10.4060/cc3017en).

Malnutrition significantly impacts women worldwide. In 2021, around 170 million women (9.1 percent) were classified as underweight, while three times as many women (610 million or 32.5 percent) were categorized as overweight. The global mean body mass index (BMI) for women stands at 24.4 kg/m^2, just below the threshold for being classified as overweight, indicating a growing trend of overweight among women. (United Nations Children's Fund. *UNICEF Programming Guidance. Prevention of malnutrition in women before and during pregnancy and while breastfeeding.* New York: UNICEF, 2021). It is crucial to remember that the nutritional well-being of women directly impacts the health, growth, and development of their children. Thus, safeguarding women's nutrition at every stage of life is paramount, particularly before and during pregnancy, as well as during breastfeeding, when they are most vulnerable nutritionally (United Nations Children's Fund. *UNICEF Programming Guidance. Prevention of malnutrition in women before and during pregnancy and while breastfeeding.* New York:

UNICEF, 2021). Additionally, undernutrition seems to have intergenerational origins. Women who experienced undernutrition during their own childhoods are more likely to give birth to newborns with low birth weight (124).

Nutritional interventions continue to be the primary approach in combating malnutrition. While therapeutic food interventions have effectively decreased mortality rates among children with severe acute malnutrition (SAM), they may not always be adequate for promoting healthy growth (125). Therefore, undernutrition cannot be solely attributed to the lack of food or nutrient deficiencies but rather stems from a combination of intricate biological and environmental influences. Insufficient maturation of the gut microbiota is linked to impaired growth and development during early life (43). Undernourished children tend to possess gut microbial communities resembling those found in younger, healthy individuals. These observations imply a potential cause-and-effect relationship between the underdeveloped gut microbiota in malnourished infants and stunted growth. Furthermore, when healthy microbiota was introduced into the intestines of undernourished mice through cohabitation, normal growth was restored in the latter. Remarkably, similar results were achieved when specific strains of two invading species, *Ruminococcus gnavus* and *Clostridium symbiosum*, were transferred. (126). These findings offer promise that modulating the gut microbiome could emerge as an effective strategy in combating malnutrition, including chronic cases.

1.5.3 Link between Helminth infection and Malnutrition.

Malnutrition and soil-transmitted helminth infections frequently occur together, leading to synergistic effects (127). Helminth infections can exacerbate malnutrition by causing symptoms such as anorexia, vomiting, diarrhoea, and intestinal blockages, which result in decreased food intake and hindered nutrient absorption (128). Consequently, intestinal helminth infections can elevate the likelihood of anaemia, undernutrition, and academic underperformance (129). Conversely, malnutrition and its associated deficiencies can increase susceptibility to helminth infections. In a study by Fuziah et al., it was observed that the severity of soil-transmitted helminth infections differed between stunted children and those with normal nutritional status, suggesting that stunted children may face a higher risk of such infections (130). Additionally, soil-transmitted helminth infections were significantly correlated with undernutrition (115). The study by Degarege et al. demonstrated the interconnection between these two health conditions. They observed a notable improvement in children's nutritional status four weeks after receiving treatment for helminth infection, administered with a single

dose of albendazole and/or praziquantel (131). Therefore, it is advisable to consider strategies that address both malnutrition and helminth infections in endemic areas.

1.6 GUT MICROBIOME TARGETED THERAPEUTIC APPROACHES

1.6.1 Probiotics, prebiotics and synbiotics

A targeted therapeutic approach focusing on the microbiome can involve the use of probiotics, prebiotics, and synbiotics.

Probiotics are defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.' This definition was established by a multinational expert group of scientists convened in 2001 by the Food and Agriculture Organization of the United Nations (132).

A **prebiotic** refers to a non-viable food component that provides health benefits to the host by influencing the composition of the intestinal microbiota. It's important to note that while prebiotics may include fibre, not all fibers qualify as prebiotics (133). When prebiotics and probiotics are used together, it's often termed as **synbiotics**, but only if the combined health benefits are greater than the sum of their individual effects.

Considerable evidence from in vitro studies indicates that both established and potential probiotics can exert strain-specific anti-inflammatory effects. Recent reports suggest that certain probiotics can enhance the quality of life for patients with inflammatory bowel disease (IBD) and potentially increase intestinal biodiversity, thereby alleviating IBD symptoms. Probiotics with the capacity to reduce inflammation and/or stimulate innate immunity hold promise for inclusion in therapeutic approaches aimed at restoring a healthy gut microbiota in the host (132,133). As resistance to existing drugs emerges in the fight against helminths, alternative control strategies like probiotics offer promising avenues for sustainable protection. Abadi's study presents an insightful overview of the application of probiotics in combating helminth zoonosis, highlighting their potential efficacy as a novel control approach (134).

1.6.2 Postbiotics

Postbiotics encompass soluble substances, including products or metabolic byproducts, which are either secreted by live bacteria or released following bacterial breakdown, such as enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins, and organic acids. These compounds have garnered attention due to their well-defined chemical composition, established safety parameters, extended shelf life, and the presence of

various signalling molecules. These molecules may exhibit a range of beneficial activities including anti-inflammatory, immunomodulatory, anti-obesogenic, antihypertensive, hypocholesterolemic, anti-proliferative, and antioxidant effects (135). Postbiotics have been acknowledged for their ability to mimic the functions and activities of probiotics without necessitating stringent manufacturing or storage conditions, rendering them particularly suitable for use in developing nations. Several highly effective postbiotics, such as cytoplasmic extracts and cell wall components, have been identified within various species of *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Bacillus*, *Streptococcus*, and *Faecalibacterium* (136). Metabolite-based therapeutics, referred to as 'postbiotics,' direct their action towards downstream signalling pathways of the microbiome. They work by mitigating the adverse effects resulting from an overabundance, deficiency, or disruption of metabolites within these pathways. Instead of directly addressing the abnormal microbial composition, the external administration or inhibition of metabolites holds promise in counteracting and rectifying the detrimental effects of dysbiosis (137).

1.6.3 Faecal Microbiota Transplantation

Faecal microbiota transplantation (FMT) involves transferring an entire microbial community from a healthy donor to a diseased recipient with the aim of replacing the disease-associated microbiome. FMT has shown remarkable efficacy in treating *Clostridioides difficile* infection, often occurring following antibiotic treatment (138). Clinical studies on FMT have demonstrated its potential effectiveness not only in treating *Clostridioides difficile* infections but also in addressing conditions such as inflammatory bowel disease, graft-versus-host disease, and cancer. Manipulating the gut microbiota via FMT presents a direct approach to replacing dysbiotic gut microbiota with healthy ones, aiming to restore normal composition and potentially achieve therapeutic benefits (139).

1.6.4 Phage therapy

Phages, also known as bacteriophages, are small viruses with the ability to target and kill bacteria while leaving cell lines of other organisms unaffected (140). They act as natural antibacterials by inducing bacterial lysis, thereby regulating bacterial populations. Phages are effective against both gram-positive and gram-negative bacteria, including multi-drug resistant (MDR) pathogens (140). Given the growing challenge of bacterial and multi-drug resistance (141) the resurgence of phage therapy offers a promising alternative to antimicrobial chemotherapy. In the face of the progressive spread of MDR bacterial pathogens and the scarcity of new antibiotics to combat these pathogens, phage therapy could provide a valuable

solution (142). However, the relatively brief lifespan of phages poses a significant challenge in phage therapy. Therefore, various experimental endeavours have been undertaken to generate phage mutants with enhanced pharmacokinetic characteristics. Merril et al. documented the discovery of a mutant strain featuring minor alterations in its capsid protein, enabling it to evade the reticuloendothelial system (RES) and thus prolong its presence in circulation, thereby enhancing its therapeutic efficacy (143).

1.7 AIM AND OBJECTIVES OF THE THESIS

The primary aim of this thesis is to investigate the interconnectedness of nutrition, gut microbiome, and intestinal parasites within the Zanzibar population, with a focus on proposing alternative or complementary strategies to combat parasites and malnutrition.

The evidence put forward is that the gut microbiome differs based on the helminth infection status and/or the nutritional condition. Additionally, inadequate nutrition is linked to dysbiosis in the intestinal microbiota which promotes helminth infections and malnutrition. Potentially beneficial microbes are those associated to a better nutritional status, better anthropometric parameters, and/or lower parasite levels.

Specific objectives include:

- To investigate the effects of *T. trichiura* on the gut microbiome of women of reproductive age (WRA) employing the shotgun metagenomic sequencing technology to compare the gut microbiomes of infected and uninfected individuals.

- To investigate the correlations between diet and gut microbiota in both healthy and helminth-infected women of reproductive age. The overarching goal is to identify specific foods or nutrients that may promote the growth of beneficial bacteria or limit the proliferation of harmful bacteria.

- The third objective is twofold. Firstly, to examine the connection between helminth infection and gut microbiota a short time after deworming treatment. Secondly, to analyse the link between nutritional status and gut microbiota to identify potential protective bacteria as candidate probiotics and potential targets in combating malnutrition in children.

1.8 OUTLINE OF THE THESIS

The People's Republic of Zanzibar (Zanzibar) is part of Tanzania, located in East Africa. It comprises numerous small islands along with two main ones: Unguja (commonly known as Zanzibar) and Pemba Island. With a population estimated at 1,6 million in 2018, most inhabitants reside in Unguja, followed by Pemba (Tanzania National Nutritional Survey 2018). The economy relies heavily on spices and tourism, with additional sectors including fishing and agriculture. Healthcare infrastructure includes the School of Health and Medical Sciences in Unguja, the Public Health Laboratory Ivo De Carneri (PHL-IDC) in Pemba, and other health research institutions coordinated by the Ministry of Health. Despite socioeconomic improvements, Zanzibar, like Tanzania, faces health challenges such as high levels of malnutrition and parasite infections. Albendazole and its derivatives are administered annually to combat these issues. In collaboration with the University of Camerino (UNICAM) in Italy and the State University of Zanzibar (SUZA), this research was conducted to characterize the gut microbiota of women of reproductive age (WRA) and children, to analyse associations between diet, helminth infections, and gut microbiota, and to identify potential probiotics and foods to enhance gut health and children's growth.

After a general introduction on the gut microbiome (**chapter I**), I selected group of infected and non-infected samples from WRA that were previously analysed using the mini Flotac for parasitological analysis and the 16S rDNA sequencing approach for bacterial relative abundances analysis. Here, I applied the shotgun metagenomics sequencing approach comparing the microbiota of infected and non-infected WRA from Pemba (**chapter II**). My goal was to compare the two sequencing approaches, to analyse the microbiota up to species level and to perform functional analysis since the metagenomics approach provides comprehensive gene data from the samples. The compositional and functional analyses of the gut microbiome confirmed that *T. trichiura* infection shaped the host gut microbiome in WRA. Certain taxa exhibited variations based on the infection status. Furthermore, functional analysis revealed significant differences in metabolic pathways ($p < 0.05$), with cholesterol metabolism and some pathogenic infections being more abundant in the infected samples than in the non-infected samples.

In the following section (**chapter III**), I performed a new sampling with the same population with more attention given to the diet of WRA. The flotation technique was used for the

parasitological analysis, and a weekly food table for each WRA was used to assess the nutrient intakes based on the Tanzanian food composition tables prepared by the Havard medical school. Additionally, I utilised the 16S rDNA sequencing to analyse the gut microbiota of infected and non-infected WRA. Spearman correlation analysis was applied to examine the relationship between food/nutrient intake and gut microbiota. Results obtained here revealed that participant's gut microbiota differs according to the location. Analysis of nutrient intake indicated micronutrient deficiencies in the diet of WRA from Zanzibar, highlighting an imbalance in their dietary patterns. Correlation analysis identified bacterial taxa consistently correlated with specific food or nutrients in healthy women from both locations, and in both type of helminth infections suggesting that the consumption of certain foods and nutrients may influence the composition of the gut microbiota, potentially enhancing resilience against helminth infections in endemic regions.

In the **fourth chapter**, I explored how helminth infection and nutritional status relate to gut microbiota after a deworming campaign. Using 16S rDNA sequencing in children and women from Pemba, no significant differences in gut microbiota diversity were observed among participants. Some taxa, statistically significantly found negatively associated with both helminth infection and malnutrition, emerged as promising candidates for addressing these health issues.

In the **general conclusion**, I emphasized the pivotal role of the gut microbiota in addressing both helminthiasis and malnutrition. I highlighted that microbiota-directed interventions, particularly during early life and among women of reproductive age, present promising avenues for combating helminth infection and malnutrition. Furthermore, I pointed out that the imbalanced diet prevalent in the Zanzibar population poses a threat to both the host and gut microbiota. As a solution, I recommended incorporating locally available foods or nutrients into the diet to promote a healthier and more resilient gut microbiota. Additionally, I identified specific bacteria that could serve as candidate probiotics to prevent helminth infection, childhood malnutrition, or both conditions.

CHAPTER II: TRICHURIS TRICHIURA INFECTION IS ASSOCIATED WITH CHANGES IN GUT MICROBIOME COMPOSITION AND FUNCTION AMONG WOMEN OF REPRODUCTIVE AGE FROM PEMBA, TANZANIA

The content of this chapter aligns with the article that I co-authored with Mozzicafreddo M, Chen H, Piersanti A, Salum SS, Ali SM, Zhang J, and Miceli C (2024), titled "*Trichuris trichiura* infection is associated with changes in gut microbiome composition and function among women of reproductive age from Pemba, Tanzania," published in Frontiers in Tropical Diseases, Volume 5, under the identifier doi: 10.3389/fitd.2024.1276210. My contributions to this research encompassed conceptualization, investigation, data collection, methodology, and drafting the original manuscript.

Furthermore, the findings presented in this chapter were also delivered as a concise oral presentation at the “9th world Congress on Targeting Microbiota 2022”, which took place in Paris from October 19th to October 21st, 2022.

2.1 Abstract

Large intestine-dwelling helminths affect microbiome composition. In sub-Saharan Africa, where helminth infections are endemic, the use of chemotherapeutic drugs is the primary strategy for controlling soil-transmitted helminthiases (STHs). However, the emergence of anthelmintic resistance necessitates the urgent exploration of alternative and complementary treatments to achieve the World Health Organization’s goal of eliminating STHs. One promising avenue involves the manipulation of gut microbiota in at-risk populations. This study aimed to enhance the understanding of the interplay between Trichuris trichiura and the gut microbiome. In this study, we used the Mini-FLOTAC technique for parasitological analyses and a shotgun metagenomic sequencing approach to investigate the effect of *T. trichiura* on the gut microbiome by comparing infected and non-infected women of reproductive age (WRA) from Pemba. Structural and functional analyses of the gut microbiome revealed that *T. trichiura* infection shaped the host gut microbiome in WRA. Some taxa vary according to infection status. *Prevotella* genus was more abundant in healthy participants, whereas species such as *Weissella cibaria*, *Leuconostoc citreum* (new emergent probiotics), and *Leuconostoc lactis* (starter) decreased in infected individuals, suggesting the use of

potential probiotic treatments to mitigate dysbiosis induced by STHs. Furthermore, the overall number of common fungi, irrespective of species, was significantly higher in the mycobiome of *Trichuris* infected participants. Functional analysis revealed significant differences in metabolic pathways ($p < 0.05$), with cholesterol metabolism and pathogenic infections being more abundant in the infected samples than in the non-infected samples. In conclusion, this study sheds light on the intricate interactions between helminth infections and the gut microbiome in the WRA, particularly in STH endemic regions. The identified associations between specific gut microbial changes and *T. trichiura* infection may pave the way for innovative complementary treatments to effectively combat STHs.

KEYWORDS: helminths, women of reproductive age, Trichuris trichiura, gut microbiome, mycobiome, fungi.

2.2 INTRODUCTION

Soil-transmitted helminth (STH) infections are among the most common infections worldwide, with an estimated 1.5 billion infected people, or 24% of the world's population. The main species that infect humans are roundworms (*Ascaris lumbricoides*), whipworms (*T. trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale).* According to the World Health Organization (WHO), heavy infections can cause a variety of symptoms, including intestinal manifestations (diarrhoea and abdominal pain), malnutrition, general malaise and weakness, impaired growth, and physical development. School-going children are most affected by STH infections, resulting in poor school performance and impaired cognitive function, among other detrimental effects (118). Recent estimates indicate that > 880 million children require treatment for these parasites. The population at risk in the African region according to the World Health Organization is estimated to be 350 million (www.who.int/health-topics/helminthiasis). Polyparasitism involving STHs and Schistosoma blood flukes is common in low- and middle-income countries. These helminths affect the gut environment and may cause changes in the gut microbiome composition (144,145). Gastrointestinal pathogens can alter the host gut microbiome and affect bacterial diversity and abundance, brain function, digestive health, immune function, and development (146).

Studies on the human intestinal microbiota have been neglected for several years (147), although they are at the interface between ingested food and the gut epithelium. They are also in contact with the first pool of immune cells and the second pool of neural cells in the body. The gut microbiota is now gaining recognition as an organ that plays a major role in health and disease (148). However, it remains true that many structural analyses are limited on gut bacteria ignoring other gut microbial members for which important roles during health and disease have become increasingly more appreciated (149). A pilot study revealed that changes in gut microbial composition and structure occur in *T. trichiura* infected individuals compared to uninfected individuals (150). Regarding the relationship between *Trichuris sp* and the gut microbiota, studies reveal that the hatching of some *Trichuris* eggs is favoured in presence of specific bacteria such as *Escherichia coli* and *Lactobacillus reuteri* (151). A recent study by Rosa et al. (2021) showed significant positive associations for seven taxa, including *Escherichia,* which has been shown to induce whipworm egg hatching and *Bacteroides*, which has previously been identified as a major component of the whipworm internal microbiome (121). Another study revealed a relationship between success- and failure-associated enterotypes. Using a survival analysis, this investigation confirmed that patients presenting an enterotype rich in *Eubacterium coprostanoligenes* and *Ruminococcus torques* before the treatment are more likely to be more efficiently cured from the *T. trichiura* infestation by using the albendazole and ivermectin-based treatment than those presenting an enterotype rich in *Prevotella, Roseburia* and *Coprococcus,* or rich in *Faecalibacterium* and *Escherichia/Shigella* (152).

With the emergence of resistance to deworming drugs (153), it is necessary to better understand the relationship between the gut microbiota and helminths to optimise the fight against STH/ *Trichuris* infections. This study was conducted in an endemic area of helminth infections. Results of a survey published in 2021 revealed that the prevalence of STH was evaluated at 80% (95% CI 78.1–81.5) and most of the STH cases were due to *T. trichiura* (154). In Unguja and Pemba, the two main islands that form the Zanzibar Archipelago, STH infections were recognised as a major public health problem in the early 1990s. In 1994, the Ministry of Health and Social Welfare of Zanzibar, in collaboration with the WHO, established an action plan for controlling STHs and urinary schistosomiasis (116). Chemotherapy consisting of albendazole has yielded interesting results by reducing the intensity of STH infections and morbidity. However, the burden of STH infection remains a public health challenge in Unguja and even more so in Pemba. Hence, it is imperative to explore complementary strategies for their use in

conjunction with the administration of chemical drugs to combat helminth infections. One promising avenue involves manipulation of the gut microbiota of at-risk populations. This study aimed to enhance our understanding of the interplay between *T. trichiura* and the gut microbiome. This study aimed to investigate the effects of *T. trichiura* on the gut microbiome of women of reproductive age (WRA), where soil-transmitted helminths (STHs) are highly prevalent. This study employed shotgun metagenomic sequencing technology to compare the gut microbiomes of infected and uninfected individuals.

2.3 MATERIAL AND METHODS:

2.3.1 Ethic statement:

The project was approved by the Zanzibar Health Research Institute (ZAHRI; protocol number: ZAMREC/001/SEPT/018)) and written informed consent was obtained from all participants enrolled in the study.

2.3.2 Study area and design:

This pilot cross-sectional study was conducted as secondary analysis of previously collected samples. Participants were categorised into two groups each consisting of 5 individuals classified as either positive or negative for *T. trichiura* infection (All samples were coded as shown in Table 2.1). As previously reported (150), women aged from 23 to 45 years were enrolled from sanitary centres in Pemba Island (Tanzania) where helminth infection is endemic and interviewed in Swahili with the help of nurses and personnel of the Public Health Laboratory Ivo de Carneri (PHL-IdC). Each participant completed a questionnaire (reported in **153**), signed the informed consent, and provided faecal samples. The inclusion criteria for all the individuals were as follows: very similar origin of food and diet, mainly consisting of banana fruit, cassava, rice, cassava leaves as vegetable and dagaa fish, no HIV infection, no diarrhoea, no fever, no diabetes, no malaria, and no antibiotics or anthelmintic treatment in the previous 3 months.

2.3.3 Parasitological analysis and selection of participants.

Parasitological analyses aimed at detecting helminth infections, employed flotation techniques, and microscopic observations were independently conducted by two experts. The samples included in this study were randomly chosen based on the criteria of absence of co-infection

with helminth species, non-pregnant, and having not used any antiparasitic treatments including herbal medications.

Table 2.1. Characteristics of the selected samples in relation to *Trichuris* infection status and detected number of eggs/grams of feces.

No	Sample code	Age (Years)	Infectious status	Number of eggs/g
1	M 06	25	Infected	22
2	M 07	33	Non infected	0
3	M 09	38	Infected	450
4	M11	28	Infected	20
5	M22	35	Non infected	0
6	M 30	26	Infected	150
7	M 61	30	Infected	70
8	M 67	45	Non infected	0
9	MS10	30	Non infected	0
10	MS11	32	Non infected	0

2.3.4 DNA extraction, library construction and shotgun sequencing.

Genomic DNA was extracted from frozen faecal samples using the NORGEN BIOTEK Stool DNA Isolation Kit, according to the manufacturer's instructions. DNA quality and quantity were determined using a NanoDrop spectrophotometer and direct gel electrophoresis, respectively. DNA integrity was confirmed by analysing 300 ng of DNA by electrophoresis and visualisation under ultraviolet (UV) light. The extracted DNA was stored at -20 °C. The purified DNA was sent to the SynBiotec laboratory (spin-off of the University of Camerino) for shotgun sequencing, which was carried out using the Illumina Miseq platform 2×150PE. Prior to library construction, the DNA was quantified using a Qubit 4.0 Fluorometer. A DNA library was prepared according to the Illumina DNA Prep Guide for Illumina paired-end-indexed sequencing. The extracted DNA (150–500 ng) were used as the library input. Bead-Linked Transposomes (BLT) were used to fragment and tag DNA with adapter sequences. The adapter-tagged DNA was washed with BLT before polymerase chain reaction (PCR) amplification, using a limited-cycle PCR program comprising five cycles. The amplified library was subjected to double-sided bead purification. To confirm the size distribution, the resulting libraries were validated using an Agilent Bioanalyzer 2100 (High-Sensitivity DNA kit). The library concentrations were quantified using a Quibit 4.0 Fluorometer. The indexed DNA libraries were normalised to 4 nM and combined into equal volumes. The samples were

then sequenced using an Illumina Miseq, 2×150bp V2 paired end run. On average, each sample yields 1,282,162.5 reads with an average read length of 146.825 bp.

2.3.5 Bioinformatics and statistical analyses

Bioinformatics and statistical analyses were performed using the OmicsBox software (version 3.0.30). The step of cleaning from contaminants such as the human DNA was performed. All read data were cleaned of human and phiX DNA using the Bowtie2 tool. Next, kraken2 classifier, a taxonomic classification system that allows high accuracy and fast classification (database version 2019.06), was used for taxonomic profiling. Paired-end reads were selected as the type of input data, and a confidence filter was enabled and set at 0.05. A Stacked bar chart was used to provide a view for inter-sample comparison separated at the main taxonomic level. The average operational taxonomic units (OTUs) were ordered by abundance from high to low. Only the 500 largest OTUs are shown for each sample; the remaining were grouped into an extra group called others. OTUs groups were also studied by analysing Krona pie charts using the same software. A rarefaction curve was generated to determine whether the sequencing coverage was sufficiently deep to obtain an accurate estimate of the total number of OTUs present in a specific sample. A Diversity Curve was generated and used to evaluate the benefits of microbial diversity, including additional samples, in the dataset. Principal Coordinate Analysis (PCoA plot), a two-dimensional plot reporting the Bray-Curtis distances between samples, was generated to perceive the distance between samples according to *Trichuris* infection status. Differential Abundance Analysis of Taxa was performed by dividing samples into two groups and comparing features using the edgeR module of OmicsBox. Non-infected samples were assigned to the reference group and compared with infected samples set as the contrast group. A ((false discovery rate) < 0.05 was considered significant. For each sample, a genome assembly was generated using the assembly pipelines of meta-SPAdes, based on the de Bruijn graph included in OmicsBox, and setting the K-mer sizes as automated. In the following step, gene prediction was performed for each assembly using the application FragGeneScan, also available in OmicsBox, which is capable of predicting intact and incomplete ORFs in short sequencing reads. For functional investigation, Clusters of Orthologous Genes (COG) and protein family (Pfam) databases were used for metabolic pathway analysis and gene annotation. The contrast-infected group was compared with the reference uninfected group to determine the differences in terms of over- or under-represented

metabolic pathways. Statistical significance was set at $P < 0.05$. T test analysis and plotting were performed using GraphPad Prism (version 9.5.1).

2.4 RESULTS

2.4.1 Sample diversity according to the *Trichuris* infection status

Principal coordinate analysis (PCoA) mapping was performed to better understand the relationships between the participants' gut microbiota and to identify similarities and differences. PCoA facilitated the visualisation of clusters based on the infection status, as highlighted in Figure 1. In addition, PCoA allowed the identification of subclusters emerging in response to parasitic loads. Notably, subjects M06 and M11, with similar loads (22 and 20 eggs/g, respectively), showed a close relationship, implying greater similarities in their gut microbiota composition. In addition, subjects M61 and M30, who carried slightly elevated parasitic loads (70 and 150 eggs/g, respectively), formed another subcluster. Intriguingly, subject M09, bearing the heaviest load (450 eggs/g), appeared to be isolated, although closer to the group of non-infected subjects. This suggests a different impact of the worm on the gut microbiota depending on the infection load. Samples from non-infected individuals showed more cohesive clustering than those from infected individuals. This suggests that the gut microbiota of the non-infected samples shared greater similarity.

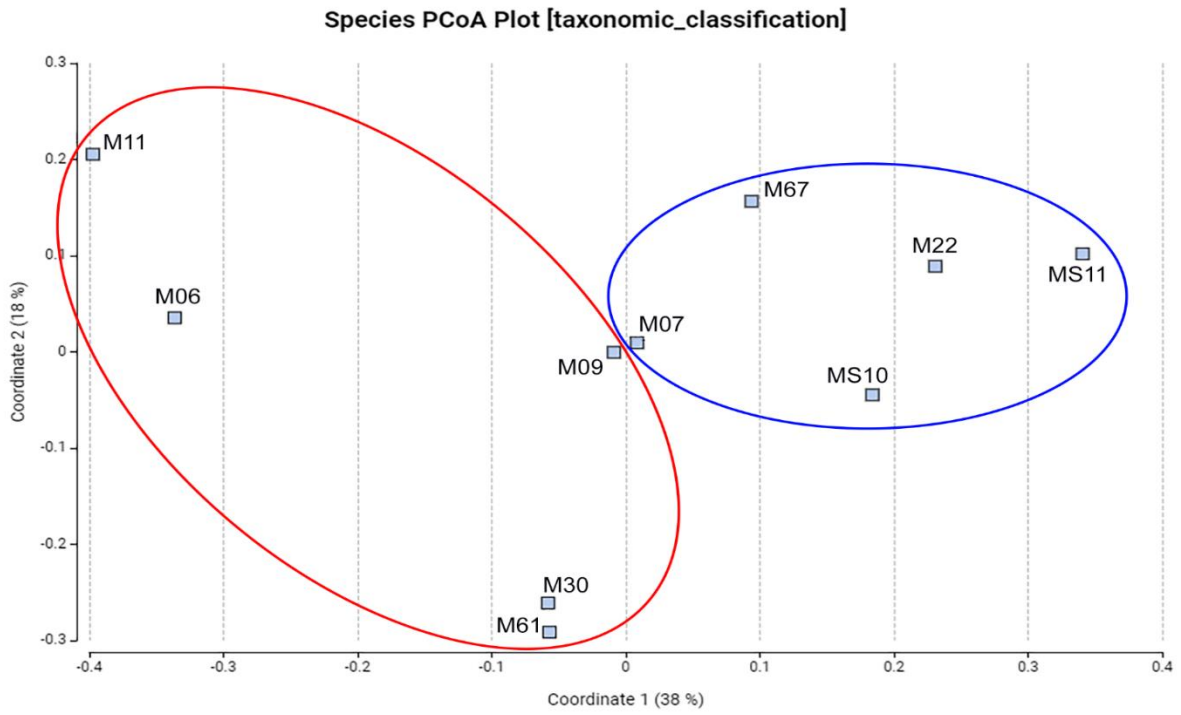


Figure 2. 1. Class PCoA plot showing different clusters between non-infected and infected samples. Non-infected samples M07, M67, M22, MS10, MS11 are indicated by blue color circle and infected samples M06, M09, M11, M30, M61 by red color circle.

2.4.2 Taxonomic profiling

Shotgun metagenomic sequencing of ten selected samples yielded a total number of 12,652,028 reads. On average, each sample contained 1,265,203 reads, with an overall average read length of 146.87. Distribution of the top 10 hits revealed that bacteria accounted for more than 90 percent of each sample, followed by eukaryotes, archaea, and viruses. The microbiome composition, visualised up to the species level in each sample using Krona charts, remained unclassified in approximately 50% of the reads. This indicates that a considerable number of sequenced genes remained unannotated. This phenomenon is well known and can be attributed to different reasons, such as limitations in the databases with respect to the high microbial biodiversity of the gut, specifically for eukaryotic microbes that may not have matches in the existing databases. This can also be attributed to the software's limitations in accommodating (annotating) genes and taxa with a limited presence, which cannot be easily interpreted by sequencing with short read lengths.

In addition, an intriguing observation emerged: the percentage of classified reads was relatively higher in non-infected samples than in infected samples (see Supplementary Material, Table l: Percentage of classification). The rarefaction curve provided evidence that the richness was adequate in the sequenced samples (Supplementary Material, Figure 1). The diversity curve used to assess and compare the diversity across populations confirmed that the number of samples was sufficient to obtain the expected diversity (Supplementary Material, Figure 2).

2.4.2.1 Taxonomic composition at the phylum level

Taxonomic analysis revealed that, at the phylum level (Figure 2.2), the community was dominated by Firmicutes (also named Bacillota), Bacteroidetes, Proteobacteria, and Actinobacteria. Notably, the Firmicutes/Bacteroidetes ratio (as shown in Figure 2.3A), a widely used indicator for assessing the state of intestinal microbiota health, was significantly low in subjects without *T. trichiura* infection. In infected samples, a higher relative abundance of Firmicutes was observed than in noninfected samples, whereas noninfected samples showed a higher relative abundance of the phylum Bacteroidetes, except for sample M09. Proteobacteria, known to include more pathogenic species, displayed a higher relative abundance in *Trichuris* infected samples (Figure 2. 4B).

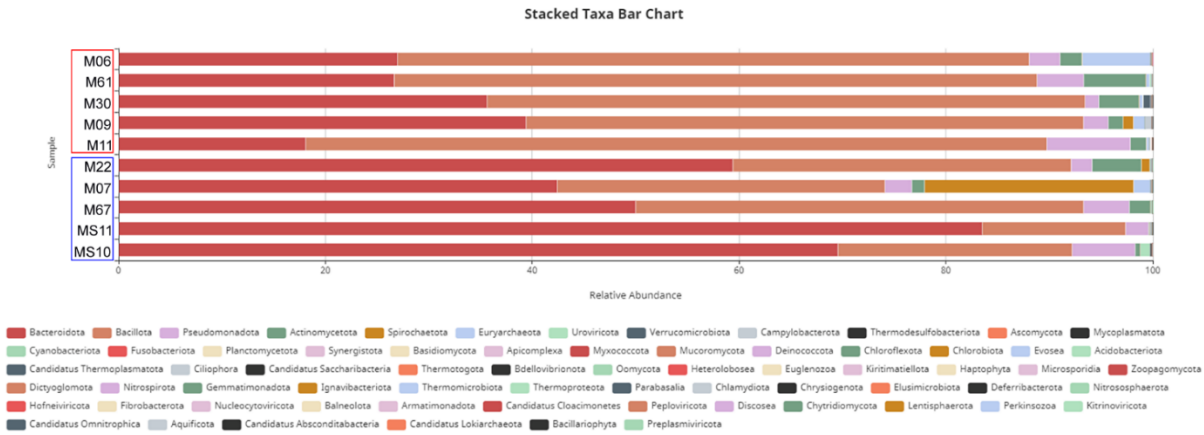


Figure 2.2. Taxa bar chart analysed at the phylum level**.** Non-infected samples M07, M67, M22, MS10, MS11 are indicated by blue color rectangle and infected samples M06, M09, M11, M30, M61 by red color rectangle.

2.4.2.2 Taxonomic composition at the genus level.

Analysis of the community composition at the genus level unveiled *Prevotella* as the most abundant genus, followed by *Faecalibacterium, Bacteroides* and *Roseburia* (Figure 2. 4). The ratio of *Prevotella/Bacteroides* (Figure 2. 3C), both members of the phylum Bacteroidetes, was only slightly higher in *Trichuris* infected samples.

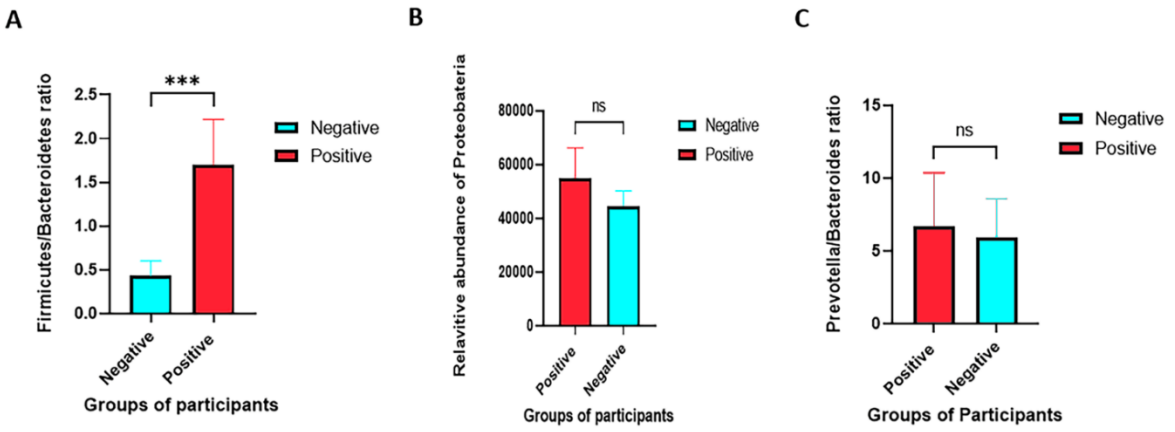


Figure 2. 3. Analysis of markers of the healthy condition of the gut microbiota. (A) Firmicutes/ Bacteroidetes ratio in *Trichuris*-negative vs *Trichuris*-positive samples (unpaired t. test, P = 0.0009); (B) relatively higher abundance of Proteobacteria in *Trichuris*-positive samples (P =

0.1099); (C) the *Prevotella*/*Bacteroides* ratio is higher in infected samples (P = 0.6979). *** stands for a P value ≤ 0.001 and ns stands for P value ≥ 0.05.

The abundance of these two bacterial genera is driven by distinct dietary preferences and contributes to the classification of different enterotypes. Furthermore, this analysis also revealed that some bacteria responsible of short chain fatty acids (SCFAs) production, including *Prevotella, Prevotella 9,* and *Ruminococcus* showed a lower relative abundance in infected samples in comparison to healthy samples (as illustrated in Figure 4 for samples MS10, MS11, M07, M22, and M67). In contrast, *Faecalibacterium* was significantly more prevalent in infected samples than in non-infected samples.

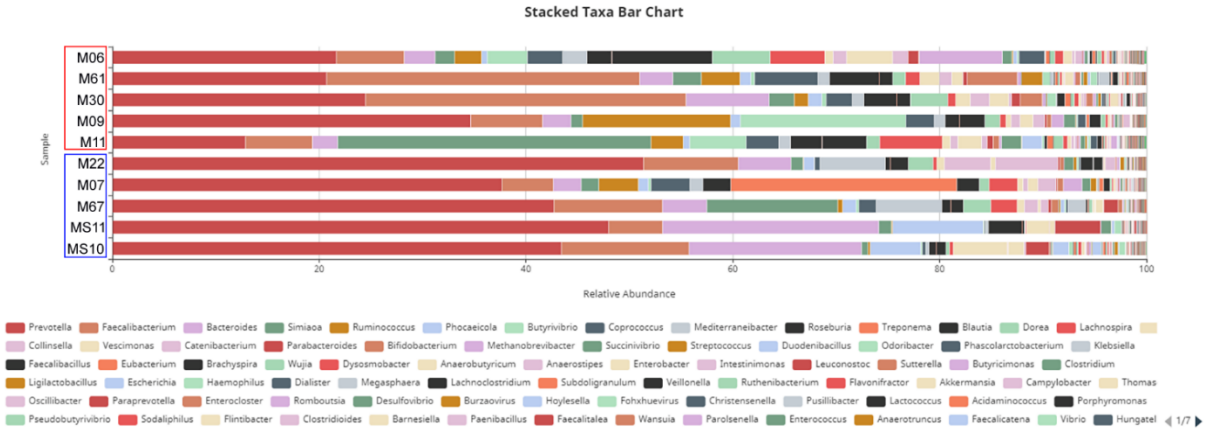


Figure 2.4. Taxa bar chart analysed at the genus level. Infected and non-infected samples are indicated by red and blue columns, respectively.

2.4.2.3 Analysis of the differential abundance of taxonomy at the species level.

Species level analysis revealed that *Prevotella copri, Simiaoa sunii* and *Faecalibacterium prausnitzii* were the predominant species (Figure 2. 5). These were followed by other species, including *Ruminococcus torques*, *Ruminococcus SP.JE7A12*, *Roseburia intestinalis*, *Lachnospira eligens,* and *Treponema succinifaciens.*

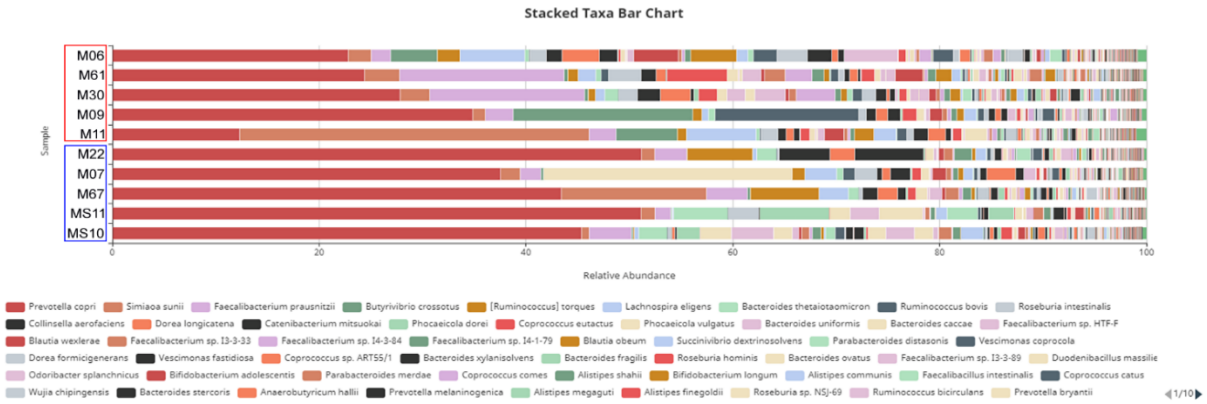


Figure 2.5. Taxa bar chart at the species level. Infected and non-infected samples are indicated by the red and blue rectangles, respectively.

The differential taxonomic abundance analyses conducted at the species level yielded distinct taxonomic compositions based on the *Trichuris* infection status in the WRA. Species such as *Weissella cibaria, Weissella paramesenteroides* and *Leuconostoc citreum* (emerging probiotics) and *Leuconostoc lactis* (used as a starter in fermentation) were underrepresented in infected samples compared with healthy samples. Species of *Bacteroides* genus, such as *B. stercoris* and *B. fragilis* were also underrepresented in the same group. Conversely, other species displayed contrasting patterns, such as *Butyrivibrio crossotus, Bifidobacterium bifidum, Ligalactobacillus salivaris, and Methanobrevibacter woesei* that were more abundant in *T. trichiura* infected subjects when compared to healthy individuals (Figure 2.6). Potentially pathogenic species, such as *Treponema succinifaciens* and *Streptococcus gallolyticus,* the latter being the main causative agents of septicaemia and infective endocarditis in elderly and immunocompromised individuals, and strongly associated with colorectal cancer (155), were notably present in infected samples.

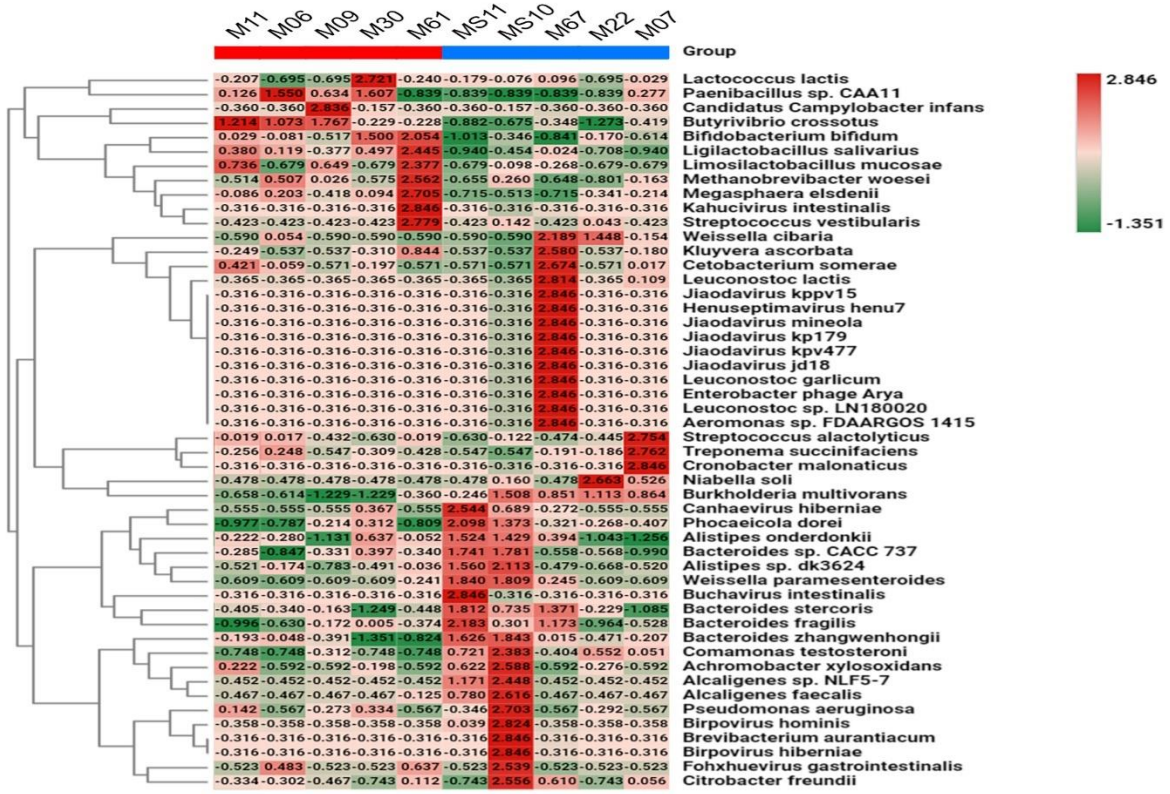


Figure 2. 6. Heat map of relative abundances of species. The bar at the top indicates the infected (in red) and non-infected (in blue) samples. Numbers indicate the differential abundances between the two groups.

2.4.3 Functional analysis of the shotgun sequencing data: differential abundance analysis of metabolic pathways and protein families

The differential abundance analysis of metabolic pathways (Table 2.2) using COG and Pfam databases showed significant over-representation and no under-represented pathways in *Trichuris* infected compared to non-infected women ($P < 0.05$). Additionally, eight COG metabolic pathways were significantly overrepresented. These pathways include Human T-cell leukaemia virus 1 infection, cholesterol metabolism, and pathogenic *E. coli* infection. This finding indicates that *Trichuris* infection not only influences the composition of the gut microbiome, as highlighted by structural analysis, but also has a discernible impact on its functional pathways.

Table 2.2. Differential abundance analysis of metabolic pathways (COG). Top ten differentially abundant features (Overrepresented), P-value < 0.05. No underrepresented features were detected.

Feature	Description (overrepresented)
map04742	Taste transduction
map04723	Retrograde endocannabinoid signalling
map04080	Neuroactive ligand-receptor interaction
map04979	Cholesterol metabolism
map04075	Plant hormone signal transduction
map05166	Human T-cell leukaemia virus 1 infection
map05130	Pathogenic Escherichia coli infection
map04392	Hippo signalling pathway-multiple species

The effect of *Trichuris* infection on the function of the gut microbiome was further confirmed by differential analysis of protein families. This analysis revealed several protein families that were either over-represented or under-represented based on the *Trichuris* infection status of the participants (Table 2.3). Notably, most of these proteins remain functionally uncharacterized, except for the endothelial cell-specific chemotaxis regulator ATP synthase subunit C, Restriction endonuclease, and putative tight adherence pilin protein family, which are overrepresented in infected WRA. In contrast, certain protein families were less abundant in infected samples. They are nodavirus V-methyltransferase, four C-terminal TMM regions of protein-O-mannosyltransferase, and the GldH Lipoprotein families (as detailed in Table 2.3).

Table 2.3. Differential abundance analysis of pfam families. The top ten differentially abundant features are reported (FDR < 0.05).

Overrepresented features		**Underrepresented features**	
Feature	**Description**	**Feature**	**Description**
PF15820	Endothelial cell-specific chemotaxis regulator	PF20089	Family of unknown function (DUF6481)
PF00137	ATP synthase subunit C	PF19222	Nodavirus Vmethyltransferase
PF14018	Domain unknown function (DUF4234)	PF17320	Family of unknown function (DUF5363)
PF04471	Restriction endonuclease	PF16192	C-terminal four TMM region of protein-O-mannosytransferase
PF16964	Putative tight adherence pilin protein F	PF15889	Domain of unknown function (DUF4738)
PF11026	Protein of unknown function (DUF2721)	PF14109	GldH Lipoprotein
PF19992	Family of unknown function (DUF6427)	PF13642	Protein structure with unknown function
PF19700	Family of unknow function (DUF6198)	PF11554	Protein of unknown function (DUF3232)

PF17461	Family of unknown function (DUF5423)			PF1434	Chemotaxis-inhibiting protein CHIPS
PF14017	Protein of unknown function (DUF4233)			PF11369	Protein of unknown function (DUF3160)

2.4.4 Mycobiome analysis of most common fungal species

To further investigate the microbiome composition and influence of *Trichuris* infection, we conducted an analysis of the mycobiome, with specific attention directed toward common gut fungi, including *Candida* spp*, Aspergillus* spp., *Saccharomyces* spp*,* and *Malassezia* (Figure 2.7B). Candida albicans was the dominant species (Figure 2.7B). The cumulative fungal content, irrespective of species, displayed a higher abundance in the mycobiome of *Trichuris* non-infected individuals (see Figure 2.7A). Although we did not observe beneficial *Saccharomyces boulardii,* known for its positive effects on human health, our findings showed distinct trends. *Candida albicans, Candida dubliniensis* and *Aspergillus fumigatus* were more abundant in *T. trichiura* infected samples than in non-infected samples (Figure 2. 7B). Conversely, *Saccharomyces cerevisiae, Saccharomyces paradoxus* were more abundant in healthy individuals (Figure 2. 7B). No significant differences were observed between groups.

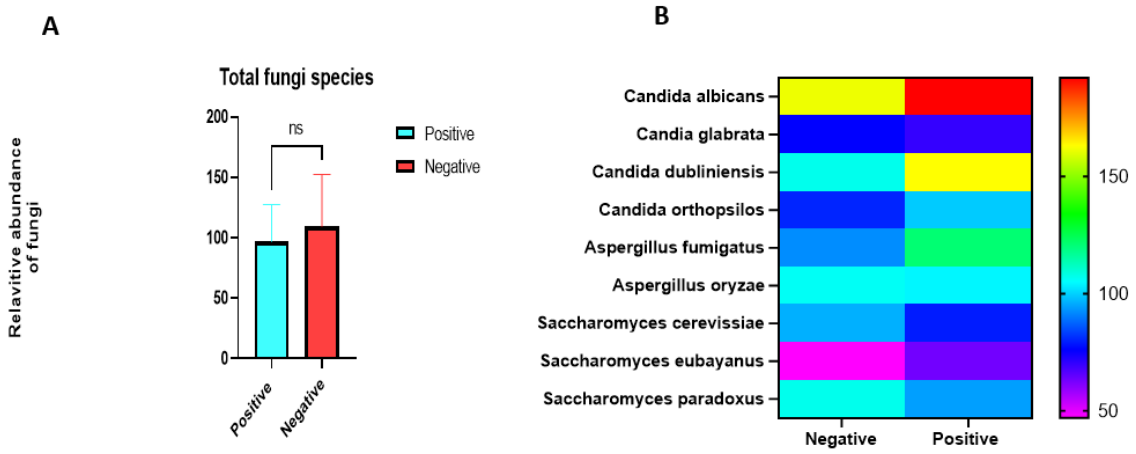


Figure 2. 7. Selective mycobiome analyses. (A): Relative increase of the total fungal species in negative samples (unpaired t test P = 0.4875); (B): Heatmap showing variation of selected common gut fungal species in positive and negative samples; positive and negative refer to *T. trichiura*-infected and non-infected samples, respectively.

2.5 DISCUSSION

This study highlights the distinct nature of gut microbiota composition according to *T. trichiura* infection status. Principal coordinate analysis revealed the subdivision of the samples into two distinct clusters: infected and non-infected. In particular, samples within the latter cluster had a tighter grouping than the *Trichuris* infected samples, implying a higher degree of similarity in their gut microbiota composition. This is in agreement with a previous study, in which significant differences in both alpha and beta diversities were found between helminth-positive and-negative groups (150). Another study demonstrated microbial shifts associated with helminthiases in endemic regions of Thailand, manifested in both faecal and saliva microbiota (156). Another study also explored the interplay between *Trichuris* and gut microbiota, showing interconnections (157). Furthermore, the exploration of potential interactions between *Trichuris* and the gut microbiota revealed that embryonated eggs incubated with gut explants containing caecal bacteria or bacterial cultures of *E. coli* and/or *Staphylococcus aureus,* provided the microbial cues necessary for successful worm larval hatching at the onset of infection. Moreover, investigations on antibiotic treatment prior to *T. muris* infection showed a significant reduction in the gut microbiota, with a consequent decrease in hatching rates. This provides evidence of a symbiotic relationship between *Trichuris* and the gut microbiota, which is crucial for initiating infection (158).

The Firmicutes/Bacteroidetes ratio observed in this study is higher in the microbiota of infected participants in contrast to a lower ratio in individual not affected by the parasite. This ratio has attracted attention as a marker for assessing the health status of gut microbiota and has been extensively investigated in relation to obesity and gut inflammation. Alterations in the dominant phyla Firmicutes and Bacteroidetes depletion have been described in obese animals (159). A calorie-restricted diet for 1 year has shown an increase in Bacteroidetes abundance and the restoration of the Firmicutes/Bacteroidetes ratio (160). For patients with irritable bowel syndrome (IBS), an increased Firmicutes/Bacteroidetes ratio has been reported (161). The divergence in the Firmicutes/Bacteroidetes ratio between *Trichuris*-infected and non-infected

individuals holds potential significance. This finding suggests that modulation of the gut microbiota of infected individuals through dietary interventions can potentially reduce this ratio, promoting the development of a resilient and healthy gut microbiota.

Comparative analysis at the genus level revealed that some SCFA-producing bacteria, *Prevotella*, *Prevotella 9,* and *Ruminococcus*, were relatively decreased in infected individuals and were overrepresented in healthy subjects. Moreover, the *Prevotella/Bacteroides* ratio was higher in healthy samples and lower in *Trichuris* infected samples, which is in agreement with previous research on the same population (150) and another study on the Bantu population in Central Africa (162), where *Prevotella* was found to be the most representative genus in the gut microbiome. *Prevotella* and *Bacteroides* are members of the phylum Bacteroidetes and are known to be driven by different types of diets. A diet rich in carbohydrates is associated with an elevated representation of *Prevotella,* whereas a diet rich in protein and animal fat is associated with an elevated proportion of *Bacteroides* (163). Schneeberger et al. (2022) reported that knowing the pretreatment enterotype or gut microbiota composition can be predictive of anthelminthic treatment outcomes (152)**.** In that study, a *Prevotella* rich enterotype was associated with a lower efficiency of the albendazole-ivermectin treatment. In a context where helminth infection is endemic and the effect of treatments is limited owing to drug efficacy issues (164), the *Prevotella/Bacteroides* ratio could be better investigated with the objective of modulating the gut microbiota to facilitate drug efficacy. Thus, it is possible to investigate the effects on drug treatment by different *Prevotella* species. In this study, *Prevotella copri* appeared to be the dominant species in both infected and non-infected samples.

Differential abundance analysis also revealed that some species known as emergent probiotics, such as *Weissella cibaria*, *Leuconostoc citreum* and *Leuconostoc lactis,* were significantly underrepresented in infected samples and overrepresented in healthy samples. Bacteria belonging to the genus *Weissella* may be important in controlling foodborne diseases through the production of bacteriocins and hydrogen peroxide. This genus has a great potential for use in the food industry (165).

Functional analysis of the metagenomic data showed significant differences in metabolic pathways (P-value < 0.05), such as cholesterol metabolism, pathogenic *E. coli* infection, and human T-cell leukaemia virus 1 infection, which were overrepresented in the infected samples.

An association between gut microbiota and cholesterol metabolism has been reported in the literature. However, there is little evidence on how and which bacteria are involved in this metabolism. Some researchers have reported coprostanol-forming bacteria such as *Eubacterium coprostanoligenes, Bacteroides dorei*, *Lactobacillus sp.,* and *Bifidobacterium* spp. (166–169). According to Lawson et al. (157), helminths require bacteria to hatch eggs. The absence of microbiota (germ-free mice) prevents *Trichuris* hatching and infection, whereas the presence of highly diverse microbiota enhances host susceptibility to infection. Further investigation is required to understand how bacterial metabolism affect*s Trichuris* infections. Differential analysis of the protein families revealed significant differences. This confirms that *Trichuris* infection not only modulates the composition, but also the function of the gut microbiome.

Mycobiome analysis revealed that *Candida albicans* was the dominant species in the population. This agrees with a previous study in which *Candida spp* were the dominant fungal species in the human gut (170). However, Hoffman et al. (171) found *Saccharomyces* to be the most prevalent genus, followed by *Candida* and *Cladosporium*. On the other hand, the discrepancy regarding the abundances of *Candida* and *Saccharomyces* might be simply explained by the fact that fungi are poor gut colonizers, but transient in the gut that rapidly changes according to the diet (171). In a non-human primate study, Barelli et al. (172) found that in red colobus monkeys and yellow baboons, *Trichuris* infection was associated to different gut fungal compositions. These findings suggest that greater attention should be given to gut fungi.

To the best of our knowledge, this is the first human gut microbiome analysis, including functional investigations, in the Zanzibar population, an area with a high prevalence of STH infection. The differences in the Firmicutes/Bacteroidetes and the *Prevotella*/*Bacteroides* ratios suggest that the modulation of the gut microbiota with diet and/or probiotics could be used as complementary approach to fight against helminths infections in Pemba. This approach may also produce a higher efficacy for deworming drug administration.

Although shotgun metagenomic sequencing generates a large amount of data, we must acknowledge that this approach has limitations, such as the missed characterisation of approximately 50% of genomic sequences that remain unannotated, and that they may include viruses and protozoan parasites, for which the contribution to the gut microbiome is less studied

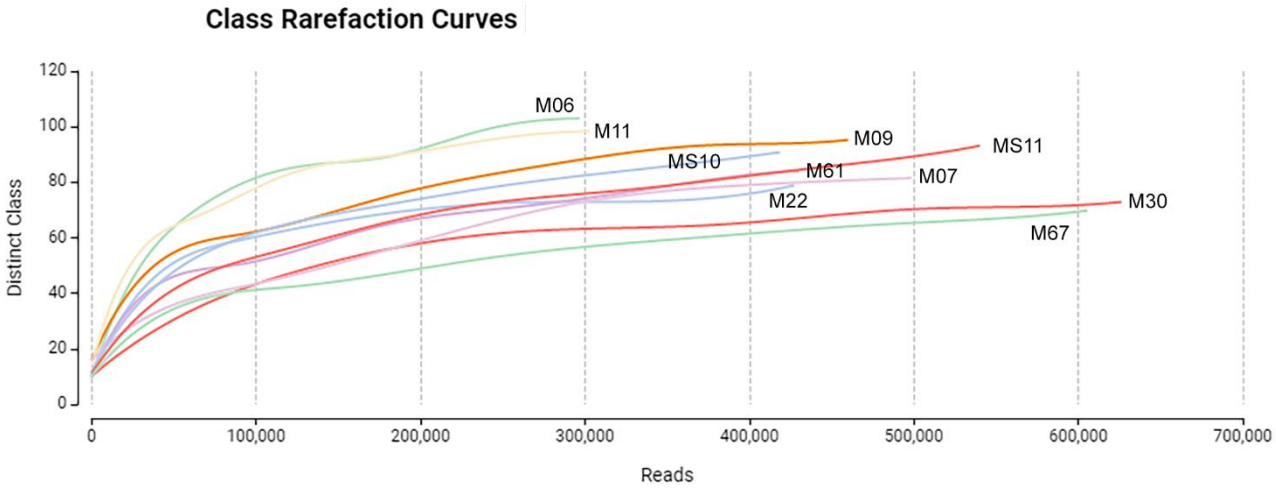
and less present in databases. We cannot anyway exclude that the limitation in the detection of viruses and protozoan parasites may be due to inefficiency of the DNA extraction kit used. Therefore, before approaching the next study, I intend to test several kits with the purpose to obtain a better DNA yield, possibly including more DNA from parasite.

2.6 CONCLUSION

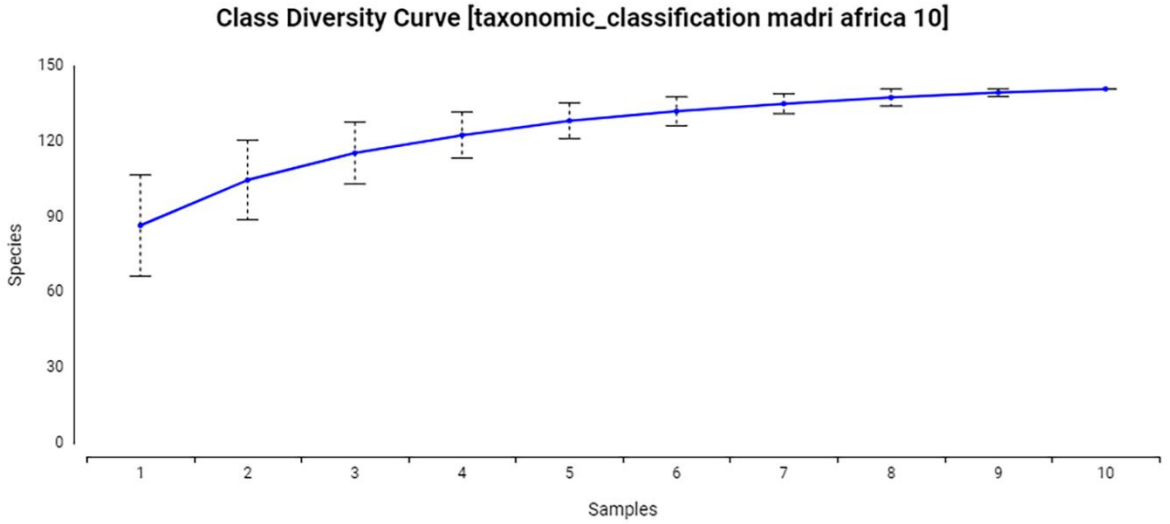
The purpose of this study was to assess the influence of *Trichuris* infection on the composition and function of the microbiome in women of reproductive age using a shotgun metagenomic sequencing approach. Our results confirmed that *T. trichiura* infection significantly shapes the gut microbiome structure and function in WRA. Notably, we identified markers of the gut microbiome health that were changed in infected participants. These indicators can be used as targets for the modulation of gut microbiota in this population. Some taxa known for beneficial characteristics and less abundant in infected participants require further attention in order to see if they can be used as probiotics in prevention or therapeutic support against helminth infection. Therefore, a well-elaborated and sustainable diet with the aim of modulating the gut microbiome and favouring the development of beneficial species can be used as a preventive and complementary approach against *T. trichiura* infection in Pemba and potentially in other areas where this infection is endemic. Since this was a pilot cross-sectional study with a relatively small sample size, a larger cohort investigation with the same population is needed to better understand the results found so far.

2.7 Supplementary Figures and Tables of the second chapter

Supplementary Figures



Supplementary Figure 1: Rarefaction curves depicting the count of distinct class relative to normalized reads across the ten samples (reported as identification code) to check the sequencing depths for all samples.



Supplementary Figure 2: Diversity curve among all samples to evaluate species diversity for the whole dataset.

Supplementary Table 1. Percentage of reads classification in each sequenced sample. Data in red are for non-infected participants.

PERCENTAGE OF CLASSIFICATION							
Sample	Superkingdom	Phylum	Class	Order	Family	Gender	Species
M06	34.78%	33.69%	32.83%	32.59%	30.81%	30.65%	28.80%
M07	43.90%	43.19%	42.59%	42.41%	41.03%	40.96%	39.37%
M09	41.50%	40.71%	40.01%	39.83%	38.36%	38.28%	36.72%
M11	31.50%	30.60%	29.78%	29.43%	28.11%	27.71%	25.69%
M22	45.80%	45.25%	44.68%	44.57%	42.88%	42.93%	41.34%
M30	42.60%	41.67%	41.02%	40.86%	39.22%	39.15%	37.24%
M61	48.32%	47.49%	46.82%	46.70%	45.01%	44.40%	42.40%
M67	50.61%	50.08%	49.57%	49.54%	47.79%	47.86%	45.74%
MS10_L001	36.77%	35.38%	34.57%	34.42%	32.48%	30.49%	25.99%
MS11_L001	55.76%	54.94%	54.45%	54.13%	52.67%	51.95%	47.20%

CHAPTER III: ASSOCIATION BETWEEN FOOD OR NUTRIENTS AND GUT MICROBIOTA IN HEALTHY AND HELMINTH-INFECTED WOMEN OF REPRODUCTIVE AGE FROM ZANZIBAR, TANZANIA

The content of this chapter corresponds to an article that I co-authored with Carrara C, Mozzicafreddo M, Chen H, Piersanti A, Salum SS, Ali SM, and Miceli C (2024), titled "Association between food or nutrients and gut microbiota in healthy and helminth-infected women of reproductive age from Zanzibar, Tanzania." Currently, this article is under review in the journal Nutrients. My contributions to this research encompassed conceptualization, data collection, investigation, formal analysis, methodology, drafting the original manuscript, editing, and visualization.

Additionally, I presented the findings of this chapter during the Second Edition of the "Microbiome: From Benchtop to Bedside" symposium, which convened in Ghent on November 9th, 2023.

3.1 Abstract:

Modulating the gut microbiota is recognised as one strategy for preventing and fighting diseases. While the significant impact of diet on the gut microbiota's composition and function has been extensively researched, there is a notable lack of studies on the interactions between diet, microbiota, and helminth infections. Here, we used a combination of self-reported food intake and a 16S rDNA sequencing approach to analyse the composition of the gut microbiota in women of reproductive age from the two main islands of the Zanzibar archipelago, where helminth infections are endemic. We also applied a Spearman correlation analysis to food/nutrients and gut microbiota. Our results reveal that, despite close ethnic and cultural ties, the participants' gut microbiota differs depending on their location. A nutrient intake analysis revealed deficiencies in minerals and vitamins, indicating an imbalanced diet. A correlation analysis identified bacterial taxa consistently correlated with specific food or nutrients in healthy women from both locations, and in two types of helminth infections. Escherichia/Shigella abundances, usually associated with Trichuris trichiura infection, consistently correlated with insufficient levels of vitamins B2 and B12. In conclusion, our findings suggest that the increased consumption of specific food like cassava and fish, as well

as essential nutrients such as calcium, B vitamins, and vitamin A, may modulate the gut microbiota of populations residing in regions where helminth infections are endemic.

Keywords: nutrients; diet; vitamins; minerals; food and gut microbiota correlations; helminths; *Ascaris*; *Trichuris*

3.2 INTRODUCTION

The composition and role of the gut microbiota are widely acknowledged to have a significant impact on host characteristics, including metabolism, immune function, and defence against infections. Therefore, disturbances in the balance of gut microbiota and the subsequent disruption of host-microbiota interactions are known to be implicated in the development of numerous intestinal and non-intestinal diseases (173). Gut bacteria also play a crucial role in supporting the host's well-being by enhancing their intestinal defence mechanisms and aiding in the maintenance of normal gut functions. They provide various benefits for the host, including the regulation of gut movement, the synthesis of essential vitamins, the conversion of bile acids and steroids, the metabolism of foreign substances, the absorption of minerals, and the activation or neutralization of toxins, genotoxins, and mutagens (174). The gut microbiota also plays an important role in energy harvesting. Thus, Bacteroidetes are recognized to have a positive correlation with reduction in body fat, whereas the positive correlation between Firmicutes and obesity can be associated with a greater energy harvest (175). Hence, the gut microbiota is associated with obesity and other nutrition-related diseases (176), and associations between diet and gut microbiota are being widely researched since diet is recognized to be a key determinant in shaping the composition and function of the gut microbiota (177). According to De Filippis et al. (85), different individuals may have distinctive metabolic responses to the same food, depending on the microorganisms that they harbour. Individuals with a higher *Prevotella*/*Bacteroides* ratio (P/B) experienced greater weight loss after consuming a high-fibre diet for six months compared with the low P/B group, whereas obese individuals with higher levels of *Akkermansia muciniphila* showed better metabolic outcomes (lower insulin resistance and LDL cholesterol) compared to those with a lower baseline concentration of this microbe, when treated with a hypocaloric, high-protein, and high-fibre diet (85). Additional evidence suggests that germ-free rats harvest less energy from a polysaccharide-rich diet, and germ-free mice have a reduced adiposity despite an

increased intake of food in comparison with their colonized counterparts (81). Thus, the gut microbiota is an important environmental factor that affects energy harvest from the diet and energy storage in the host(178). Those findings can justify why correlations between diet and microbiota are widely investigated. Interactions between microbes and vegan, vegetarian, and omnivore diets have been explored in details (179,180). Similarly, the Mediterranean diet has been linked to a healthy gut microbiota and changes in circulating metabolites (82). Studies have shown that strict adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome (181). Some bacterial taxa have thus been associated with specific nutrients, groups of foods, or dietary patterns. It was proven that individuals mainly obtaining nutrients mainly from plant-food sources tended to have a more diverse gut microbiota than individuals obtaining nutrients from animal-food sources (84,182). *Prevotella* was found to be associated with a high consumption of carbohydrates and with both vegetarian and vegan diets, whereas *Bacteroides* was associated with a high consumption of animal protein, amino acids, and saturated fats (80). Despite the widespread adoption of this type of analysis, the presence of abnormalities (183) or inconsistencies in the associations between some taxa and diet makes it difficult to draw conclusions. Moreover, while many studies have explored the associations between diet and the microbiota in the context of obesity (184) or inflammation (185), few investigations have been conducted in the context of infections with soil transmitted helminths (STHs) (186). STHs infect millions of people in subtropical and tropical countries. The infection particularly affects children, resulting in malnutrition, growth stunting, intellectual disability, cognitive deficits and other manifestations (187)**.** In some African endemic regions, such as the Zanzibar archipelago (Tanzania), preventive chemotherapy has been conducted for many years. However, this approach is not effective enough efficacious to control STH morbidity (154). Therefore, finding complementary strategies in the fight against helminth infection is an urgent priority. One approach could be the modulation of the gut microbiota since a relationship between gut microbiota and unsuccessful helminth therapies has been found (150,152,157).

In developing countries, women play a pivotal role in sustaining and nurturing the family unit by procuring and preparing food, as well as providing care to dependent family members, especially children (188). Additionally, the initial colonization of bacteria in the gastrointestinal tract of naturally born infants is primarily attributed to the mother through the faecal-oral route (8). Recognizing the significance of these factors, we argue that a fundamental step in the development of complementary strategies to limit children malnutrition and fight

against helminth infection is understanding how to improve diet and gut microbiota in women of reproductive age.

Consequently, this study aimed to investigate the correlations between diet and gut microbiota in both healthy and helminth-infected women of reproductive age. The research was conducted among women residing in Pemba and Unguja, the two main islands of Zanzibar, where child stunting and malnutrition are monitored by the World Health Organization (WHO). The overarching goal was to identify specific foods or nutrients that may promote the growth of beneficial bacteria or limit the proliferation of harmful bacteria.

3.3 MATERIALS AND METHODS

3.3.1 Ethic statements

This study was approved and authorized by the Zanzibar Health Research Ethical Committee (ZAHREC/03/REC/MARCH/2022/16). After an explanation provided in Swahili with the help of a nurse and a local community worker, all participants accepted and signed an informed consent before enrolment in the study.

3.3.2 Study design and recruitment of participants:

This cross-sectional study was conducted in the Zanzibar archipelago (Tanzania) where helminth infection and malnutrition are endemics. In total, 75 women of reproductive age (WRA) were recruited the islands of Pemba (58 participants), and Unguja (17 participants). From each island, WRA were categorised as either healthy or infected by helminths following microscopical examination. The composition of microbiota was analysed, and a Spearman correlations analysis was applied to analyse food/nutrients and gut microbiota associations.

All participants were between18 and 45 years old, had not taken antibiotics or probiotics within the last two months, had no symptoms of disease. A detailed questionnaire was filled in by the WRA and used for collecting information about their lifestyle, family, health, and nutritional conditions. Finally, nutritional-related anthropometric parameters were measured. Participants meeting the inclusion criteria were provided with stool containers to collect stool samples.

3.3.3 Faecal sample collection and parasitological analysis

We strongly recommended that participants provide stool samples to the health facilities as soon as possible after emission. The contact with the participants was maintained by local

nurses. In both islands, we worked with well-equipped research institutions, specifically the Public Health Laboratory Ivo-de-Carneri (PHL) in Pemba, and the State University of Zanzibar (SUZA) in Unguja. In Pemba, the sample collection was in sanitary centres (equipped with blue ice containers) located directly in the villages where participants were recruited. In Unguja, recruited participants were hosted in the Mnazi Moja Hospital for the regular sanitary checking of their children, and their samples were immediately stored at −20 by the nurses. After collection, stool samples were sent to PHL-IDC in Pemba and to SUZA in Unguja for parasitological analysis. There, each sample was divided in aliquots and stored at −20 °C before shipment to the University of Camerino, Italy, for DNA studies. The Mini–FLOTAC technique was utilized for microscopic examination. Briefly, two grams of stool samples were sufficiently homogenized with the flotation solution (saturated sodium chloride). After homogenization, the samples were added to the two flotation chambers. Finally, after waiting for 10 min, the number of eggs per gram of feces was determined under a microscope. Analytic sensitivity allowed up to ten eggs to be identified per gram of feces. This analysis was repeated twice for each sample by two well-trained laboratory technicians. The shape of the eggs is different and peculiar to each helminth species. Therefore, it was possible to detect the presence of *Ascaris lumbricoides* eggs and *Trichuris trichiura* eggs, or the concurrent presence of eggs from both parasites. After parasitological analysis, the results were delivered to the enrolled female participants to ensure that they could go to the sanitary centre to receive anti-helminth treatment, where necessary.

The sample collection was all performed in about one month, specifically in May.

3.3.4 Recording and evaluation of food and nutrients intake.

All participants were asked to record their food consumption for one full week using a seven-day nutritional table provided by the research team. Then, the Tanzanian food composition tables prepared by the Haward medical school in collaboration with the Tanzanian Ministry of Health were used to evaluate the average intake of food and nutrients (Lukmanji Z et al. 2008; Appendix A). The WHO guidelines for the nutrient profiles of African women (WHO. 2018; Appendix A) was used to evaluate the nutrient profiles of participants. When WHO information was insufficient, we used EFSA recommendations for nutrient intakes of women (EFSA. 2017; Appendix A).

Although the structure of the diet was studied in all 75 participants, as well as the microbiota diversity and taxonomy, we excluded pregnant (12 WRA), obese (11 WRA), undernourished

(3 WRA), and helminth-infected women (17 WRA) for the analysis of food/nutrient–microbiota correlations, to avoid specific conditions that could have an influence on the relationship between food and gut microbiota. One participant from which we could not get complete anthropometric parameters was also excluded from this analysis. The remaining were divided into healthy women from Pemba (18 WRA) and healthy women from Unguja (13 WRA). In addition, to detect the correlations be-tween food and gut microbiota in the presence of helminth infections, we considered 8 WRA infected by Ascaris lumbricoides and 8 WRA infected by Trichuris trichiura (all from Pemba).

3.3.5 DNA extraction, PCR, and sequencing.

DNA was extracted using the QIAamp Fast DNA Stool Mini Kit of QIAGEN. DNA concentration and the ratio of two absorbances (A260/A280) was measured using the NanoDrop™ One/One C Microvolume UV-Vis Spectrophotometer, Thermo Fisher Scientific Waltham, USA.

Before sending the samples for sequencing, all extracted DNA was amplified through the conventional PCR. The primers (150) used for this were the following:

Pro 341F: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG-3′

Pro 805R: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC-3′

The PCR amplified products were run at 120-124 volts for 24 minutes using gel electrophoresis and checked in a UV light room.

Next, 50 ng of purified DNA from each sample was subsequently prepared and sent to the BMR Genomics company (Padova, Italy) for sequencing. Sequencing libraries were generated using the NEBNext® Ultra™ DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendations. Library quality was assessed and sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA).

3.3.6 Bioinformatic and data analysis.

The software QIIME2 (Quantitative Insights into Microbial Ecology, version 2023.5) was used to analyse 16S rDNA gene sequences generated using NGS technologies. Briefly, after filtering out low-quality reads (minimum quality score of 25, minimum/maximum length of 200-250, no ambiguous bases allowed, no mismatches allowed in the primer sequence, and no phiX

reads/chimeric sequences), all remaining sequences were subsequently clustered into Operational Taxonomic Units (OTUs) on the basis of similarity following the DADA2 pipeline and using the QIIME 2 Plugin '*dada2*' version 2023.5.0. Samples were evaluated for alpha diversity (microbial diversity within samples) and beta diversity (community diversity divergence between samples) calculations in QIIME2. We assessed the statistical significance of alpha diversity metrics using a two-sample t-test and a Kruskal-Wallis as implemented in QIIME2. Taxonomic analysis was performed by matching OTU sequences with both the Silva and Greengenes databases. The raw reads were deposited into the NCBI Sequence Read Archive database (SRA accession number: SRP495566, BioProject accession number: PRJNA1088637).

3.3.7 Statistical analysis.

A differential analysis of taxonomy between two groups was performed using STAMP (Statistical Analysis of Metagenomic and other Profiles) software with Welch's t-test (version 2.1.3) and two-sided 95% confidence interval. p-values < 0.05 were considered significant. From each island, WRA were categorised as either healthy or infected by helminths following microscopical examination. The composition of micro-biota was analysed based on the location. The associations between food/nutrient in-takes and gut microbiota were explored using Spearman's correlation analysis, implemented through the 'cor' function of the R system. Then, we converted the correlation matrix into an adjacency matrix, setting a cutoff of 0.3 for both positive and negative correlations by comparing the absolute values. Spearman correlation heatmaps were generated using the R package ggplot2 (https://ggplot2.tidyverse.org/ (accessed on 26 August 2023)). The software GraphPad prism 9.1.5 was used to compare food intake between women from Pemba and Unguja. A Student's t-test was also used and a p-value < 0.05 was considered significant.

3.4 RESULTS

3.4.1 Characteristic of participants

In total, 58 women of reproductive age without any pathological symptoms were recruited from Pemba and 17 women were recruited from Unguja, the most urbanised and touristic island of the Zanzibar archipelago. Details of participant characteristics are reported in supplementary Table S1 and in additional table S2 (Appendix B). Anthropometric parameters are presented in

Table 3.1. The average ages of selected participants were 30.04 ± 6.31 years and 29.79 ±7.36 years in Pemba and in Unguja, respectively; and the average BMI was significantly high in Unguja compared to Pemba (p-value < 0.001). The calorie intakes were nearly equivalent, with 2047 kcal in Unguja and 2076 kcal in Pemba. Consequently, the disparity in BMI can be attributed to variations in other anthropometric parameters, notably height, which differed significantly between the two groups (138.41 ± 16.79 cm in Unguja and 155.89 ± 5.95 cm in Pemba; p-value < 0.001). Ten participants from Pemba were obese and twelve were pregnant. Three of them were underweight. The microscopic examination of stools revealed that six participants exclusively carried *Ascaris lumbricoides,* six carried *Trichuris trichiura,* and two participants had a co-infestation (*Ascaris*/*Trichuris*) in Pemba.

Table 3.1. Anthropometric characteristics of selected participants from Pemba.

Characteristics	Pemba (Mean ± Std)	Unguja (Mean ± Std)
Age (years)	30.04 ± 6.31	29.79 ±7.36
Weight (kg)	59.29 ± 13.30	59.8 ±11.19
Height (cm)	155.89 ± 5.95	138.52 ±16.46
BMI (kg/m^2)	24.34 ± 4.88	32.22 ± 9.13

3.4.2 Structure of the diet of women of reproductive age from Pemba.

The analysis of recorded food intake for one week (Figure 3.1.) revealed that women from Pemba (Figure 3.1A) consume a limited variety of foods: rice, cassava, and bread as sources of carbohydrate. They also consume fish, beans, and vegetables. However, fish consumption was not common among all recruited participants. Other foods consumed by this population are tea, banana, ugali (stiff cassava flour) and porridge. The heatmap of food consumption for women from Pemba revealed that fruits, milk and dairy products, eggs, and poultry are either absent or consumed by very few numbers of participants. Women from Unguja (Figure 3.1B) presented a slightly different trend of dietary habits.

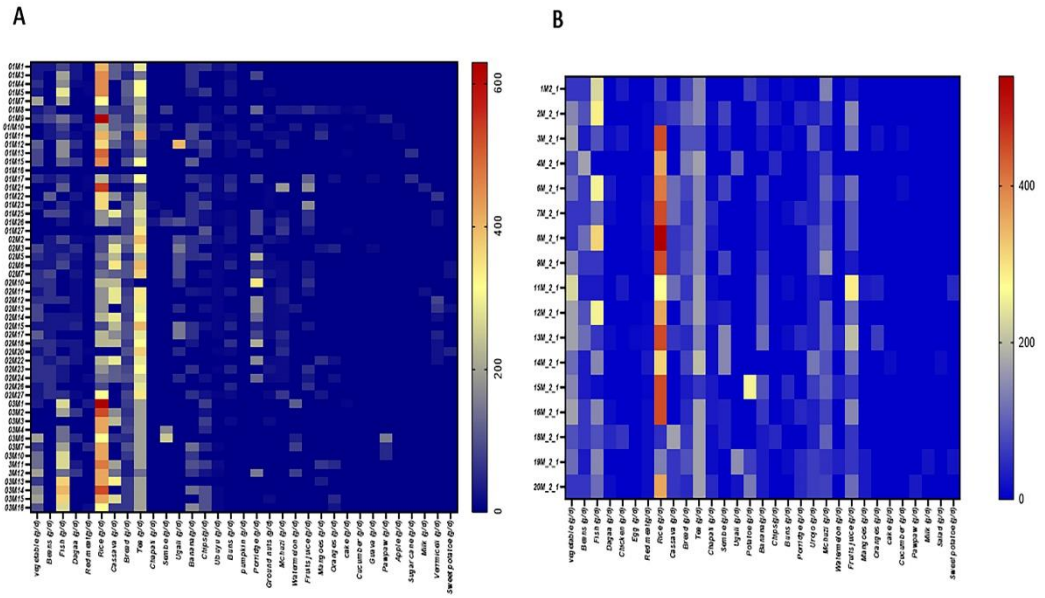


Figure 3.1. Heatmap of the foods consumed by women in the Zanzibar archipelago. (A): Pemba cases. (B): Unguja cases. The food intake is evaluated in term of number gram eaten per day.

To further our understanding of the dietary trends of the two islands, we performed a comparative analysis of the intake of commonly eaten foods. In Unguja, there was a significantly high consumption of beans, vegetables, and red meat, as well as a relative high consumption of fish, banana, and rice (Figure 3.2). Moreover, women from Unguja tended to very frequently consume urojo (also called Zanzibar mix, an energy-dense food characterized by a mixture of many food items) and fruit juice. They also eat significantly less cassava than women from Pemba. Although there was a low consumption of dagaa (an affordable, small, dried fish available in both Islands), there was a relatively higher consumption of this food in Pemba than in Unguja. In general, it appears that, despite the availability of a wide variety of foods, women from Pemba and Unguja had a limited consumption of fruits, poultry, and eggs, and almost no consumption of milk and dairy products.

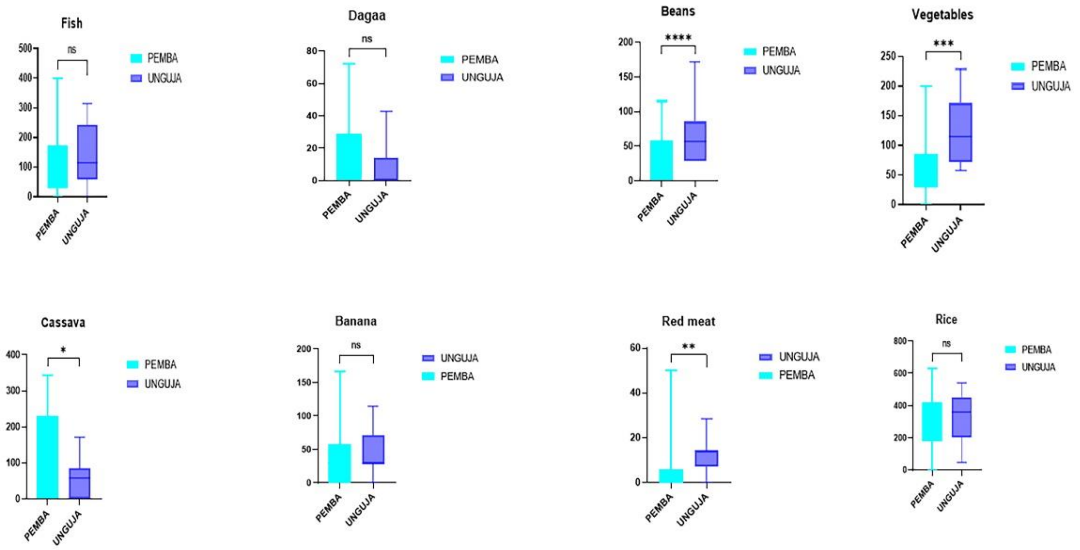


Figure 3. 2. Comparative analysis of the average intakes (in gram) of the most consumed foods in Zanzibar. An unpaired t-test was used. Fish (p-value = 0.331), dagaa (p-value = 0.096), beans (p-value < 0.0001), vegetables (p-value =0.0001), cassava (p-value = 0.015), banana (p-value = 0.418), red meat (p-value = 0.004), rice (p-value = 0.448), bred (p-value = 0.754). A p-value < 0.05 was considered as significant. * means p-value < 0.05; ** means p-value < 0.01; *** means p-value < 0.001; **** means p-value < 0.0001; ns means p-value > 0.05.

3.4.3 Analysis of nutrient intake for Women in Pemba and Unguja

Following the WHO guidelines for the nutrient intake of African women (WHO. 2018; Appendix A) and the EFSA guideline for micronutrients (EFSA. 2017; Appendix A), we calculated that many women achieved the necessary intake of macronutrients, specifically for proteins, fats, and carbohydrates (supplementary Table S3; Appendix B). The average protein intake in grams per day is shown in Table 3.2 with the corresponding recommended nutrient intake value (RNI), and the percentage of women who achieved this in the two islands. The intake of fibre did not reach the 25 grams per day as recommended, in both Pemba and Unguja women (37.20% and 17% in Pemba and Unguja women, respectively).

Concerning vitamin intakes (Table 3.2), fewer than 50% of women from Pemba reached the recommended intake for all vitamins except for vitamin B1 (thiamine), with 69.81% of women reaching the daily RNI in Pemba. The situation was worse for vitamin B2 (riboflavin) and vitamin B5 (pantothenic acid), the percentage of participants reaching the daily RNI was 0% for both vitamins. Only 29.30 % and 37.73% of women reached the RNI of vitamin A and folate, respectively. In Unguja, there was a relatively improved condition regarding some

vitamins. The recommended intake was reached for vitamin A, vitamin C, vitamin B1, and folate by 76.47%, 52.94%, 94.11%, and 88.23 % women, respectively. For vitamin B2, no participant reached the RNI. A comparison between the two islands revealed that there was a relatively high intake of vitamins in Unguja compared to Pemba, except for vitamin B12, the RNI of which was reached by the same 41% of women on both islands.

Table 3.2. Macro and micronutrient intakes of women from Zanzibar archipelago. Recommended micronutrients are established according to the WHO guidelines for the nutrient profiles of African women that define the ranges for each nutrient intake per day. Macronutrients are measured in grams(g) while micronutrients are measured in milligrams (mg) or micrograms (µg).

Nutrient	Intake (Pemba) (g/d)	Intake (Unguja) (g/d)	p value	RNI (g/d)	% of women reaching the RNI (Pemba)	% of women reaching the RNI (Unguja)
Protein (g)	67.59 ± 21.19	76.87 ± 19.46	0.114	50 – 75	84.61	88.24
Fat (g)	72.59 ± 26.95	71.16 ± 18.68	0.809	33.33 – 66.66	96.15	88.24
Carbohydrates (g)	290.84 ± 52.85	274 ± 60.23	0.340	275 – 375	75	58.82
Fibres (g)	18.94 ± 7.82	21.60 ± 5.29	0.122	25 g/d	37.20	17
Vitamin A (ug)	499.89 ± 467.43	1124.12 ±449.41	0.0002	650ug/d	28.30	76.47
Vitamin E (mg)	4.4 ± 1.77	6.91 ± 4.40	0.034	11mg/d	0.0	11.76
Vitamin C (mg)	85.23 ± 44.28	91.54 ± 41.30	0.594	95mg/d	43.39	52.94
Vitamin B1 (Thiamine) (mg)	0.91 ± 0.23	1.08 ± 0.21	0.007	0.83mg/d	69.81	94.11
Vitamin B2 (riboflavin) (mg)	0.78 ± 0.22	1.04 ± 0.23	0.0005	1.6mg/d	0.0	0.0
Vitamin B3 (Niacin) (ug)	8.34 ± 3.13	10.17 ± 2.69	0.026	14ug/d	7.54	11.76
Vitamin B6 (pyridoxine) (mg)	1.18 ± 0.36	1.55 ± 0.47	0.006	1.6mg/d	11.32	29.41
Folate (ug)	264.79 ± 109.07	428.21 ± 95.90	<0.0001	330ug/d	37.73	88.23
Vitamin B12 (Cobalamin) (ug)	2.76 ± 2.53	2.55 ± 1.61	0.696	2.4 ug/d	41.50	41.17
Vitamin B5 (pantothenic acid) (mg)	2.51 ± 0.83	4.25 ± 2.75	0.019	5 mg/d	0.0	17.64

Calcium (mg)	512.34 ± 360.05	599.45± 219.83	0.014	950 mg/d	13.20	11.78
Magnesium (mg)	323.51 ± 112.05	367.85 ± 68.07	0.056	300 mg/d	49.05	94.11
Iron (mg)	10.81 ± 5.01	12.25 ± 2.57	0.128	16 mg/d	13.20	5.88
Phosphorus (mg)	1258 ± 451.03	1253.49±336.45	0.963	550 mg/d	94	100
Zinc (mg)	7.12 ± 2.26	7.64 ± 1.39	0.262	7.5 mg/d	41.50	47.05
Copper (mg)	1.46 ± 0.60	1.49 ± 0.39	0.804	1.3 mg/d	50.94	70.64
Potassium (mg)	2574.25 ± 825	2912.70 ±545.41	0.059	3500 mg/d	15	17.64
Manganese (mg)	5.15 ± 2.12	4.58 ± 1.11	0.162	3.0 mg/d	88.67	94.11

Mineral intake was higher than vitamin intake. The average intake reached the RNI for magnesium, phosphorous, copper and manganese in both islands. However, looking at the proportion of participants reaching the RNI, many women did not take enough calcium (13.20% in Pemba and 11.78 in Unguja), iron (13.20 % in Pemba and 5.88% in Unguja) and potassium (15% in Pemba and 17.64% in Unguja).

3.4.4 Women from Pemba and Unguja harbour different gut microbiota

The analysis of the 16S rDNA sequencing data revealed that the gut microbiota of Zanzibar women differs according to their location (Pemba and Unguja) (Figure 3.3), despite being ethnically and culturally close, and there is a regular flow of the population between the two islands, as well as genetic connections due to mixed marriages. The alpha diversity (Figure 3.3A) was statistically different regarding the number of observed OTUs, as well as the Shannon, Evenness_pielou and phylogenetic diversity indexes. The beta diversity (Figure 3.3B), that is, the qualitative measure of community dissimilarities incorporating phylogenetic relationships between different groups or different ecological environments, also revealed a significant difference regarding the Unweighted UniFrac distance (P = 0.032 *).

The composition of the gut microbiota at the phylum and genus levels was different according to the locations. The differential analysis of taxonomy considering the effect size using the STAMP software showed that at the phylum level (Figure 3.3C), Bacteroidetes and Desulfobacterota were significantly more abundant in the gut microbiota of women from Unguja (Zanzibar), while Cyanobacteria and Proteobacteria were significantly more present in the gut microbiota of women from Pemba**.**

The analysis of taxonomy at the genus level (Figure 3.3D) showed that *Blautia*, *Catenibacterium*, *Intestinibacter*, and *Romboutsia* (all members of the Firmicutes), as well as *Collinsella*, *Gastranaerophilales*, *RF39*, and *Sutterellla* were significantly more present in Pemba. On the other hand, members of the Bacteroidetes phylum, specifically *Prevotella*, *Rikenellaceae_RC9_gut group*, and some members of the Firmicutes phylum, specifically *Dorea*, *UCG-002, UCG-003*, were more abundant in Unguja. Despite the high diversity found in Unguja, we noticed that *Escherichia-Shigella,* belonging to the Proteobacteria was more abundant there.

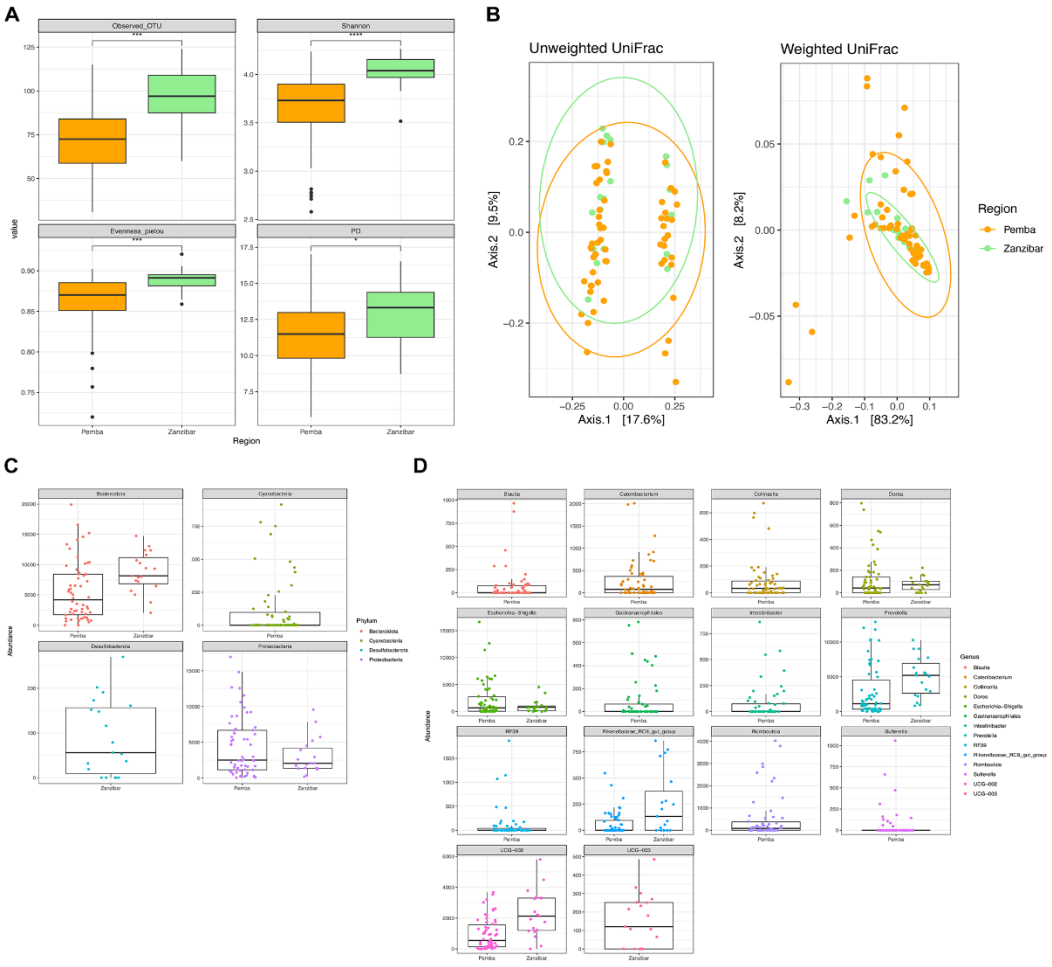


Figure 3. 3. Diversity and taxonomy analyses of the gut microbiota of women of reproductive age from the Zanzibar archipelago according to the location. (A): Alpha diversities; (B): Beta diversities (p-value = 0.032 * for the Unweighted UniFrac, p-value = 0.776 for the Weighted UniFrac). (C): Taxa that significantly changed at the phylum level. (D): Taxa that significantly changed at the genus level. In this figure, Zanzibar refers to Unguja Island. If only one bar is

shown in the plot, it indicates that the values for the other variables are all zero. * means $p \leq 0.05$; *** means $p \leq 0.001$; **** means $p \leq 0.0001$.

3.4.5 Correlations analysis of foods and gut microbiota in non-pregnant and healthy women from Pemba and Unguja

Spearman's correlation analysis between the food and gut microbiota of healthy participants revealed consistent correlations in both Pemba (Figure 3.4A) and Unguja (Figure 3.4B). We can underline some of these relevant correlations, such as *Clostridium sensu stricto* (potential protective bacteria), which was positively correlated with cassava in Pemba and Unguja; *Faecalibacterium,* encompassing a major actor of human intestinal health; and the specie *Faecalibacterium prausnitzii,* consistently and positively correlated with vegetables in both locations. On the other hand, *Escherichia/Shigella* (known to favour the *Trichuris* infection) was positively correlated with banana and negatively correlated with cassava in both contexts.

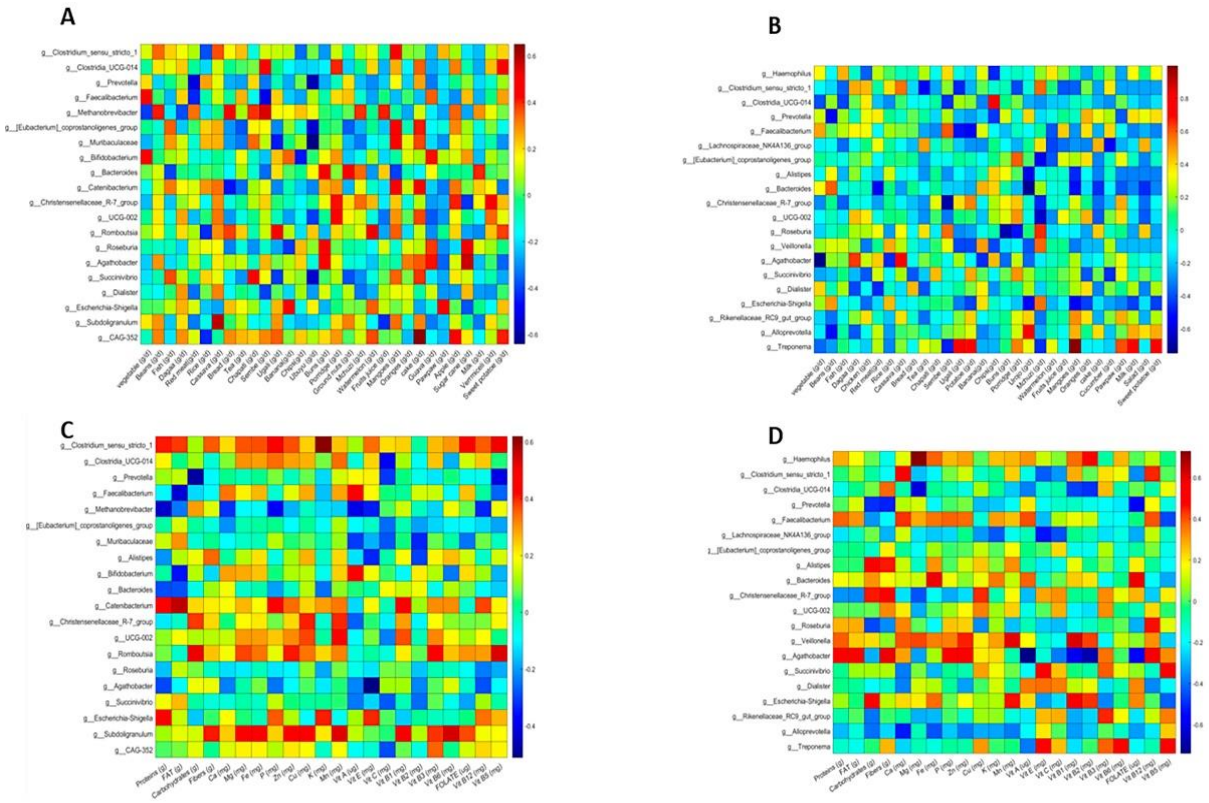


Figure 3. 4. Heatmaps showing food-gut microbiota correlations in non-pregnant and healthy women in Pemba (A) and Unguja (B). Heatmaps of nutrients and gut microbiota correlations in Pemba (C) and Unguja women (D).

Moreover, we noticed that some correlations were only found in one location. Among correlations found only in Unguja (Figure 3.4B): *Haemophilus* which correlated positively with

fish and negatively with chips, and *Treponema,* which was positively correlated with mango and negatively correlated with sembe (corn flour stiff porridge) and vegetables. Likewise, some associations were found only in Pemba (Figure 3.4B), such as *Methanobrevibacter;* which positively correlated with sembe, bread, and beans, and negatively correlated with vegetables and dagaa, and *Subdoligranulum*, a potential, protective butyrate-producing bacterium, which was positively correlated with cassava, beans, and negatively correlated with red meat, milk, and fruit juice in Pemba women.

Furthermore, we noticed that there were some bacteria that had different or inconsistent associations with the same food item according to the location. For example, *Clostridia_UCG-014* was positively correlated with sembe (corn flour stiff porridge) in Pemba (Figure 3.4A), but it showed an opposite correlation with the same food in Unguja (Figure 3.4B). Likewise, *Faecalibacterium* and sembe were negatively correlated in Pemba, but they showed a positive correlation in Unguja. This suggests that the correlation between gut microbiota and diet may vary according to the host environment or context.

3.4.6 Nutrients and gut microbiota correlations in non-pregnant and healthy women from Pemba and Unguja.

The analysis of the associations between nutrients and gut microbiota in non-pregnant and healthy women showed consistent correlations in both locations and some inconsistent correlations (Figure 3.4C and 3.4D). Firstly, as consistent correlations, *Clostridium_sensu_stricto-1* positively correlated with vitamin B12 in both islands indicating that increasing the intake of vitamin B12 may favour the development of this taxa; in the same way, *Faecalibacterium* was consistently positively correlated with calcium and vitamin A; and *UCG-002* was consistently positively correlated with copper. Unexpectedly, *Prevotella* was negatively correlated with carbohydrates and fibre in both locations.

On the other hand, we found correlations that were observed in only one location. In healthy women from Unguja (Figure 3.4D), *Haemophilus* was positively correlated with magnesium, iron, and vitamin B2; *Clostridium_sensu_stricto-1* was strongly positively correlated with calcium in Unguja. In Pemba (Figure 3.4C), *Bifidobacterium* had a strong positive correlation with vitamin A. It was also positively correlated with calcium and magnesium, and negatively correlated with fats. Only in Pemba, we observed that *Subdoligranulum* was positively

correlated with fibre, as well as several minerals (Mg, Fe, Zn, Cu, Mn) and vitamins (B1, B3, B6.)

Finally, we searched for inconsistent correlations and noticed that some associations vary depending on the location. For example, *Clostridium_sensu_stricto-1* was positively correlated with vitamin E in Pemba but had a negative correlation in Unguja; similarly, *UCG-002* and vitamin B1 were positively correlated in Pemba (Figure 3.4C) but negatively correlated in Unguja (Figure 3.4D).

3.4.7 Correlation analysis of foods and gut microbes in helminth-infected participants

Since it has been demonstrated that helminth infection has an impact on the composition of the gut microbiota, we conducted a teste to determine whether the food/nutrients-microbiota correlations were altered in the guts of infected women. Figure 3.5 reveals new consistent associations between bacteria and foods in *Ascaris* (Figure 3.5A) and *Trichuris* (Figure 3.5B) infections. Amidst those correlations, we noticed that *Prevotella*, the most abundant genus of the African population was positively correlated with banana and negatively associated with vegetables in both types of helminth conditions; *Eubacterium coprostanoligenes_group* was invariably positively correlated with cassava and negatively with buns (bread rolls). *Bacteroides*, one the most abundant genus of Bacteroidetes, was consistently positively correlated with porridge and vermicelli. *Escherichia/Shigella*, an undesirable genus since it is generally associated with *Trichuris* infection, was consistently and negatively correlated with banana and dagaa in both *Ascaris* and *Trichuris* infection conditions.

We also looked for correlations found only in one or the other helminth condition. In *Ascaris* infection, The *Lachnospiraceae-NK4136-group* was strongly and positively correlated with sembe (stiff corn flour made porridge) and negatively correlated with ugali; *Subdoligranulum* was positively correlated with fish and cassava in *Ascaris* infection (Figure 3.5A). In the context of *Trichuris* infection (Figure 3.5B), we noticed that *Bifidobacterium*, a well-characterised genus in children and healthy gut microbiota, was positively correlated with beans, vegetables, porridge, and oranges, and negatively correlated with buns and ubuyu; other relevant associations were as follows: *Alloprevotella,* which was positively correlated with dagaa, bananas, oranges and negatively correlated with breads and ground nuts, *Subdoligranulum,* which was positively correlated with beans, porridge, and oranges in the same context.

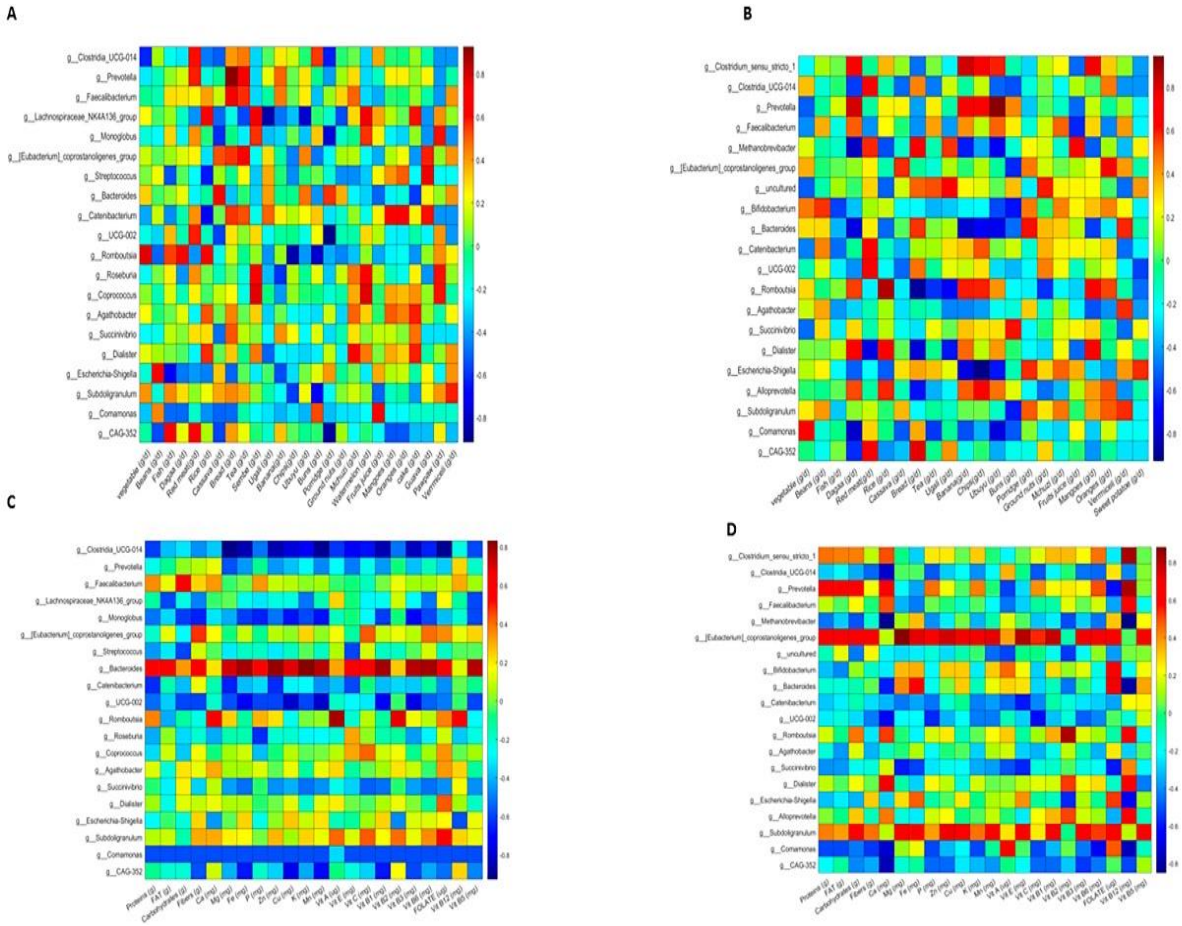


Figure 3.5. Heatmaps of the correlations between food or nutrients and gut microbiota in infected participants. (A): food-microbiota correlations in Ascaris lumbricoides-infected participants. (B): food-microbiota correlations in Trichuris trichiura-infected participants. (C): nutrient-microbiota correlations in Ascaris lumbricoides-infected participants, (D): nutrient-microbiota correlations in Trichuris trichiura-infected participants.

3.4.8 Correlation analysis of nutrients and gut microbes in helminths infected participants

Finally, we analysed the Spearman's correlations of nutrients and microbiota in *Ascaris* and *Trichuris*-infected participants (Figures 3.5C and 3.5D) and observed the existence of new and consistent correlations. Here, *Faecalibacterium* which was positively correlated with calcium as in healthy participants maintained this correlation and showed a new correlation with vitamin B12 in both conditions. The *Eubacterium coprostanoligenes-group* was positively and consistently correlated with fibres, vitamin C, vitamin B6, and folate in both *Ascaris* and *Trichuris*-infected participants. We also observed that *Bacteroides* was positively and

consistently correlated with iron under both conditions. Interestingly, *Escherichia-Shigella* was negatively and invariably correlated with vitamin B12 and vitamin B2 indicating that a supplementation of these vitamins may be useful for limiting the growth of *Escherichia-Shigella* which is usually associated with *Trichuris* infection. We also noticed that *Subdoligranulum* presented more positive correlations with minerals and B vitamins, suggesting the affinity of this genus with healthy diets rich in micronutrients.

On the other hand, some correlations were found either only in *Ascaris* or only in *Trichuris* infection. As examples of correlations appearing only in *Ascaris* infection (Figure 3.5C), *Roseburia* was positively correlated with vitamin E and negatively correlated with phosphorus; *Coprococcus* was positively correlated with vitamin E, vitamin C, and folate, and negatively correlated with protein, carbohydrates, and phosphorus.

Similarly, some correlations were observed only in the context of *Trichuris* infection, as follows: *Clostridium sensu_stricto-1* (Figure 3.5D) was positively and strongly correlated with vitamin B12 and negatively correlated with iron; *Bifidobacterium*, in the same context, was positively correlated with folate and vitamin A, and it was negatively correlated with fat and protein; *Alloprevotella* appeared to positively correlated with calcium, vitamin B2 and vitamin B12, and negatively correlated with vitamin E.

An unexpected finding was that the *Prevotella* genus, an important member of the core microbiota of the African population, showed unusual patterns of correlations. It showed strong positive correlations with protein, fat, carbohydrates, and vitamin B12 in *Trichuris* infection (Figure 3.5D).

3.5 DISCUSSION

The analysis of the diets of Zanzibar women revealed that food they consume is not diverse enough, despite the availability of several varieties of locally produced foods, such as fruits and vegetables. According to the World Health Organization, eating plenty of vegetables and fruits is recommended since they are important sources of vitamins, minerals, dietary fibre, plant protein, and antioxidants; people with diets rich in vegetables and fruit have a significantly lower risk of obesity and other diseases. Moreover, the low or absent consumption of milk products should be considered not favourable for the health of this population, since milk and dairy products are recognised as nutrient-dense foods, supplying energy and high-

quality proteins with a range of essential micronutrients (189). Milk and dairy products can play important roles in human nutrition in developing countries, where the diets of poor people frequently lack diversity and the consumption of animal-source foods may be limited (190).

Since their diet was not diversified enough, most women of Pemba and Unguja reach the recommended nutrient intakes (RNI) for macronutrients but have serious deficiencies in vitamins and minerals. Participants had significant deficiencies regarding the recommended intake of B vitamins (B2, B3, B5, B6, and B12). The RNI of vitamin A was not reached. The importance of B-vitamins is clear, they are precursors of essential cofactors used in many metabolic pathways. This makes them essential for the development of the host and gut microbiota. Mammalian hosts are not able to produce vitamin B de novo. Therefore, they are dependent on the intake from diet and gut microbiota activity (191). In research using in silico analysis, Rodionov et al. showed that 20-30% of gut bacteria lack the capacity to produce essential B-vitamins (192). This deficiency may therefore affect not only host health in general but also the gut microbiota.

The deficiency in minerals is worrying, specifically calcium, iron, and potassium. Only 13.20 % and 5.88 % of women reached the daily RNI of iron in Pemba and Unguja, respectively. This condition has been a concern in Zanzibar since 2018, when the prevalence of anaemia among women ranged from 37.5% in Stone Town to 49.2% in Pemba South (see Tanzania National Nutrition Survey 2018; Appendix A). It has been demonstrated that iron deficiency impacts not only the host, but also microbiota efficiency (193) . A previous study conducted on rats showed that iron-deficient rats had considerably lower concentrations of butyrate and propionate (194). The deficiency in calcium intake is also critical, since calcium the is essential for the activities of muscles, nerves, and blood vessels and the release of hormones that affect many other functions in the human body, and for the gut microbiota function. Chaplin et al. found that calcium acts in a prebiotic manner to influence the gut microbiota in mice (195). Calcium-fed animals exhibited increased levels of *Bifidobacterium* spp., *Bacteroides,* and *Prevotella*. Whisner et al. found that the daily consumption of galacto-oligosaccharides increases calcium absorption, which may be mediated by the gut microbiota, specifically by *Bifidobacterium* (196).

The alpha and beta diversity of the gut microbiota of women from Pemba and Unguja differ according to the location even though they are ethnically close. This agrees with the study by

Mehta et al., which demonstrated that residence location was associated with differences in gut microbiome composition in Indian adults (55). This difference can be explained by the possible influence of the environment (197), more rural in one case, or the difference in their diets (84). We found that women from Unguja (Figure 1B) had slightly different dietary habits with some specifications. They had significantly high consumption of beans, vegetables, and red meat, and relatively high consumption of fish, banana, and rice (Figure 2). Moreover, they tend to consume urojo very frequently (an energy dense food made from a mixture of many food items) and fruit juice compared to women from Pemba. They also eat significantly less cassava compared to women from Pemba.

The relative abundance analysis, which revealed that Bacteroidetes were more abundant in Unguja, can be explained by their diet being more varied than in Pemba participants. At the genus level, the dominant taxa in Pemba compared to Unguja are mostly members of the Firmicutes phylum (*Blautia, Romboutsia, Intestinibacter and Catenibacter*), while in Unguja, the most dominant taxa are either from the Bacteroidetes (*Prevotella* and Rikenellaceae_RC9_group) or the Firmicutes (*Dorea*, *UCG-002*, *UCG-003*). Our findings agree with a study conducted by Elsherbiny et al. in which the abundance of many genera was strongly dependent on geographical location (54).

Spearman's correlation analysis showed that some bacterial taxa were consistently associated with the same food or nutrients in healthy women from both locations or in both types of helminth infection. Many studies demonstrated that diet influences the composition (198) and function (199) of the gut microbiota. In our study, *Clostridium sensu stricto*-1, which was previously negatively associated with *Trichuri*s infection in the studies of Rosa et al. (121) and Cooper et al. (200), was consistently and positively correlated with cassava in both Pemba and Unguja. It was also consistently and positively correlated with vitamin B12 for both islands. This indicates that increasing consumption of cassava, rich in dietary fibre, can favour the development of this taxa. Additionally, vitamin B12 may also increase the abundance of *Clostridium sensu stricto*-1. Vitamin B12 (Cobalamin) uptake by *Bacteroides thetaiomicron* has been observed to be able to limit shiga toxins, acting as an immunomodulator to promote cellular immunity (191). Likewise, *Faecalibacterium*, recognised as a producer of short chain fatty acid (SCFA), was consistently and positively correlated with vegetables, vitamin A, and calcium in healthy women from both locations but conserved only the positive correlation with calcium in both *Ascaris* and *Trichuris* infection, suggesting the strong affinity of this genus

with calcium. The prebiotic properties of calcium have been recognized in humans and mice (201). Importantly, *Escherichia-Shigella,* which is associated with *Trichuris* infection (121,152,157), was invariably and negatively associated with cassava for both locations, indicating that the increased consumption of this fibre reach food may limit the proliferation of this taxa and consequently reduce infections. This also justify why we found a high abundance of *Escherichia-Shigella* in the microbiota of women from Unguja who consume significantly less cassava compared to women from Pemba. Moreover, we noticed that *Escherichia-Shigella* was also negatively correlates with vitamin B2 and vitamin B12 in both *Ascaris* and *Trichuris* infections. A preliminary study showed that supplementation of riboflavin (vitamin B2) increased *F. prausnitzii* and concomitantly reduced *Escherichia-Shigella* in a small group of adults (202). Another relevant observation was the positive correlation of *Eubacterium coprostanoligens* with cassava and negative correlation with buns in healthy women. *Eubacterium coprostanoligens* was found to be part of the enterotype that favourably respond to the Albendazole and Ivermectin-based treatment (152). This means that an increase in the abundance of *Eubacterium* may be helpful in fighting against helminths for these population. Likewise, *Bacteroides,* which is usually not associated with *Trichuris* infection in human (150,203,204) was positively correlated with porridge and iron in both infections. Our findings here are in agreement with a study on Korean adolescents, which also found that *Bacteroides* was associated with iron, especially plant iron (182). *Subdoligranulum*, which might contribute to resistance to helminth infection in self-clearing individuals (120,123) was positively correlated with cassava, beans, porridge, and vermicelli. It was also positively and strongly correlated with magnesium, zinc, copper, vitamin B1, vitamin B3, and folate. This suggests that *Subdoligranulum* abundance is an indicator of a balanced micronutrient diet.

Moreover, the results of Spearman's correlation analysis also revealed that helminths may affect the relationships between diet and gut microbiota in a helminth-specie dependant manner. This was illustrated by correlations that were only found in either *Ascaris* infections or *Trichuris* infection. It has already been demonstrated that helminth infection influences the response of the gut microbiota to dietary interventions (205). Our analysis revealed that this influence may differ according to the helminth species.

Finally, the *Prevotella* genus, an important member of the gut microbiota associated with African diets (206) which predominantly contain carbohydrates (163), showed a consistent

positive correlation with bananas and negative correlation with vegetables. Unexpectedly, our analysis revealed that the association between diet and this genus is not as simple as originally thought. *Prevotella* genus, in our investigation, consistently correlated negatively with carbohydrates and fibre in both locations. A study conducted by De Filippis et al. (183) revealed that some *Prevotella* oligotypes were significantly associated with plant-based diets while others were associated with animal-based nutrients. This highlights the importance of considering the sub-genus level when studying the diet-microbiota interactions.

Our study revealed that, although diet/nutrients and gut microbiota associations can vary based on several factors such as the host health and environment, associations between bacteria and food/nutrients that are consistent in healthy women from both locations meant that the increase consumption of specific foods and nutrients can modulate the gut microbiota and potentially improve host health by either increasing good bacteria or limiting the proliferation of unwanted bacteria that promote helminth infections.

However, this study had some limitations, such as the relatively limited number of participants and the fact that the dietary data were derived from self-reported food intake. Correlations observed between food and genera are insufficient since we know that, in the same genus, different species or strains may present different metabolic responses to the same foods. Moreover, this study analysed the associations, but not the causation relationships between food/nutrients and microbiota. Furthermore, the differences in microbiota composition according to origin of participants, as well as the differences in pattern of association between diet and microbiota depending to location, limit the potential to globalise our findings.

3.6 CONCLUSION

This study This study used a combination of self-reported food intake from women of reproductive age and the 16S rDNA sequencing approach to evaluate diets, nutrient intakes, the gut microbiota composition, and its correlations with food or nutrients for women living on the two main islands of the Zanzibar archipelago, where helminth infections are endemic. We found that, despite the availability of many locally produced foods, the diet of women of reproductive age in Zanzibar was not diverse enough. Consequently, most participants had vitamin and mineral deficiencies, particularly for B vitamins, vitamin A, and calcium. This

sufficiently demonstrated the need for the nutritional education of the population on the composition of a balanced diet. The analysis of correlations between food/nutrients and gut microbiota revealed consistent associations between food or nutrients and gut bacteria. Associations between specific food, such as cassava, beans, dagaa, fish, and porridge, lead us to presume that in-creasing the consumption of these food items with the supplementation of B vitamins, vitamin A, iron, and calcium can support the growth of beneficial bacteria or limit the proliferation of undesirable bacteria, and thus make the participants more resistant to helminth infections. Of course, we cannot ignore the socioeconomical conditions of the populations and the difficulties to obtain micronutrient supplementation. However, we had evidence that the specific food that we recommend after this investigation is sustainable for the population living in villages in both islands.

A nutritional intervention in a large cohort with the same population is required to further support our understanding and to confirm results obtained in this cross-sectional study.

3.7 Supplementary Figures and Tables of the third chapter

Additional table S1. Details of characteristics of participants from Pemba.

Code	Region	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Helminth	Ascaris	Trichuris	EPG
01M_11A_1	Pemba	32	52.6	152.5	22.5	No	No	No	0
01M_11B_1	Pemba	32	52.6	152.5	22.5	No	No	No	0
02M_10A_1	Pemba	30	48	150	21.33	No	No	No	0
02M_10B_1	Pemba	30	48	150	21.33	No	No	No	0
02M_11A_1	Pemba	24	47	156	19.31	No	No	No	0
02M_11B_1	Pemba	24	47	156	19.31	No	No	No	0
02M_19B_1	Pemba	35	73.4	153.2	31.18	No	No	No	0
03M_16A_1	Pemba	42	67	155.4	27.74	No	No	No	0
03M_16B_1	Pemba	42	67	155.4	27.74	No	No	No	0
03M_3B_1	Pemba	23	60	155.7	24.75	No	No	No	0
03M_15B_1	Pemba	38	83.7	151	36.7	No	No	No	0
01M1B	Pemba	30	75	158	30.04	TT	No	Yes	60
01M3A	Pemba	26	45.3	158	18.14	No	No	No	0
01M4B	Pemba	34	75	149	33.78	No	No	No	0
01M5B	Pemba	23	63.3	163	23.82	No	No	No	0
01M7B	Pemba	20	68.3	168	24.19	TT	No	Yes	50
01M8B	Pemba	37	65	154.2	27.4	AS	Yes	No	40
01M9B	Pemba	32	68	162.5	25.91	No	No	No	0
01M10B	Pemba	27	67	157	27.18	No	No	No	0
01M12B	Pemba	35	67	167	24.02	No	No	No	0
01M13B	Pemba	25	45.4	146.6	21.11	No	No	No	0
01M15B	Pemba	40	68	151	29.82	AS	Yes	No	70
01M16B	Pemba	27	43	151.2	18.85	No	No	No	0
01M17B	Pemba	27	43	155	17.89	No	No	No	0
01M21A	Pemba	27	46	147	21.28	No	No	No	0
01M22B	Pemba	20	60.3	162	24	No	No	No	0
01M23B	Pemba	38	76	158	30.44	No	No	No	0
01M25B	Pemba	25	52	153	22.21	No	No	No	0
01M26B	Pemba	25	67	163	25.21	No	No	No	0
01M27B	Pemba	23	61	158.1	24.43	No	No	No	0
02M2A	Pemba	33	82	168	31.24	AS/TT	No	No	60&100

02M3A	Pemba	35	75	153	32.03	AS	Yes	No	110
02M5A	Pemba	31	45	146	21.11	AS	Yes	No	90
02M6B	Pemba	21	60	154	25.3	TT	No	Yes	30
02M7B	Pemba	23	86	156	35.33	No	No	No	0
02M12B	Pemba	32	49	161.6	18.9	AS/TT	No	No	20&40
02M13B	Pemba	30	56.3	156.3	23.01	No	No	No	0
02M14B	Pemba	28	77	157.3	31.23	No	No	No	0
02M15B	Pemba	30	43	151	18.85	No	No	No	0
02M17B	Pemba	32	50	158	20.02	No	No	No	0
02M18B	Pemba	38	53	153	22.64	No	No	No	0
02M20B	Pemba	40	57	155.3	23.72	No	No	No	0
02M22A	Pemba	28	47	153.4	20.07	No	No	No	0
02M23B	Pemba	37	42	146.6	19.43	No	No	No	0
02M24A	Pemba	27	86	162.5	32.58	TT	No	Yes	50
02M26A	Pemba	30	69	160	26.95	AS	Yes	No	150
02M27A	Pemba	22	50	160	19.53	TT	No	Yes	180
02M28A	Pemba	25	54	158	21.63	No	No	No	0
03M1B	Pemba	40	NA	NA	NA	No	No	No	0
03M2B	Pemba		53	155.5	21.91	No	No	No	0
03M4B	Pemba	28	79	166	28.66	No	No	No	0
03M6B	Pemba	37	40	147	18.51	AS	Yes	No	360
03M7B	Pemba	45	76.5	166	27.76	No	No	No	0
03M10B	Pemba	22	50	162	19.05	No	No	No	0
03M11B	Pemba	27	65	163	24.46	TT	No	Yes	170
03M12A	Pemba	28	38	141	19.11	No	No	No	0
03M13A	Pemba	20	39.1	151	17.14	No	No	No	0
03M14A	Pemba	30	56	155	23.3	No	No	No	0

Additional table S2. Details of characteristics of participants from Unguja. Nd=Not defined

Code	Region	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Helminth	Ascaris	Trichuris	EPG
1M_2	Unguja	26	44.5	148	20.31	No	No	No	0
2M_2	Unguja	32	73	158	29.24	No	No	No	0
3M_2	Unguja	36	60.5	135	33.19	No	No	No	0
4M_2	Unguja	24	61.7	120	42.85	No	No	No	0
6M_2	Unguja	20	35.1	136	18.98	No	No	No	0
7M_2	Unguja	36	60.5	139	31.31	No	No	No	0
8M_2	Unguja	23	52	119	36.72	No	No	No	0
9M_2	Unguja	27	76	120	52.78	No	No	No	0
11M_2	Unguja	45	55	120	38.19	Yes	No	Yes	Nd
12M_2	Unguja	38	72	121	49.18	No	No	No	0
13M_2	Unguja	27	55	126	34.64	Yes	No	Yes	Nd
14M_2	Unguja	25	58.4	137	31.12	No	No	No	0

15M_2	Unguja	25	60	154	25.3	No	No	No	0
16M_2	Unguja	45	80	159	31.4	No	No	No	0
18M_2	Unguja	32	54	153	23.07	No	No	No	0
19M_2	Unguja	22	74	174	24.44	No	No	No	0
20M_2	Unguja	24	56	134	29.84	No	No	No	0

Additional table S3: Macronutrients profiles for woman from Pemba and Unguja

Nutrients. Recommended intakes		% PEMBA	% UNGUJA	% TOTAL
Proteins (g) 50 -75	Normal	48.07	29.41	43.48
	Above	36.53	58.82	40.02
	Below	15.38	11.76	14.50
Fats (g) 33.33 -66.66	Normal	42.30	47.06	43.48
	Above	53.84	52.94	53.62
	Below	3.85	0	2.90
Carbohydrates(g) 275 – 375	Normal	69.23	52.94	65.21
	Above	5.77	5.88	5.79
	Below	25	41.18	29

CHAPTER IV: ANALYSING THE INTERPLAY BETWEEN HELMINTH INFECTIONS, NUTRITIONAL STATUS, AND GUT MICROBIOTA IN CHILDREN AND WOMEN OF PEMBA, TANZANIA

I wrote this chapter for preparing a manuscript for journal submission. Its content is still under discussion with the following researchers as collaborators: Mozzicafreddo M, Carrara C, Piersanti A, Salum SS, Ali SM, and Miceli C. Furthermore, the findings presented in this chapter were also featured as a poster, with a poster pitch presentation that I did at the Microbiome Interactions in Health and Disease 2024 conference, organized by Wellcome Connecting Science (WCS), held at Hinxton Hall Conference Centre, Wellcome Genome Campus, Hinxton, Cambridge, UK, from February 14th to February 16th, 2024. My contribution to this research, in addition to writing the first draft, was conceptualization, data collection, investigation, formal analysis, and methodology.

4.1 Abstract:

Malnutrition and soil–transmitted helminth infections in sub–Saharan Africa persist besides the efforts made in frame of the Sustainable Development Goal to end malnutrition by 2030. Inadequate understanding of effective intervention methods contributes to the limited success. Research on the gut microbiota's role in health, mainly conducted in developed nations, lacks in low-income countries making it an urgent matter to expand studies on underprivileged populations' gut microbiota to tackle these public health issues. Here, we employed 16S rDNA sequencing to assess the composition of the bacterial gut microbiota in children and women from Pemba, considering both helminth infection and nutritional status. Our analysis of differential bacterial abundance revealed certain taxa not linked to either helminth infection or malnutrition. Crucially, some of these taxa showed a negative association with both helminth infection and malnutrition, suggesting the potential for microbiota-directed interventions to cope with these health issues simultaneously. Taxa like *Akkermansia, Haemophilus*, *Blautia, Dorea*, and *Odoribacter* show promise for microbiota-directed interventions, potentially interfering with both health problems. This study indicates that microbiota-directed interventions in children and among women of reproductive age, in conjunction with established approaches, deserve attention as promising avenues for combating helminth infection and malnutrition.

Key words: Malnutrition, Stunting, Wasting, Underweight, Helminth, *Ascaris, Trichuris Trichiura*, gut microbiota, children, women of reproductive age.

4.2 INTRODUCTION

Malnutrition remains one of the important world health challenges pointing to the immense challenge of achieving the Sustainable Development Goal (SDG) that aims to eliminate hunger by 2030. Millions of children under five years of age continue to suffer from stunting, wasting and overweight (207). Along with the increase proportion of undernourished people, there is also increasing prevalence of overnutrition leading to obesity and related health problems. This is called the double burden of malnutrition (208).

Growth faltering in children in the form of stunting, a sign of chronic malnutrition, and wasting, an indicator of acute malnutrition, are common among young children in low- and middle-income countries (LMIC) and may contribute to child mortality and adult morbidity. Three out of five subregions with high rates (more than 30%) of child stunting are found in sub-Saharan Africa: western Africa, middle Africa and eastern Africa (208).

In the specific case of Tanzania, in 2018, the level of stunting was considered "very high" (≥30%) in 15 regions out of 26. In Zanzibar, the 2018 survey indicated prevalences of 21.5 %, 5.3 %, 13.6% for stunting, wasting and underweight respectively **(*Tanzania National Nutrition Survey (TNNS) 2018).***

Another public health challenge that rages in developing countries and particularly in sub-Saharan Africa is the soil transmitted helminth (STH) infection. STH infections impact on human health, nutrition, and worker productivity and hence aggravate poverty (116). Parasitic infections have been recognized for decades to be major public health problems in Zanzibar (119). Many programs against parasite infections have been going on in that area with encouraging results in reducing the intensity and the morbidity due to helminth infections. Unfortunately, the ultimate goal of eliminating the STH infections has not yet been achieved (154).

The above-mentioned public health problems are interconnected with synergistic consequence (127). Helminth infections have been reported to play an important role in malnutrition by causing protein-energy malnutrition, anaemia and physical complications as a result of increased nutrient squandering, blood loss, intestinal obstruction and rectal prolapse (209).

Many studies report that soil-transmitted helminths infection are significantly associated with under-nutrition (115,129,210). On the other hand, being malnourished is considered as a risk factor for intestinal parasite infection (130). Another important point to mention is that malnutrition has an intergeneration character (211). Research suggests that the impact of stunting on development continues in the next generation of children (212). Thus, intervention in young age and in childbearing age women is the key for stopping this vicious circle.

The modest effects of interventions to prevent malnutrition may be due to an incomplete understanding of the most effective way and timing of these interventions (213). With the development of the high throughput sequencing tools and approaches, the study of the gut microbiota and its connection with healthy condition and disease is now widely adopted. Therefore, the gut microbiota has been recognized to play important role in nutritional conditions (214,215). Studies revealed that malnourished children have distinct microbiome compared to healthy counterparts (216,217). The gut microbiota of children who are undernourished is usually immature, that is, more similar to the microbiota of younger children than to that of age-matched healthy controls (218). Laursen et al. demonstrated that inadequate maturation of the gut microbiota is associated with poor growth and development in early life (43). These findings suggest a causal relationship between the immature gut microbiota from undernourished infants and impaired growth phenotypes. This was illustrated by the fact that the transfer and invasion of healthy microbiota into the guts of the undernourished mice by co-housing restored normal growth in the latter (126). Hopefully, some interventional studies targeting the gut microbiota had positives outcomes. One experimental study using the probiotic *Lactobacillus plantarum* revealed that the gut microbiota enhances sensitivity to growth hormone (219). Similarly, Michael et al. found that restoring *Bifidobacterium infantis* to the gut of malnourished infants boosts weight gain and reduces inflammatory markers (220). Furthermore, we (150,221) and others have already demonstrated that helminth infections shape the gut microbiota composition and its function (123). Helminth colonization in the gut require support from the gut microbiota. Helminth eggs hatching is supported by some gut microorganisms such *Escherichia/Shigella* and *Salmonella typhimuriun* (157,158,204). On the other hand, success of the chemotherapy again helminth infection may be dependent of the composition of the gut microbiota prior to treatment (152).

Despite increasing interest in the study of gut microbiota and its associations with health and disease, still the research in the less developed countries remain scarce. Browne et al. (222) reported that around 85% of the 25,000 high-resolution gut metagenomes from children under

four that are publicly available come from individuals living in wealthy regions (Europe and Nord America). According to the research, the gut microbiota vary according to regions where people live (55,206), creating an emergency to study the microbiota of less favoured populations for the effectiveness of any microbiota directed interventions involving them.

This study was conducted in area with vulnerable population to malnutrition and parasite infections with a twofold objective. Firstly, it aimed to explore how helminth infection affects the gut microbiota of mothers and children post-deworming, aiming to identify potential probiotic candidates or intervention targets. Given shared environments and diets between mothers and children, we could compare the impact of helminth infections on both groups. Secondly, it sought to analyse the link between nutritional status and gut microbiota to identify potential protective bacteria as candidate probiotics and potential targets in combating malnutrition.

4.3 MATERIALS AND METHODS

4.3.1 Ethic statements

This study was authorized by the Zanzibar Health Research Ethical Committee (ZAHREC/03/REC/MARCH/2022/16) and all participants signed the informed consents for themselves and their children to participate in the study.

4.3.2 Study design and recruitment of participants:

This cross-sectional study was conducted in Zanzibar archipelago (Tanzania) where helminth infection and malnutrition are endemics. A total of 58 women of reproductive age (WRA) previously included in a study that analysed correlations between foods or nutrients and the intestinal microbiota (223), and 60 children were recruited from three health facilities in Pemba. Given that mothers and children share environments and dietary habits—children often consuming the same foods as their mothers within the age range considered in our study— we could observe and compare the impact of helminth infections on both groups. All participants were either healthy or infected by helminths after microscopical examination of their stool samples. A comparative analysis of their gut microbiota was done considering the helminth infection and the nutritional status.

All participants were 18 to 45 years old for mothers, and 1.5 to 3 years old for children, not taking antibiotics or probiotics within the last two months, not presenting any symptoms of disease. A detailed questionnaire was filled in by WRA for collecting information about their lifestyle, family, health, and their nutritional conditions. Nutritional-related anthropometric parameters were measured. Participants meeting the inclusion criteria were provided with stool containers to collect stool samples.

4.3.3 Faecal sample collection and parasitological analysis

After collection, stool samples were sent and processed at the Public Health Laboratory Ivo De Carneri (PHL-IDC) in Pemba for parasitological analysis. Each sample was divided in aliquots and stored at -20°C before shipment and for the DNA extraction at the University of Camerino, Italy. The Mini–FLOTAC technique was used for microscopic examination. Briefly, two grams of stool samples were homogenized sufficiently with the flotation solution (saturated sodium chloride). After homogenization, the samples were added to the two flotation chambers. After 10 min, the numbers of eggs per gram of feces were determined under a microscope. Analytic sensitivity could reach ten eggs per gram of feces. This analysis was repeated twice for each sample by two well trained laboratory technicians. After the parasitological analysis, results were delivered to the enrolled participants to ensure that they could receive from the PHL-IDC team or go to the sanitary centre to receive anti-helminth treatment**.**

4.3.4 Recording and evaluation of nutritional conditions.

With the assistances of nurses, anthropometric parameters such as height, weight, abdominal circumference were collected for women and their children. We used the WHO indicators to evaluate the nutritional conditions of children (**WHO. 2024. malnutrition-in-children**). For children, these indicators are defined as follows:

- *stunting* - height-for-age <-2 SD of the WHO *Child growth standards* median,
- *wasting* - weight-for-height <-2 SD of the WHO *Child growth standards* median,
- *overweight* - weight-for-height >+2 SD of the WHO *Child growth standards* median,
- *underweight* - weight-for-age <-2 standard deviations (SD) of the WHO *Child growth standards* median.

For women (**WHO. 2024. Malnutrition in women)**, the nutritional conditions were evaluated based on their BMI values as follows:

- BMI <18.5: underweight; BMI 18.5-24.9: normal weight; BMI ≥25.0: overweight; BMI ≥30.0: obesity.

4.3.5 DNA extraction, PCR, and sequencing.

DNA was extracted using the QIAamp Fast DNA Stool Mini Kit of QIAGEN. DNA concentration and absorbance of each sample was evaluated by using the NanoDrop™ One/One C Microvolume UV-Vis Spectrophotometer.

Before sending the samples for sequencing, all the extracted DNA was amplified through the conventional PCR. The primers (150) used for the purpose were:

Pro
341F: 5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCA G-3′

Pro
805R:5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCT AATCC-3′

The PCR amplified product was run by the gel electrophoresis at 120-124 volts for 24 minutes. Then checked in UV light room. 50 ng of purified DNA of each sample was subsequently prepared and sent to the BMR Genomics company (Padova, Italy) for the 16S sequencing. Libraries were generated using the NEBNext® Ultra™ DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendations. Library quality was assessed and sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA).

4.3.6 Bioinformatic and data analysis.

The software QIIME2 (Quantitative Insights into Microbial Ecology, version 2023.5) was used to analyse 16S rDNA gene sequences generated from NGS technologies. Briefly, after filtering of low-quality reads (minimum quality score of 25, minimum/maximum length of 200-250, no ambiguous bases allowed, no mismatches allowed in the primer sequence, and no phiX reads/chimeric sequences), all remaining sequences were subsequently clustered into Operational Taxonomic Units (OTUs) based on their similarity (>97%) following the DADA2 pipeline and using the QIIME 2 Plugin 'dada2' version 2023.5.0. Samples were evaluated for

alpha diversity (microbial diversity within samples) and beta diversity (community diversity divergence between samples) calculations in QIIME2. We assessed the statistical significance of alpha diversity metrics by two-sample t-test and Kruskal-Wallis as implemented in QIIME2. Taxonomic analysis was performed by matching OTU sequences with both the Silva and Greengenes databases.

4.3.7 Statistical analysis.

Differential analysis of taxonomy between groups was performed using STAMP with Welch's *t*-test (version 2.1.3). *p*-values < 0.05 were considered as significant. A student t-test was also used with P < 0.05 considered as significant.

4.4 RESULTS:

4.4.1 Characteristics of participants

Participant characteristics are summarised below in **Table 4.1** and detail are found in the supplementary table 1 and supplementary table 2 for WRA and children respectively. A total of 58 WRA and 60 children were scrutinized for their nutritional condition and carriage of helminth parasites. The parasitological analysis revealed a prevalence of helminthiasis of 24.13% and 33.33% in WRA and children, respectively. Notably, these individuals had undergone treatment with anti-helminthic drugs just one to two months prior to stool collection. This suggests either a failure of the treatment or a fast reinfestation.

Analyses on the nutritional conditions of participants revealed a prevalence of obesity of 16.66% in mothers and a prevalence of children malnutrition (all types taken together) of 56.66 % in Pemba.

The investigation of a link between helminth infection and malnutrition revealed an increased odds of being stunted (OR = 1.21; 95% CI 0.4 to 3.65) with *Trichuris trichiura* infection. Similarly, there was an elevated risk of underweight status associated with *Trichuris* infection (OR = 2.333; 95% CI 0.70 to 7.75) among children from Pemba.

Table 4.1. Characteristics of participants

Features	Mothers (Pemba)	Children (Pemba)
Number of participants	58	60

Mean age	30.03 ±6.31 (years)	26.63 ±6.36 (months)
Mean BMI (mothers)	24.34 ± 4.88 (kg/m2)	/
Helminth infected	14	20
Ascaris infected	8	5
Trichuris infected	8	18
Co-infected	2	3
Obesity (mothers)	16.66% (10/60)	/
Underweight (mothers)	6.66 % (4/60)	/
Overall children malnutrition	/	56.66% (34/60)
Stunting	/	46.66 % (28/60)
Wasting	/	23.33% (14/60)
Underweight Children	/	26.66 % (16/60)

4.4.2 Study of the associations between helminth infection and bacterial abundances

The analysis of diversity of the gut microbiota according to the helminth infection revealed no significant differences in the alpha and beta diversity of WRA and children gut microbiota analysed a short time after deworming (**see additional figure 1**). Since previous analyses (…) on the same population with stool collection at almost one year from the drug treatment, revealed a modulation of the gut microbiota diversity in the infected individuals with respect to non-infected. The present finding suggests that the administration of the anthelminthic drug may have contributed to the modulation of gut microbiota reducing the differences between infected and uninfected individuals.

However, the examination of the taxonomic composition differences between infected and non-infected children and WRA, on the base of the advanced statistical analysis with STAMP, revealed four taxa negatively associated with *Ascaris* infection in both WRA and children: *Akkermansia*, *Haemophilus*, *Alloprevotella*, and Verrucomicrobiota phylum (see Table 4.2). *Akkermansia*, typically associated with a healthy gut microbiota and metabolic activity, is a member of the Verrucomicrobiota phylum. Additionally, several other genera showed significantly different abundances based on helminth infection status. In children, seven other

taxa including two members of the clostridia class (*Eubacterium ruminatium* and *Flavonifractor),* and the genus *Streptococcus,* which includes the commonly used probiotic *Streptococcus thermophilus* were more abundant in healthy children compared to *Ascaris*-infected children. Similarly, in WRA from Pemba (**Table 4.2**)*,* six other taxa among which three belonged to the Clostridia class *(Eubacterium eligens, Lachnospira* and *UCG-005), Odoribacter,* and *Phascolarctobacterium* were also more abundant non-infected participants than in those infected with *Ascaris.* These results suggest the potential significance of the Clostridia class in microbiota targeted approach to fight *Ascaris* infection.

Looking at the association between *Trichuris trichiura* and gut microbiota of WRA and children, we identified notable differences. In children, four taxa belonging to the Clostridia class (*Eubacterium_coprostanoligens*, *Christenellaceae_R_7* group, *Clostridia viadin BB60_*group, and *UCG-002*, along with *Paraprevotella* from the Bacteroidia class were more abundant in the non-infected group. Conversely, 16 taxa, including *Eubacterium eligens*, *Eubacterium siraeum*, *Ruminococcus torques*, *Blautia*, *Lachnospiraceae NK4A136*, *UCG-005*, *Roseburia*, and *Odoribacter* (which was also more abundant in non-infected children by *Ascaris* lumbricoides), were more prevalent in non-infected WRA (Table **4.2**). Interestingly, the genus *Blautia* has been found negatively associated with *Trichuris* infection (150) in a study conducted in the same population in 2018. This suggests it potential utility as candidate probiotics or targets for microbiota-directed therapy.

Table 4.2. Taxa that are significantly more abundant in healthy participants compared to *Ascaris* or to *Trichuris* infected children and women. The analysis was done using the software STAMP that considers the effect size. $p < 0.05$ was considered as statistically significant.

Healthy vs *Ascaris* (children)	P. value	Healthy vs *Ascaris* (Mothers	P. value	Healthy vs *Trichuris* (children)	P. value	Healthy vs *Trichuris* (mother)	P. value
Eubacterium ruminatium	0.015	*Eubacterium eligens*	<0.001	*Eubacterium _coprostano ligens*	0.045	*Eubacterium eligens*	<0.001

Akkermansia	0.015	*Akkermansia*	0.020	*Christensenellaceae_R_7 group*	0.030	*Eubacterium siraeum*	<0.001
Alloprevotella	0.034	*Alloprevotella*	<0.001	*Clostridia viadin BB60*_group	0.034	*Ruminococcus torque*	0.014
Haemophilus	0.020	*Haemophilus*	<0.001	*Klebsiella*	0.048	*Barnesiella*	0.019
Megamonas	<0.001	Lachnospira	0.0037	*Paraprevotella*	0.034	*Bilophila*	0.048
Barnesiella	0.012	*Odoribacter*	0.029	*UCG-002*	0.034	*Blautia*	0.013
Clostridia UCG-014	0.027	*Phascolarctobacterium*	0.037			*Butirivibrio*	0.024
Erysipelotrichaceae UCG-003	0.017	*Sutterella*	<0.001			Cyanobacteria	<0.001
Flavonifractor	0.017	*UCG-005*	0.024			Desulfobacterota	0.042
Streptococcus	0.035	Verrucomicrobiota	0.020			*Eryipelotrichaceae UCG-003*	<0.001
Verrucomicrobiota	0.012					Gastranaerophilale	<0.001
						Klebsiella	<0.001
						Lachnospiraceae NK4A136	<0.001
						Odoribacter	0.015
						Roseburia	0.015
						Sutterella	0.017
						UCG-005	0.025

In conclusion, this study revealed that the light helminth infection detected a short time after deworming did not significantly affect the diversity of the gut microbiota of children and women from Pemba, but it did lead to a significant shift in the abundance of specific taxa.

4.4.3 Analysis of the diversity of the gut microbiota according to nutritional status.

We conducted inquiries regarding the feeding practices of children and found that all children included in this study had been breastfed, with nearly all of them (50 out of 60) having ceased breastfeeding entirely by the time of sample collection. Breastfeeding is a prevalent practice for infant nutrition in this region. We then proceeded to analyse the gut microbiota diversity in children based on their nutritional status. The diversity analysis uncovered no significant difference in alpha and beta diversity based on children nutritional status. However, alpha diversity was relatively lower in cases of stunting and underweight conditions, suggesting potential disparities in taxa abundance (**Figure 4.1**). Similarly, the analysis did not reveal a significant difference in alpha and beta diversity between the gut microbiota of obese and non-obese WRA (**additional Figure 2**).

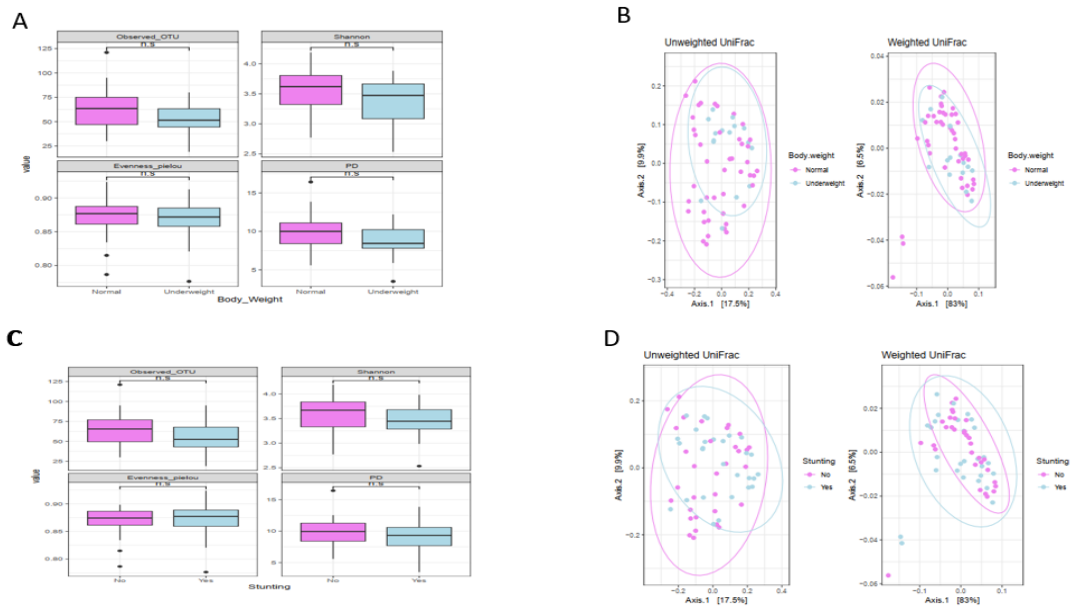
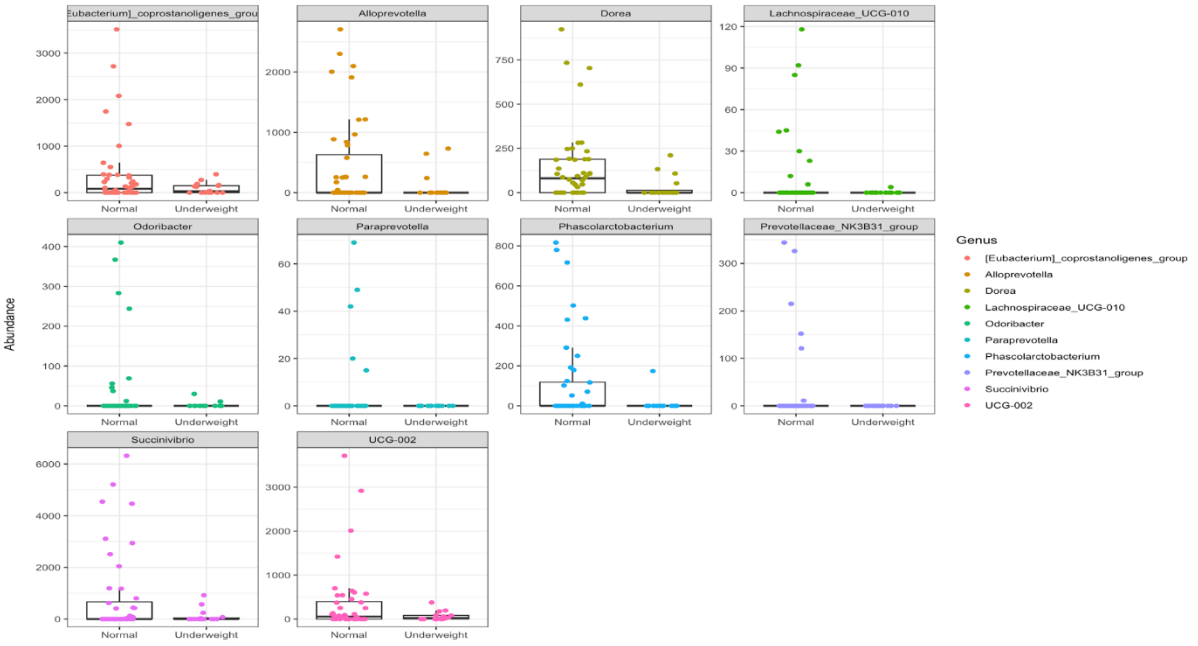


Figure 4.1. Analysis of the diversity of the gut microbiota of children from Pemba**.** (A) Alpha diversity according to the body weight, (B) Béta diversity according to the body weight, (C) Alpha diversity according to stunting; (D) Béta diversity according to stunting. “No” refers to “no stunting” while “Yes” indicates “stunted group.”

4.4.4 The taxonomic analysis revealed differences in the abundance of taxa based on nutritional conditions.

The analysis of the microbiota based on the **bodyweigh**t of children **(Figure 4.2A)** showed that ten genera were significantly more abundant in children with normal weight compared to the underweight group. Most of these taxa belonged to the Bacteroidia class such as *Prevotellaceae_NK3B31*_group (p = 0.03), *Alloprevotella* (p = 0.039), *Paraprevotella* and *Odoribacter* (p = 0.039), or to the Clostridia class: *Dorea* (P= 0.00923), *Eubacterium_coprostanoligenes_group* (P= 0.019), UCG-002 (p = 0.015), and *Lachnospiraceae_010* (p = 0.019). Importantly, some of these taxa were also found negatively associated with helminth infection in this study: *Alloprevotella* which were negatively associated with Ascaris; *Eubacterium_coprostanoligenes_group, UCG-002 and Paraprevotella* which were negatively associated with *Trichuris* infection. Therefore, it can be inferred that, a gut microbiota therapeutic approach can be utilised to address both health challenges of helminth infection and malnutrition in children.

A



B

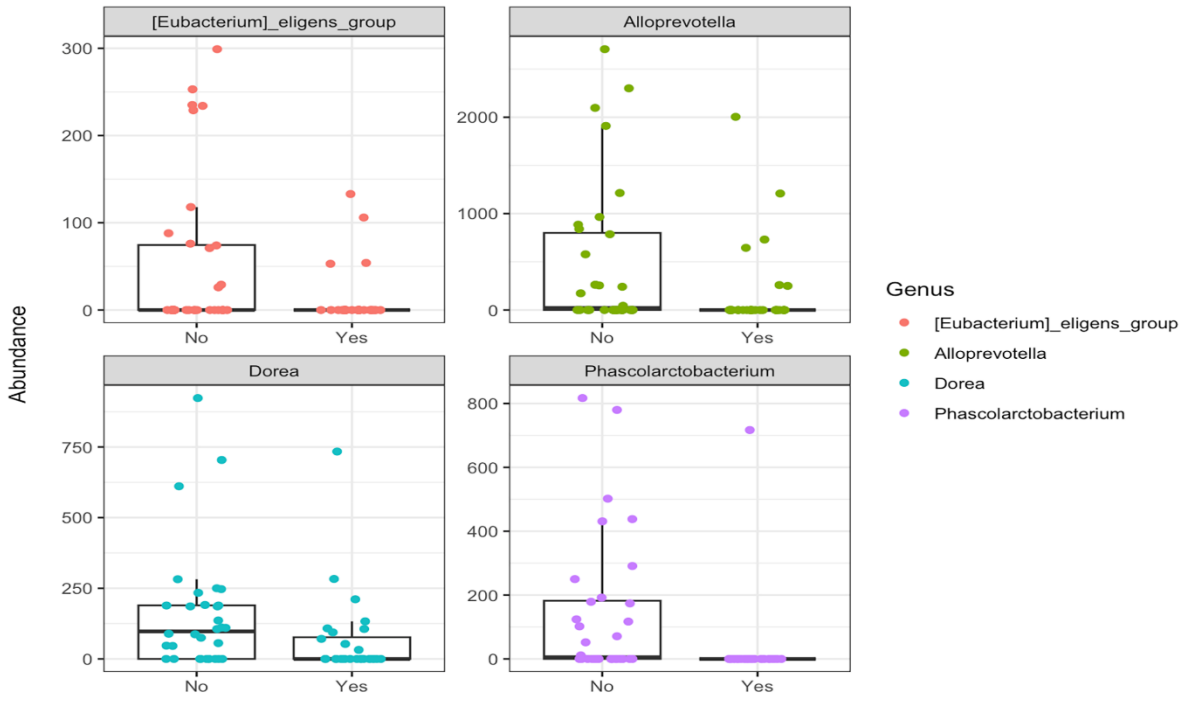


Figure 4.2. Differential abundance of taxa considering the body weight (A) and stunting (B) of children from Pemba. "**No**" refers to "no stunting" while "**Yes**" indicates the "stunting group."

Secondly, we conducted a comparative analysis of taxonomy between children with stunting (growth retardation) and those with normal growth. Our analyses identified four taxa that were significantly more abundant in children with normal growth than in the stunted group (see **Figure 4.2B**). The genus *Dorea* (p = 0.022), which was also found to be less abundant in underweight children, was notably less abundant in stunted children, suggesting its potential as an indicator of healthy nutritional conditions in children (normal weight and height). *Alloprevotella* (p = 0.041), less abundant in *Ascaris*-infected children, was also found to be lower in stunted children but significantly more abundant in non-stunted children. Additionally, two other genera, *Phascolarctobacterium* (p = 0.012) and *Eubacterium_eligens_group* (p = 0.021), showed similar patterns of abundance.

Thirdly, we analysed the taxonomy regarding the **wasting** condition in children (see **Additional figure 3.**) and found that six taxa were significantly more abundant in the group of

children not suffering from wasting compared to children with wasting. The phylum Verrucomicrobiota (p = 0,005) was notably higher in the group without wasting, a finding supported by an increased abundance of the genus *Akkermansia*, a preeminent member of this phylum, in the group of children with normal weight. Additionally, four other taxa negatively associated with wasting were identified: *RF39* (p = 0.011), Christensenellaceae_*R-7_group* (p = 0.014), *Lachnospira* (p = 0.020), *Haemophilus* (p = 0.010).

Finaly, we analysed the the significantly differences in bacterial abundances regarding the WRA nutritional status (obesity) (**Figure 4.3**). *Alloprevotella* (p = 0.003) and *Bilophila* (p = 0.008) were significantly more abundant in obese participants than in non-obese individuals.

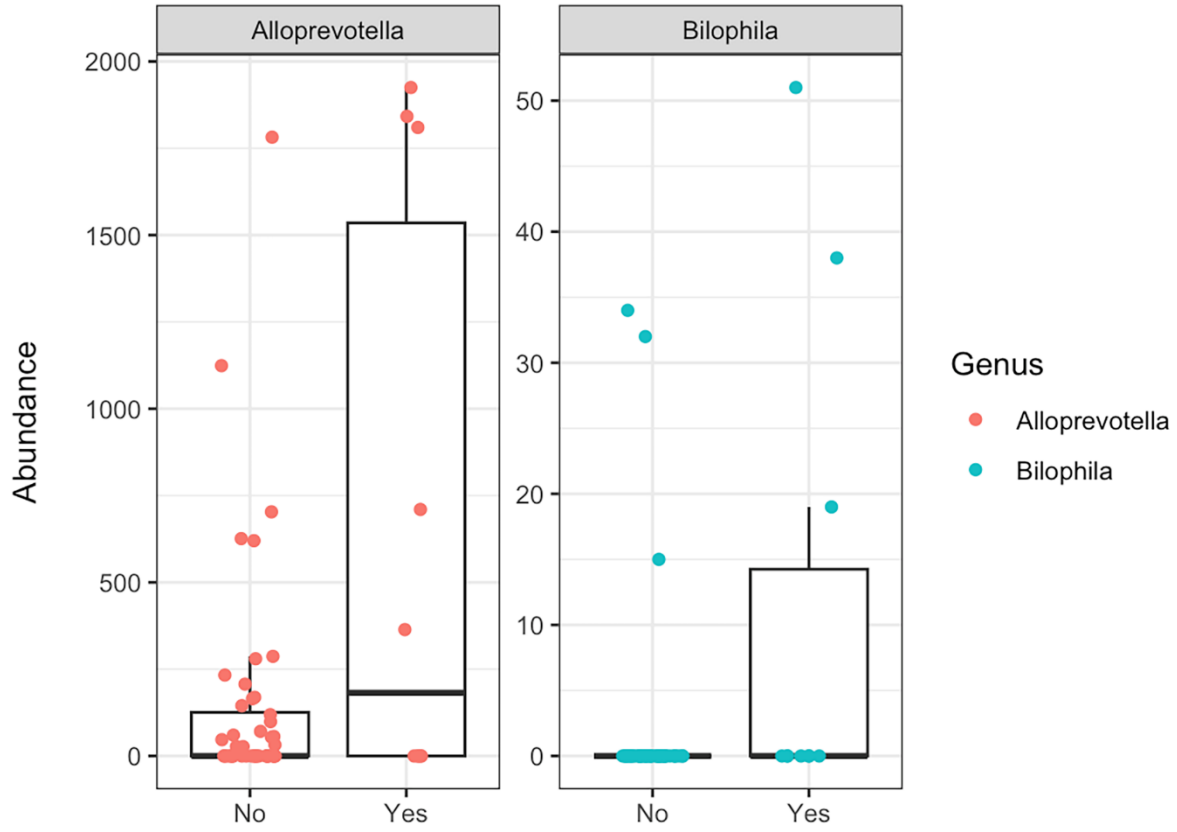


Figure 4.3. Differential abundance of taxa according to obesity in women from Pemba. "No" refers to "not obese" while "Yes" indicates "obese group."

4.5 DISCUSSION:

Our findings shed light on the interplay between gut microbiota, helminthiasis, and malnutrition. Here, we observed a relatively high prevalence of helminth infection despite a recent deworming campaign, alongside a high prevalence of malnutrition among children in Pemba. While we did not detect significant differences in the biodiversity of the gut microbiota among participants based on helminth infection and nutritional status, differential abundance analyses uncovered taxa with negative associations with either helminth infection or malnutrition, and taxa that exhibited negative associations with both helminth infection and malnutrition, suggesting that microbiota-directed interventions could serve as a dual approach to fighting these health issues simultaneously.

The relatively high prevalence of helminth infection observed shortly after deworming campaigns suggests either rapid re-infestation or a failure of chemotherapy to effectively cure the helminth infections in this population. In a cross-sectional study conducted on the same population in 2018, in which samples were collected at almost a year distance from the deworming campaign, Chen et al. (150) found a prevalence of helminth infection (37.93% and 22.22% in mother and children respectively) that closely mirrored the prevalence found in our study conducted shortly after deworming campaign. Previous studies with the same population highlighted the limited efficacy of chemotherapy interventions and restricted sensitivity of *Trichuris trichiura* to commonly used anthelminthic drugs (154,187,224). The current situation reinforces the need in identifying complementary strategies to address the issue of helminth infection in this population.

In addition, our analysis unveiled a high prevalence of malnutrition among children. Specifically, the overall prevalence of child malnutrition was 56.66% in Pemba. This situation that is similar to what was found in Ghana where nearly half of all the schoolchildren were malnourished (209). This result clearly underscores the seriousness of the issue of child malnutrition in sub-Saharan Africa. The prevalences of children malnutrition in Pemba, characterized by stunting (46.66%), underweight (26.66 %) and wasting (23.33 %) appear to be relatively high compared to those reported in the Tanzanian national nutritional survey in 2018 (21.5 %, 5.3 %, 13.6% for stunting, wasting and underweight respectively). The difference observed here can be attributed to the age range covered in our study, which was 18 to 36 months, compared to the broader range of 0 to 59 months in the national survey. However, it's noteworthy that in the national survey, the prevalence of stunting among children aged 24 to 35 months was similar (43.3%) to the one found in our study (46.66%). This suggests a

heightened vulnerability of children aged 1.5 to 3 years—a period of nutritional transition when children gradually shift from maternal feeding to more adult-like feeding habits. This result also alerts that the Global Nutrition Targets by 2025 that includes a 40% reduction in the number of children aged under 5 years who are stunted (208) might not be reached if a more appropriate and more integrative approach is not being developed. Moreover, a prevalence of stunting in childhood may results into obesity later in women life. Henriques et al. have demonstrated that being stunted predispose to obesity in the adulthood (225). Therefore, fighting against stunting does not only save children life but prevents later onset of obesity and its related complications.

Our analysis revealed an elevated odd of being stunted and underweight for children who had *Trichuris trichiura* infection. Previous studies have established that the presence of helminth affect nutritional status of children (127,130,226–228). Helminth infections can affect the nutrition of children through different mechanisms such as anorexia (226,229), mucosal damage, vomiting, diarrhoea (128,230). A study in Brazil revealed that helminthiasis in early childhood was associated with a reduction of 4.6 cm in the height by the age of seven years in children (231). Conversely, the use of anthelmintic drugs has been linked to improvement in weight, height, mild-upper arm circumference (131,232). These findings indicate the importance of combating helminth infections as a critical step in reducing childhood malnutrition.

The analysis of bacteria co-occurrences indicates a strong negative co-abundance between *Escherichia/Shigella* and anti-inflammatory and SCFA producers like Faecalibacterium and Dialister. This finding aligns with Chen et al. 2020 who observed a similar pattern, where a species within Escherichia genus showed positive co-abundance with species with pro-inflammatory properties, and negative co-abundance with species with anti-inflammatory properties, like F. prausnitzii (233). Additionally, positive co-occurrences of Lactobacillus and healthy gut bacteria such as Bacteroides and Prevotella offers promise for the potential supportive role of Lactobacillus probiotics in children's gut microbiota health, as evidenced by studies in mice (234) and humans (173). Nevertheless, it is crucial to further investigate these correlations at the species and subspecies levels, as microbial relationships can vary depending on the taxonomic level chosen (235).

Although previously investigations have reported significant changes in the alpha and beta diversity of the gut microbiota in the presence of helminth infection (149,150,200), our study did not find a significant impact of helminth infection on the biodiversity of the gut microbiota of children. However, a comparative analysis between non-infected and helminth-infected participants indicated significant changes of the abundance of specific taxa**.** Similarly to our finding, Gobert et al. (156) observed altered abundance of specific bacterial taxa but not the overall gut microbiota diversity. Interestingly, in line with our results, they also noted a reduction in the *Haemophilus* genus in the group of helminth infected participants indicating the potential protective properties of some members of this group of bacteria against helminths. We observed that two other taxa consistently exhibited higher abundance in *Ascaris* non-infected children and mothers: *Akkermansia* and *Alloprevotella. Akkermansia muciniphila* has shown promise as a probiotic treatment for improving insulin sensitivity and metabolic dysfunction (236) and has been negatively associated to the helminth infection in other studies (236,237). Additionally, two members of the Clostridia class (*Eubacterium ruminatium* and *Flavonifractor)* were significantly reduced in children while three other members of the same class in mothers *(Eubacterium eligens, Lachnospira* and *UCG-005)* exhibited similar reduction in abundance in *Ascaris* infected participants. Members of this class also dominated in group of bacteria negatively associated with *Trichuris trichiura.* Among five other taxa that were more abundant in children without *Trichuris*, four belongs the Clostridia class *(Eubacterium_coprostanoligens, Christenellaceae_R_7 group, Clostridia viadin BB60_group* and *UCG-002),* suggesting a protective property of bacteria belonging to this group. One previous research has observed a decreased proportional abundance of genera from the Clostridia class and noted that *Clostridium sensu stricto* genus was significantly more abundant in faecal samples from non-infected children (200). Likewise, *Lachnospiraceae incertae sedies* belonging to the same class was found negatively associated with soil transmitted helminth infection by Rosa et al. (123). Other taxa negatively associated with the helminth infection belong to the Bacteroidia class (*Odoribacter, Alloprevotella* and *Paraprevotella*) within the Bacteroidetes phylum. Consistent with our finding, Kupritz et al observed elevated abundance of the class Bacteroidia among participants not infected by helminths (145). All these findings suggest that a microbiota-directed intervention, such as dietary modifications aimed at increasing the abundance of these bacterial groups, may offer protective benefits for vulnerable populations. Some of these bacteria may also serve as candidate probiotics. For instance, *Odoribacter* that we found significantly more abundant in helminth non-infected

women, has already been investigated as potential probiotic in humans (238) and has been recognised to be able to improve glucose control and inflammatory profile in obese mice (239,240).

While no significant changes were observed in the alpha and beta diversity of malnourished participants compared to those with normal nutritional status, the analysis of differential abundance revealed that some taxa, particularly within to the Clostridia class were significantly more abundant in the group of children with normal weight and low in children with underweight. Previous studies, differing from ours, have explored the relationship between the gut microbiota and malnutrition and have identified significant differences between case and control (214,241). We observed that some of these taxa, such as *Alloprevotella*, *Eubacterium_coprostanoligenes_*group, *UCG-002*, and *Paraprevotella*, which were described as negatively associated with children malnutrition, were also negatively associated with helminth infection. This suggests that intestinal parasites may play an essential role in the chronicity of children's malnutrition (242). Among taxa that differ according to the nutritional condition in children, *Dorea* drew our attention as it appears to be negatively associated with underweight and stunting. *Dorea formicigenerans*, a specie belonging to *Dorea* genus was among taxa associated with weight gain in a microbiota directed food intervention in children (243). Two other studies found that species of the same genus were positively correlated to weight and lean mass gain (244,245). A study by Fontaine et al. (214) revealed that the most discriminative species between healthy and undernourished children were *Bifidobacterium longum* for young children in their first six months while *Dorea* species, *Faecalibacterium Prausnitzii* and *Ruminococcus* species were for children from 6 to 24 months. Therefore, bacteria from the genus *Dorea* may be considered as candidate probiotics for addressing childhood malnutrition.

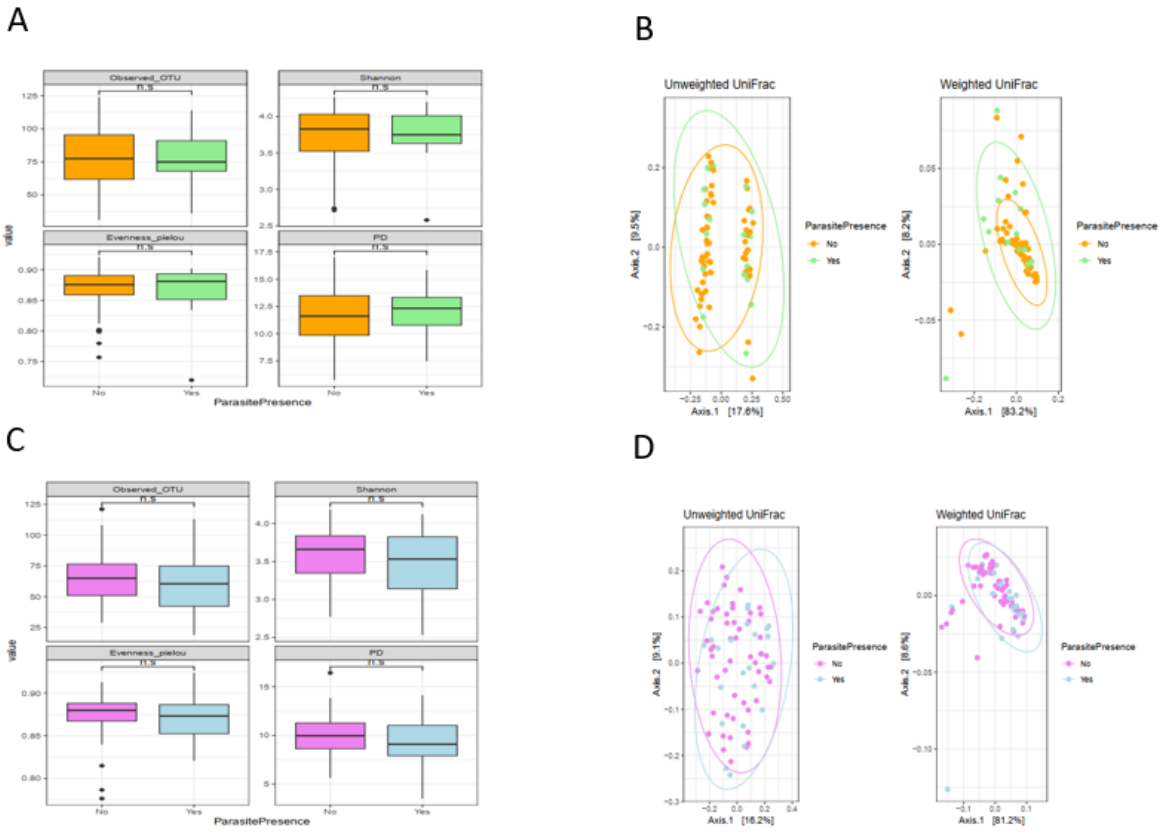
The abundance *Akkermansia* was significantly higher in the group without wasting and negatively correlated with *Ascaris* infection. A reduced abundance of this genus is compromising for the maturation of the gut microbiota of children with wasting condition. Roswell et al.(217) discovered that *Akkermansia* along with other bacteria usually found in healthy gut microbiota of adults, increased with age in children. Therefore, helminth infection may delay the maturation of children gut microbiota. Fortunately, it was demonstrated that early life consumption of polyphenol (246–248) and omega-3 supplementation (249) can promote the development of *Akkermansia.*

Our study offers the advantage of being the first to simultaneously investigate the connections between helminth infection, malnutrition, and the gut microbiota of young children and mothers from Zanzibar, where helminthiasis and child malnutrition are prevalent. Another strength is the acceptable total sample size. However, the limited resolution of the 16SrDNA sequencing did not allow detection for subgenus taxa. Additionally, this study did not consider functional aspects, which could be better addressed using metatranscriptomics and metabolomics approaches.

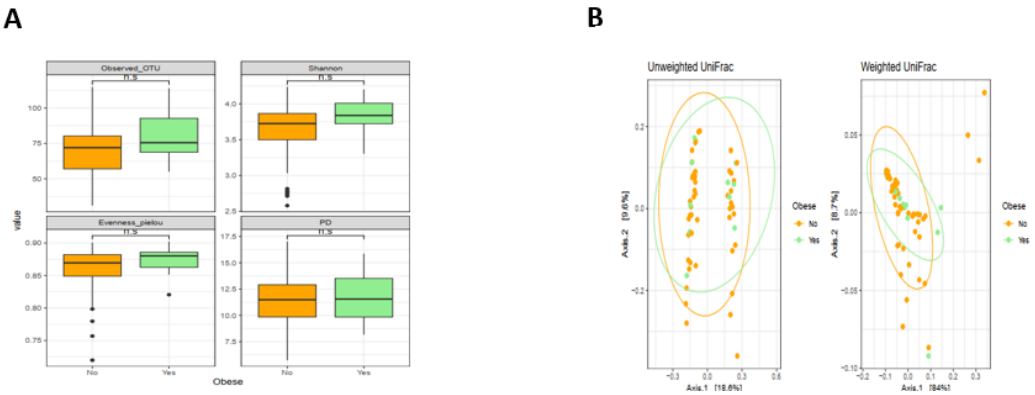
4.6 CONCLUSION

In this cross-sectional and observational study, we used the 16S rDNA sequencing approach to evaluate the composition of the gut microbiota of children and women from Pemba according to the helminth infection and the nutritional status. Our analyses showed that despite efforts made by the local government and partners to fight against soil transmitted helminth infection and malnutrition, their prevalences remain high. The differential abundance of some taxa allowed us to postulate that the microbiota directed intervention through the diet or probiotics may provide a helping hand in the fight against the two public health challenges of malnutrition and helminthiasis. Some taxa identified negatively associated with either helminth infection (*Akkermansia, Haemophilus*, and *Blautia*) or children malnutrition (*Dorea* and *Odoribacter*) emerged as candidate probiotics. Furthermore, it appears very important to promote nutritional education among the population so that they can obtain healthy nutrients for their body but also for their intestinal microbiota. This should be part of an integrated control approach that consider a regular use of certified anthelminthic drugs, improvement of water, hygiene, and sanitation, and improvement of socio-economic condition of the population particularly of women.

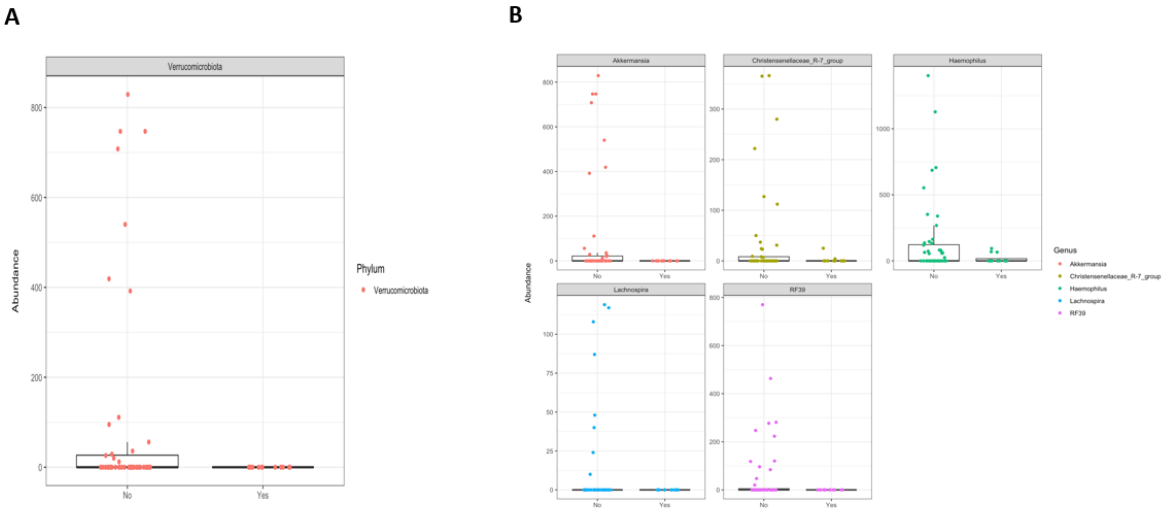
4.7 Supplementary materials for the fourth chapter



Additional Figure 1. Diversity discovery according to helminth infection. A= Alpha diversity of the gut microbiota in mothers. B= Beta diversity of the gut microbiota in mothers. C= Alpha diversity of the gut microbiota in children. D= Beta diversity of the gut microbiota in children.



Additional figure 2. Diversity analysis according to obesity in mothers. A= alpha diversity in Pemba, B= beta diversity in Pemba



Additional figure 3. Analysis of the taxonomy according to the wasting condition in children from Pemba. (A): Phylum level, (B): Genus level

Supplementary table 1: detailed characteristics of women of reproductive from Pemba. AS stands for *Ascaris* and TT stands for *Trichuris trichiura*. EPG: Egg count per gram. Where there are two values for EPG, the first is for *Ascaris* and the second for *Trichuris*.

Sample	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Bodyweight	Obese	Helminths	EPG
01M_11A_1	32	52.6	152.5	22.5	N	No	No	0
01M_11B_1	32	52.6	152.5	22.5	N	No	No	0
02M_10A_1	30	48	150	21.33	N	No	No	0
02M_10B_1	30	48	150	21.33	N	No	No	0
02M_11A_1	24	47	156	19.31	N	No	No	0
02M_11B_1	24	47	156	19.31	N	No	No	0
02M_19B_1	35	73.4	153.2	31.18	SOW	Yes	No	0
03M_16A_1	42	67	155.4	27.74	OW	No	No	0
03M_16B_1	42	67	155.4	27.74	OW	No	No	0
03M_3B_1	23	60	155.7	24.75	N	No	No	0
03M_15B_1	38	83.7	151	36.7	SOW	Yes	No	0
01M1B	30	75	158	30.04	SOW	Yes	TT	60
01M3A	26	45.3	158	18.14	UW	No	No	0
01M4B	34	75	149	33.78	SOW	Yes	No	0
01M5B	23	63.3	163	23.82	N	No	No	0
01M7B	20	68.3	168	24.19	N	No	TT	50
01M8B	37	65	154.2	27.4	OW	No	AS	40
01M9B	32	68	162.5	25.91	OW	No	No	0

01M10B	27	67	157	27.18	OW	No	No	0
01M12B	35	67	167	24.02	N	No	No	0
01M13B	25	45.4	146.6	21.11	N	No	No	0
01M15B	40	68	151	29.82	OW	No	AS	70
01M16B	27	43	151.2	18.85	N	No	No	0
01M17B	27	43	155	17.89	UW	No	No	0
01M21A	27	46	147	21.28	N	No	No	0
01M22B	20	60.3	162	24	N	No	No	0
01M23B	38	76	158	30.44	SOW	Yes	No	0
01M25B	25	52	153	22.21	N	No	No	0
01M26B	25	67	163	25.21	OW	No	No	0
01M27B	23	61	158.1	24.43	N	No	No	0
02M2A	33	82	168	31.24	SOW	Yes	AS/TT	60&100
02M3A	35	75	153	32.03	SOW	Yes	AS	110
02M5A	31	45	146	21.11	N	No	AS	90
02M6B	21	60	154	25.3	OW	No	TT	30
02M7B	23	86	156	35.33	SOW	Yes	No	0
02M12B	32	49	161.6	18.9	N	No	AS/TT	20&40
02M13B	30	56.3	156.3	23.01	N	No	No	0
02M14B	28	77	157.3	31.23	SOW	Yes	No	0
02M15B	30	43	151	18.85	N	No	No	0
02M17B	32	50	158	20.02	N	No	No	0
02M18B	38	53	153	22.64	N	No	No	0
02M20B	40	57	155.3	23.72	N	No	No	0
02M22A	28	47	153.4	20.07	N	No	No	0
02M23B	37	42	146.6	19.43	N	No	No	0
02M24A	27	86	162.5	32.58	SOW	Yes	TT	50
02M26A	30	69	160	26.95	OW	No	AS	150
02M27A	22	50	160	19.53	N	No	TT	180
02M28A	25	54	158	21.63	N	No	No	0
03M1B	40	NA	NA	NA	UW	No	No	0
03M2B		53	155.5	21.91	N	No	No	0
03M4B	28	79	166	28.66	OW	No	No	0
03M6B	37	40	147	18.51	N	No	AS	360
03M7B	45	76.5	166	27.76	OW	No	No	0
03M10B	22	50	162	19.05	N	No	No	0
03M11B	27	65	163	24.46	N	No	TT	170
03M12A	28	38	141	19.11	N	No	No	0
03M13A	20	39.1	151	17.14	UW	No	No	0
03M14A	30	56	155	23.3	N	No	No	0

Supplementary table 2: detailed characteristics of children from Pemba

Sample	Sex	Age (months)	Weight (kg)	Height (cm)	WHZ (std)	Wasting	WAZ (std)	Body weight	HAZ (std)	Stunting	Malnutrition	Helminth	EPG
02C19B	F	24	12.3	89	0	No	1	Normal	1	No	No	TT	410
02C20B1	F	36	12	87	0	No	-1	Normal	-2	MS	Yes	TT	120
02C21B	F	36	11	88	-1	No	-1	Normal	-1	No	No	AS	60
02C23B	M	31	9	83	-2	MW	-3	Underweight	-2	MS	Yes	TT	460&20
03C3B	M	24	12.2	90.4	0	No	1	Normal	1	No	No	TT/AS	4750&230
01C11A	F	18	10.1	78.5	0	No	0	Normal	-1	No	No	No	
01C11B	F	18	10.1	78.5	0	No	0	Normal	-1	No	No	No	
02C10A1	M	24	6.9	81	-3	SW	-3	Underweight	-2	MS	yes	No	
02C10A2	F	24	7.9	78	-2	MW	-3	Underweight	-2	MS	yes	No	
02C10B1	M	24	6.9	81	-3	SW	-3	Underweight	-2	MS	yes	No	
02C10B2	F	24	7.9	78	-2	MW	-3	Underweight	-2	MS	yes	No	
02C11A	F	31	13	90	0	MW	0	Normal	-1	No	yes	No	
02C11B	F	31	13	90	0	No	0	Normal	-1	No	no	No	
03C16A	M	30	12	88.5	0	No	-1	Normal	-1	No	no	No	
03C16B	M	30	12	88.5	0	No	-1	Normal	-1	No	no	No	
03C15B	F	30	14.3	87	2	No	1	Normal	-1	No	no	No	
01C1B	M	18	8.5	73	0	No	-1	Normal	-3	MS	yes	No	
01C3B	M	36	10	84.3	-1	No	-3	Underweight	-2	MS	yes	No	
01C5A	F	24	10.1	86	-2	MW	-1	Normal	0	No	yes	No	
01C6B	F	24	11.1	88.5	-2	MW	-1	Normal	1	No	yes	TT	10
01C7B	M	30	9.3	76	0	No	-3	Underweight	-3	SS	yes	TT	40
01C8B	M	18	9	75.2	0	No	-1	Normal	-2	MS	yes	No	
01C9B	M	35	13	93.2	0	No	1	Normal	1	No	no	No	
01C10B	F	30	10	80	0	No	-1	Normal	0	No	no	TT	110
01C12B	M	24	10.3	78.1	1	No	-1	Normal	-3	SS	yes	No	
01C13B	F	18	9.5	73.6	1	No	-1	Normal	-2	MS	yes	No	
01C15B	F	35	10	73.2	-1	No	-2	Underweight	-3	SS	yes	No	
01C16B	M	35	10.1	85	-1	No	-2	Underweight	-2	MS	yes	No	
01C17B	F	33	9	86.5	0	No	-3	Underweight	-1	No	yes	TT	30
01C18B	M	24	9.9	76.5	0	No	-1	Normal	-3	SS	yes	No	
01C21A	F	18	9	74.1	0	No	-1	Normal	-2	MS	yes	No	
01C22B	M	18	8.8	77.8	0	No	-1	Normal	-1	No	no	No	
01C23B	F	20	10.8	79.7	1	No	1	Normal	-1	No	no	No	
01C25B	F	30	11.2	73.9	2	No	-1	Normal	-3	SS	yes	No	
01C26B	M	30	12	82	1	No	-1	Normal	-2	MS	yes	No	
01C27B	M	25	11	89.9	-2	MW	-1	Normal	1	No	yes	No	
02C1A	M	18	9.5	79.8	-1	No	-1	Normal	-1	No	no	TT	30
02C3B	F	24	8	74.7	-1	No	-3	Underweight	-3	SS	yes	AS/TT	80&10
02C5A	F	21	13	86.2	1	No	-1	Normal	-2	MS	yes	No	
02C6B	F	31	12	89	0	No	-1	Normal	-1	No	no	No	
02C7B	F	25	9.5	80	0	No	-2	Underweight	0	No	yes	No	
02C12B	M	18	9	73.2	0	No	-1	Normal	-3	SS	yes	No	
02C13B	M	18	9.5	80.2	-1	No	-1	Normal	-1	No	no	No	
02C16B	F	24	11.5	82	1	No	0	Normal	-1	No	no	No	
02C18B	F	29	10	90	-3	SW	-1	Normal	0	No	yes	No	

02C22A	F	36	11	89	-1	No	-1	Normal	-1	No	no	AS	80
02C24A	F	35	13	88	0	No	-1	Normal	-1	No	no	No	
02C26A	M	23	9	80	-3	SW	-2	Underweight	-2	MS	yes	No	
02C27A	F	32	11.2	83.5	0	No	-1	Normal	-2	MS	no	AS/TT	250&160
02C28A	F	29	102	83	0	No	-1	Normal	-2	MS	no	TT	120
03C1B	F	24	12	83	0	No	1	Normal	-1	No	no	No	
03C2A	M	36	13.4	90	0	No	-1	Normal	-1	No	no	TT	30
03C4B	M	19	10	79	0	No	-1	Normal	-2	MS	no	TT	30
03C7B	F	22	9	75	0	No	-1	Normal	-3	SS	yes	No	
03C8B	F	30	9	79	0	No	-2	Underweight	-3	MS	yes	TT	20
03C10B	M	18	10.1	78	0	No	-1	Normal	-1	No	no	TT	50
03C11B	M	36	11	96	-3	SW	-2	Underweight	0	No	yes	TT	200
03C12A	M	36	14	94	0	No	-1	Normal	-1	No	no	No	
03C13B	F	36	10.5	84.5	-1	SW	-2	Underweight	-2	MS	yes	TT	100
03C14A	M	18	10	85	-1	SW	-1	Normal	1	No	yes	No	

CHAPTER V: GENERAL CONCLUSION AND PERSPECTIVES

In conclusion, this thesis embarked on a comprehensive examination of the intricate relationships between nutrition, gut microbiome composition, and intestinal parasitic infections within the Zanzibar population, aiming to identify candidate probiotics and food/nutrients that can be used in the microbiota directed strategies to address helminth infections and malnutrition. Using both targeted and untargeted metagenomic approaches, this research yielded significant insights.

The initial phase of my activities consisted in analysing with the shotgun metagenomic sequencing selected healthy and infected samples previously studied with the 16s rDNA sequencing. This analysis confirmed that *Trichuris* infection shapes the structure of and the function of the gut microbiota. The Principal Coordinate Analysis (PcoA) illustrated distinct clustering of samples based on the infection status, with infected samples exhibiting greater variability compared to healthy ones. The findings confirmed that the microbiota of the population of Pemba is dominated by the Firmicutes and the Bacteroidetes phyla. The genus *Prevotella* is the dominant genus while *Prevotella copri* appears to be the dominant specie. Species such as *Bacteroides stercoris* and *Bacteroides fragilis*, *Weissella cibaria*, *Weissella paramesenteroides* and *Leuconostoc citreum* and *Leuconostoc lactis* displayed underrepresentation in infected samples alongside with a significant elevation of the Firmicutes / Bacteroides ratio. Predictive functional analysis revealed that the microbiota metabolic pathways and protein synthesis were significantly different based on the infection status. Importantly this investigation revealed that about 50% of the reads were not annotated. This demonstrates that the African microbiota as other underrepresented microbiota is not yet enough explored and deserve more attention. Many studies are needed with accent given to the de novo annotation.

Subsequent sampling done in two different islands of Zanzibar revealed that participants gut microbiota composition differ according to the island of origin even though the populations are ethnically closely related. This difference might be attributable to dietary and the environmental disparities. Although the biodiversity of the gut microbiota based on the helminth status did not show significant difference, the analysis of the differential abundance of some taxa revealed significant differences. Indeed, taxa like *Akkermansia, Haemophilus*, *Alloprevotella* consistently negatively associated with *Ascaris* infection in both children and

mothers, and other taxa such as *Blautia, Eubacterium_coprostanoligens, Ruminococcus torque*, *Roseburia*, *Odoribacter* emerged as potential targets for microbiota directed interventions.

The analysis of the diet of this population revealed different dietary trends in the two islands. In Pemba, I noticed a reduced consumption of food rich in proteins and fruits despite the availability of fish, beans, and a huge variety of fruits and legumes. This evidence suggests that the nutritional behaviour is mostly related to the limited nutritional education in the population. Milk consumption was also almost absent justifying a very low intake of B vitamins. It was also characterised by serious micronutrient deficiencies. If some women reach the recommended nutrient intake for carbohydrates intake, most of them did not consume enough vitamins and minerals, particularly B vitamins, vitamin A, and calcium warranting targeted dietary interventions.

The analysis of the relationship between the gut microbiota and children nutritional condition reveals some taxa that were negatively associated with both helminth infection and malnutrition. Among these bacteria, *Dorea* and *Odoribacter* emerged as potential probiotics that should be better explored.

Correlations between food/nutrients and gut microbiota highlighted the importance of micronutrient-rich diets in maintaining a healthy microbial balance. For example, bacteria like *Escherichia*/*Shigella*, typically associated with *Trichuris* infection, consistently showed negative correlations with vitamin B2, vitamin B12, and calcium levels. This suggests a need to increase the consumption of these micronutrients in the diet of the Pemba population where they are less commonly consumed. Conversely, beneficial bacteria such as *Subdoligranulum* and *Faecalibacterium* showed positive correlations with minerals and vitamins, highlighting the importance of maintaining adequate levels of these nutrients for a healthy gut microbiota.

Although this study has the merit to be part of the few investigations on African gut microbiota, it has some limitations. Firstly, it explored the microbiome composition up to the species level just in a limited number of samples. It is known that metabolic activities of gut microbes may be strain-dependant. Secondly this study did not analyse the real time situation of the gut microbiota of the population as this can be only better investigated with the metatranscriptomics and the metabolomics that can allow to know which genes are active in the moment of sampling. Thirdly, the sampling site was limited to some areas of the Archipelago. As our results have revealed, the microbiota composition varies based on the

origin or location of participants. Therefore, our results require caution before generalisation even among Zanzibar populations and Sub-Saharan populations in general.
As future measures, organizing nutritional education sessions for the community, facilitated by nurses and community workers, will be recommended. These sessions will aim to empower the population with knowledge on composing a balanced diet using locally available foods.

Furthermore, an intervention study involving the supplementation of vitamins such as B vitamins and minerals (calcium, iron, magnesium) is proposed to enhance micronutrient intake and observe its effects on overall health and the gut microbiome. Future investigations should consider expanding the sample size and encompassing other regions within the study. This expansion warrants a multi-omics approach, integrating metagenomics, metatranscriptomics to identify active genes during sampling, and metabolomics for functional analysis. Replication of this study in other African regions where helminth infections and child malnutrition persist is crucial before generalizing the findings.

Additionally, isolating, cultivating, and characterizing candidate probiotics identified in this study before conducting trials in mice is recommended. If successfully characterized and with recognized health benefits, these probiotics should be introduced to populations at risk of soil-transmitted helminth infections and child malnutrition. These initiatives should be part of an integrated control strategy, incorporating regular use of certified anthelminthic drugs, improvements in water, hygiene, and sanitation, and enhancing socio-economic conditions, particularly for women.

Appendix A: references for tools used in the third chapter.

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