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The Demographic and Geographic Variations Mediate Fecal and Oral Microbial Dynamics Alteration in Human Population of District Faisalabad

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ABSTRACT

The gut microbiome offers numerous advantages to the hosts, through a variety of physiological mechanisms like firming up gut integrity or shaping the intestinal epithelium. Studies showed 2172 species are identified and isolated from human beings, ordered into twelve different phyla, of which 93.5% belonged to *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria*. Out of the 12, three phyla are identified. Human microbial communities are affected by the host external factors like dietary patterns, lifestyle factors, antibiotic usage, internal factors gender, host genotype, age and race. To analyze the dysbiosis and bacterial variation in different areas of District Faisalabad 10,000 individuals of age 25–35 years were recruited from two different rural and urban localities. The oral swab and fecal sample were collected according to prescribed manner. The collected samples from demographically and geographically distinct regions of District Faisalabad were subjected to DNA extraction and quantification. The amplification of 16S rRNA was carried out by PCR and visualized through a transilluminator. The results revealed significant elevated, % abundance of fecal microbial *profile Blautia spp*, *Firmicutes*, *Ruminococcaceae*, in rural population however non-significant difference % abundance of total *Bifido*, *E. coli*, *Fecali bacteria*, total *Lactobacillus* were identified in both demographically distinct regions. The pathogenic *Gamma Protebacteria*, *Fusobacterium*, *Campylobacter coli* were not detected in both study areas. Out of five orally detected microbial profiles, elevated % abundance of *Firmicutes* was detected in rural population however non-significant difference was observed in % abundance of total *Bifido* and *E. coli* in both populations. Total *Lactobacillus* and *Fusobacterium* were not detected in oral sample of both urban and rural population. Conclusively, this research accomplished the potential effects of demographic and geographical induced dysbiosis in the normal microbiome of the oral cavity and intestine that might be due to the reason for dysbiosis associated disorders.

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KEYWORDS

Gut microbiota; Dysbiosis; Demography; *Firmicutes*; *Proteobacteria*; *Actinobacteria*

Introduction

The microorganisms in human body exist as mutualistic and symbiotic relationships; known as microbiota. These microbes trillions in number together with bacteria, protozoa, viruses, and fungi are present on and in our bodies [1,2]. These microorganisms are found on the skin, in the mouth, reproductive organs, and gut. The supposed role of microbes has been linked to the analysis, medication, and administration of numerous ailments [3]. It has been reported that microbes associated by prevention and progression of numerous CNS diseases such as multiple sclerosis [4-30]. They are also associated with detection and care of cardiac problems such as hypertension [28]. Many microorganisms living in the gut, assisting to digest the indigestible foods [22]. The gut microflora offers numerous advantages to the hosts, through a variety of physiological roles like firming up gut integrity or shaping the epithelium of intestine [25], protecting against pathogens [5], harvesting energy [10], and regulating host immunity [13]. However, altered microbial composition, known as dysbiosis disrupt these mechanisms [31-35].

Numerous groups of bacteria have a crucial role in the breakdown of bile salts and suppress the pathogenicity of pathogenic microbes in gut, convert pro forms of drugs into active state and lessen the harms of xenobiotic compounds by chemical alterations [5]. Moreover, the gut microorganisms play a vital role in the production of neurotransmitters, vitamins and metabolites, the essential constituent of human health, regulate host immune system, cytokine generation, and growth of lymphoid organs in the gut, growth and development of gut-asso-

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ciated immune system [1,2]. It has been reported that intestinal microbiome also plays significant part in the improvement of whole body's immune status. These specific combinations of bacteria, viruses and fungi are not only essential for the distinctive care of health, but also in dispensation of exogenous compounds (medicine) intended to rectify homeostatic inequities [1,2]. Numerous factors involving the use of antibiotics, prebiotics and probiotics, along with geographical distribution, and environmental factors, such as air pollution, or disrupting chemicals, and hygienic conditions act as modulators, influencing the gastrointestinal microbiome functionality, makeup and diversity [1,2].

According to studies, moderate amounts of species have been found and isolated from humans, divided into twelve phyla, with *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* accounting for 93.5 percent of the total. Out of the 12, three identified phyla have only one species separated from humans, like intestinal species, *Akkermansia muciniphila*. In human being, 386 of the known species are anaerobic and henceforth will in general be found in mucosal areas like the GI tract and the oral cavity [15].

Dysbiosis of the intestinal microbiome is by far the most major cause of infection. Furthermore, bacterial infection and its treatment have a significant impact on the human intestinal microbiome that determines the manifestation of infection in host body. Invading pathogens invade the intestinal mucosa, triggering a severe inflammatory reaction and the subsequent translocation of gastrointestinal microorganisms [8]. Number of studies has reported the close link between microbial deregulation and infection, as well as the fact that disease is linked not just to the microbes but also to viruses [9].

Clostridium difficile overpopulation is commonly linked to antibiotic-related diarrhoea, which is one of the most common side effects of antibiotic therapy, and now it has become a public health concern [14]. *Clostridium difficile* is spore-forming, gram-positive, anaerobic, bacillus an important element, of the human gut micro flora. The decrease in the resistance contrary to toxin-producing *Clostridium difficile* is due to altered intestinal mucosal homeostasis and stimulation of *Clostridium Difficile* Infection (CDI) development. Studies found that either *Clostridium Difficile* Infection (CDI) is existent or not, fecal microbial diversity is reduced and intestinal bacterial diversity pompously changes in patients with antibiotics administration [14]. Another research investigated that whether CDI exist or not, antibiotic medication has been associated with reduction of butyrate-forming bacteria, an upsurge of endotoxin-producing pathogens and lactate producing phylotypes in individuals [36]. *Helicobacter pylori* bacterium is known to causes digestive problems. It has lately been related to the development of periodontitis. He looked at the link between *H. pylori* infection and inflammation, periodontal parameters, and periodontal pathogens. According to their findings, the frequencies of *Prevotella, Treponema denticola, Fusobacterium nucleatum, Aggregatibacter actinomycete* and *Fusobacterium* nucleatum, are much higher in *H. pylori*-infected patients than non-infected ones. The findings suggest that *H. pylori* patients had significantly increased probing depths or attachments failure, as the *H. pylori* may encourage the evolution of various periodontal infections and aggravate the progression of chronic periodontitis [16].

Current studies have revealed that, the gut microorganisms have impact on circadian oscillations and also affect the circadian clock [20]. Dysbiosis induces the Interruption of circadian clock, which is related to the host metabolic diseases [33]. Obesity, which is accompanied with altered metabolic pathways and gut microbiome dysbiosis, reduces intestinal epithelial barrier functioning and seems to have a significant impact on physiological functions [35], like immune homeostasis and gut [11].

The microbiota of a human stomach is shaped by numerous factors. These are (1) The way of delivery; (2) diet during infancy and adulthood; and (3) use of antibiotics and the gut commensal community [17].

Our study determined the variations in selected oral-fecal bacterial species in rural and urban regions of district Faisalabad. Also determined the variations in oral-fecal bacterial species across rural and urban individual suggested potential impact of demographic and geographic distinction on microbiome dysbiosis.

Materials and Methods

Experimental design

Ethics approval and consent to participate: The study was approved by ethical Committee on Human Research (CHR) of Koç University Institutional Review Board (IRB) (2022. 216. IRB3.122) and all methods were performed in accordance with relevant guidelines and regulations. The informed consent was obtained from all subjects and their legal guardians.

Population: Ten thousand healthy male aged between 25–35 years were recruited from the two different rural and urban localities of the district Faisalabad.

Inclusion criteria: Individuals recruited have regular excrement habits. Devoid history of gastrointestinal disease such as gastritis, colorectal cancer, gastric cancer, inflammatory bowel disease and peptic ulcer. No diarrhea for the previous month. The informed consent was obtained from all subjects and their legal guardians.

Study area: The city of Faisalabad is one of the major cities in Punjab province of Pakistan was identified as the urban location of the study and nearby areas was identified for rural location. Samples were collected from healthy

subjects (N=10,000) who lived in urban area of Faisalabad Wapda city (n=5000) located in the Faisalabad. For rural area samples were collected from subjects lived in Naimatabad (n=5000).

Dietary assessment: A self-administered evaluative food routine questionnaire was used to assess dietary patterns. All recruited individual had routine meals comprised of protein-based diets (beef, pork, poultry, eggs, fish, and cow milk) and carbohydrate-based foods (rice and rice noodle, breakfast cereal, bread, sweet potato), as well as dietary fibres (fruit and vegetables).

Oral swab sample collection and processing: Before collecting oral rinses, people were instructed to fast for nine hours. Oral swab sample was collected in a sterile cup by healthy participants. Oral swab specimens were kept cool until they arrived at the Lab, where they have been freezed and stored at -80°c in 50 mL collection tube. The oral rinsed samples were subsequently transported to laboratory on dry ice and stored at -80°c until processing. We extracted DNA and amplified it in the V4 region of the 16S rRNA using primers.

Fecal sample collection: Each subject received a stool sampling kit, which included a sample box and sterilized tissue papers, as well as a question paper regarding their eating habits and a consent paper. The questionnaires were filled in volunteers according to need of research. Participants were recruited randomly through direct approach. Participants had not taken any antibiotic for at least six months prior to sampling. The consent documents were signed by and obtained from the all participants prior to fecal sample collection. Volunteers were briefed on how to use and collect the sample with minimal contamination following the detailed instructions included in the sampling kit. After samples collection, samples were collected from participants within two hours and transported on dry ice in the cooler box in the laboratory. Samples were stored immediately at –80°C in the laboratory till the analysis occurs. A total of 10,000 samples were collected according to the grouping described in (Table 1).

Table 1. Rural and Urban areas sampling in the District Faisalabad.

Group	Number of sample
Rural area	5000
Urban area	5000

DNA extraction and quantification: The fecal samples were kept at -80°C till genomic DNA extraction. About 30 mg of samples were used for DNA extraction. In brief, each fecal sample was diluted to 1:10 (weight/ volume) in distilled water and homogenized boiling for 2 min. The sample was then transferred into a 1.5-mL screw-capped tube. One milliliter of sample obtained was centrifuged at 14,000 × g for 10 min at 4°C, after centrifugation, pallet was taken and dissolved in DNAzol 300 microliters. The solution was homogenized by using vortex machine. 300 microliter chloroform added to this solution incubated on ice for 30 minutes until three layers are formed. When three layers are formed upper layer containing bacterial DNA was collected and transferred it in Eppendorf of 1.5 mililiter, equal amount of isopropanole was added and freezed it overnight. Next day 70 percent ethanol was added then centrifuged at room temperature for 40 minutes at 14,000 × g for washing. This washing step was repeated for three times. Every washing sample was shifted into new Eppendorf. Afterwards the lid of Eppendorf was kept open for ethanol evaporation. 30µl nuclease free water was added. The quality and quantity of DNA were analyzed by using Thermo Scientific™ Nano Drop 2000.

Primer sequences: List of forward and reverse primers for amplification of 16S rRNA collected from Macrogen, South Korea.

PCR and agarose gel electrophoresis: The variable segment of the microbial 16S rRNA markers was amplified by using forward and reverses primers fallowing the sample thawed on ice. The following conditions were used for primary 16S rRNA gene PCR amplification: 95°C for 3 minutes preceded by 35 cycles of 95°C for 15 seconds each, 60°C for 30 seconds, and 72°C for 30 seconds, and a final extension step of 72°C for 5 minutes. Agarose gel electrophoresis was used to determine success of amplification. Variable regions of the 16S rRNA genes were amplified in 50µl reaction volume using 0.1 ng of template DNA with different bacterial primers given in (Table 2) in Primer sequences using the PCR thermocycler BioRad T100. The PCR amplification products were purified on 2% agarose gels. And the positive number of samples for various bacteria were counted and compared in each groups to calculate the microbial diversity in different population. Final concentration in gel/running buffer was as 40 mM Tris, 20 mM acetic acid, 1 mM EDTA and 3.6 Gel preparations.

Table 2. List of forward and reverse primers for amplification of 16S rRNA collected from Macrogen, South Korea.

Primer Name	Sequence
Total Bifido-F	TCGCGTC[Y]GGTGTGAAAG
Total Bifido-R	CCACATCCAGCRTCCAC
Blautia spp-F	TTGATGTGAAAGGCTGGGGCTTAC
Blautia spp-R	GGCTTAGCCACCCGACACCTA
Compylobacter coli- F	TGACGGTAGAACTTTCAAATCC
Compylobacter coli- R	GCAAGTGCTTCACCTTCGATA
E Coli-F	GTTAATACCTTTGCTCATTGA
E Coli-R	ACCAGGGTATCTAATCCTGTT
Faecalibacterium-F	GAAGGCGGCCTACTGGGCAC

Faecalibacterium-R	GTGCAGGCGAGTTGCAGCCT
Firmicutes-run-F	GGCAGCAGTRGGGAATCTTC
Firmicutes-run-R	ACAC[Y]TAG[Y]ACTCATCGTTT
Fusobacterium-F	KGGGCTCAACMCMGTATTGCGT
Fusobacterium-R	TCGCGTTAGCTTGGGCGCTG
Gamma-Protebacteria-F	TGCCGCGTGTGTGAA
Gamma-Protebacteria-R	ACTCCCCAGGCGGTCDACTTA
Total Lactobacillus-F	AGCAGTAGGGAATCTTCCA
Total Lactobacillus-R	CACCGCTACACATGGAG
Ruminococcaceae-F	ACTGAGAGGTTGAACGGCCA
Ruminococcaceae-R	CCTTTACACCCAGTAAWTCCGGA

Separation, purification, and identification 0.5- to 25-kb DNA fragments with agarose gel electrophoresis was carried out in three main phases: (1) a gel was prepared with an agarose concentration appropriate for the size of DNA fragments to be kept separate; (2) DNA samples were loaded into sample wells and the gel is run at a voltage and for a period of time which will achieve optimal separation; and (3) Ethidium bromide is incorporated into the gel and electrophoresis was performed. An electrical field was used to drive negatively charged DNA throughout an agarose gel matrix forward towards a positive terminal in electrophoresis. Relatively short DNA fragments go faster through the gel than longer ones. By running a DNA fragment on a gel matrix alongside a DNA ladder, we were able to establish its approximate length.

Statistical analysis

The Mann–Whitney U-test was used to compare the abundance of 16S rRNA Graph Pad Prism Software, version 5 software. P value *p<0.05, **p<0.01.

Results

In total, 10,000 volunteers were recruited from two different geographic and demographic areas. In current study 10 bacterial species were identified using PCR in different geographic and demographic regions specifically rural and urban areas of district Faisalabad, Pakistan. A total of 10 bacterias were aimed to detect in fecal and 5 in oral sample. The list of bacteria's detected in study is showed in (Table 3).

Table 3. List of fecal and oral microbial profile detected in present study.

Sr no	Bacterias	Rural	Urban
Fecal microbial profile			
1	Total *Bifido*	Present	Present
2	*Blautia spp*	Variable abundance	Variable abundance
3	*E.coli*	Present	Present
4	*Faecali bacteria*	Present	Present
5	*Firmicutes*	Variable abundance	Variable abundance
6	*Total Lactobacillus*	Present	Present
7	*Ruminococcaceae*	Variable abundance	Variable abundance
8	*Gamma Protebacteria*	Absent	Absent
9	*Fusobacterium*	Absent	Absent
10	*Compylobacter coli*	Absent	Absent
Oral microbial profile			
1	Total *Bifido*	Present	Present
2	*E.coli*	Present	Present
3	*Firmicutes*	Variable abundance	Variable abundance
4	*Total Lactobacillus*	Absent	Absent
5	*Fusobacterium*	Absent	Absent

Fecal microbial profile

Total *Bifido*: (Figure 1) and (Supplementary file 1. and Figure 2) illustrated % abundance and corresponding bands of Total *Bifido* in the fecal samples of both rural and urban populations respectively. Results demonstrated the environmental and life style variation of people living in rural and urban areas of district Faisalabad have no effect on % abundance of Total *Bifido* in the gut in these two populations.

***Blautia spp*:** The presence of *Blautia spp* in both rural and urban populations has been shown in (Figure 1) and (Supplementary file 1 and Figure 2) clearly depicted the variable abundance of bacteria in two demographically distinct areas. It implies the influence of environmental changes on the bacterial population of gut microbiota. The % abundance has been increased in rural populations.

E. coli: *E. coli* bacterial species found in fecal sample of human population of both rural and urban regions demonstrated no influence of life style variation in % abundance of *E. coli* bacterial population in gut flora. As % abundance and representative bands are depicted in (Figure 1) and (Supplementary file 1 and Figure 2).

Fecali: PCR results depicted changes in the environment and people's lifestyles in rural and urban parts of Faisalabad district have no influence on the prevalence of *Fecali bacteria* in the gut. However relative quantity of this bacterial species may decrease or increase, but found in both rural and urban areas of District Faisalabad. As % abundance and representative bands are depicted in (Figure 1) and (Supplementary file 1 and Figure 2).

Firmicutes: It has been found that *Firmicutes* bacteria are present in both rural and urban areas of human population of District Faisalabad. The results have been depicted in (Figure 1) and (Supplementary file 1 and Figure 2) illustrated the people living in rural areas of district Faisalabad revealed elevated % abundance of *Firmicutes* load in fecal samples of rural individuals.

Total Lactobacillus: Total *Lactobacillus* bacterial species is a part of normal gut microbiota. (Figure 1) and (Supplementary file 1 and Figure 2) indicated variations in the environmental and people's lifestyles in rural and urban areas of Faisalabad district have no influence on the % abundance of Total *Lactobacillus* in gut.

Ruminococcaceae: The bacterial species of *Ruminococcaceae* has been showed variable abundance in both rural and urban populations. Geographical and socio-economical differences alter the gut microbial diversity. PCR results demonstrated the % abundance of *Ruminococcaceae* in rural populations was elevated. As % abundance and representative bands are depicted in (Figure 1) and (Supplementary file 1 and Figure 2).

Gamma Protebacteria: *Gamma Protebacteria* in fecal sample of both rural and urban populations was devoid. As this bacterium is pathogenic and absent in both rural and urban areas of district Faisalabad it showed change in lifestyle and environment did not cause any *Gamma Protebacteria* associated disease. As depicted in (Figure 1) and (Supplementary file 1 and Figure 2).

Fusobacterium: *Fusobacterium* is the pathogenic bacterial species found in human gut microbiota in disease condition. This bacterium was included in the current study to observe change in lifestyle may influence *Fusobacterium* load of gut. Results showed absence of *Fusobacterium* in both rural and urban areas of District Faisalabad. As % abundance and representative bands are depicted in (Figure 1) and (Supplementary file 1 and Figure 2).

Compylobacter coli : The absence of *Compylobactor coli* in both rural and urban population has been observed in (Figure 1) and (Supplementary file 1 and Figure 2).This bacterial species is pathogenic and typically not found in both rural and urban regions of the district Faisalabad; it indicated lifestyle and environment variation did not contribute illness caused by this bacterium.

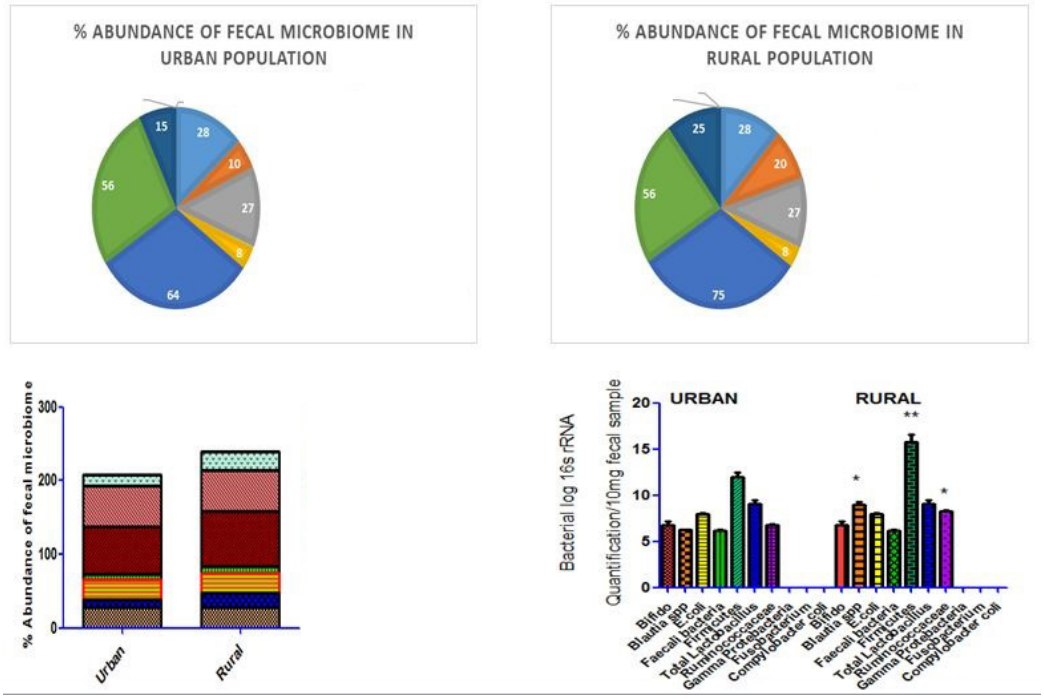

Figure 1. % abundance of fecal bacterial profile in samples collected in 5000 individuals at urban and rural areas of Faisalabad. The Abundance analysis of microbiome in urban and rural individuals 16s rRNA quantification by PCR revealed a significant elevated abundance of *Blautia spp*, *Firmicutes Ruminococcaceae*, in rural population however non-significant difference in abundance of Total *Bifido* , E.coli , Faecali bacteria , Total *Lactobacillus* were found in both urban and rural population. The Compylobacter coli *Fusobacterium* and *Gamma protebacteria* bacteria were not detect in any population of study. The abundance of 16S rRNA genes comparison was carried out by Mann–Whitney U-test in Graph Pad Prism Software, version 5 software. P value *p<0.05, **p<0.01.

Note: (■) Total *Bifido*, (■) *Blautia spp*, (■) E.coil, (■) Faecali bacteria, (■) *Firmicutes*, (■) Total *Lactobacillus*, (■) *Ruminococcaceae*, (■) *Gamma Protebacteria*, (■) *Fusobacterium*, (■) *Bifido*, (■) *Blautia spp*, (■) E. coil, (■) Faecali bacteria, (■) *Firmicutes*, (■) Total *Lactobacillus*, (■) *Ruminococcaceae*, (■) *Gamma Protebacteria*, (■) *Fusobacterium*, (■) Compylobacter coil, Urban: (■) *Bifido*, (■) *Blautia spp*, (■) E. coil, (■)Faecali bacteria, (■)*Firmicutes*, (■)Total *Lactobacillus*, (■)*Ruminococcaceae*, Rural: (■) *Bifido*, (■) *Blautia spp*, (■) E. coil, (■)Faecali bacteria, (■)*Firmicutes*, (■)Total *Lactobacillus*, (■)*Ruminococcaceae*.

Oral microbial profile

Total Bifido: Total *Bifido* normally present in oral sample of human populations. (Figure 3) and (Supplementary file 1 and Figure 4) depicted the % abundance and corresponding band of Total *Bifido* in both rural and urban regions. It indicated the variation in environment and life style of people in rural and urban areas of district Faisalabad has no influence on Total *Bifido* in the oral micro flora.

E. coli: *E. coli* bacterial species is normally present in the microbiome of human population. (Figure 3) and (Supplementary file 1 and Figure 4) depicted the % abundance and corresponding band indicated that variations in the environment and people's lifestyles in rural and urban areas of Faisalabad district have no influence on the prevalence of *E. coli* in the oral microbiota.

Firmicutes: PCR results demonstrated the variable microbial load of *Firmicutes* bacteria in rural and urban populations. It depicted the role distinct life style on abundance of *Firmicutes* bacterial population in oral microbime. The % abundance of *Firmicutes* elevated in rural population. As (Figure 3) and (Supplementary file 1 and Figure 4) depicted the % abundance and corresponding bands.

Total Lactobacillus: The results revealed the absence of Total *Lactobacillus* bacteria in both rural and urban areas population. As this bacterial species is pathogenic and normally not found in both rural and urban regions of the district of Faisalabad. It indicated lifestyle and environmental changes did not affect illness caused by this bacterium. As presented in (Figure 3) and (Supplementary file 1 and Figure 4) the % abundance and corresponding band.

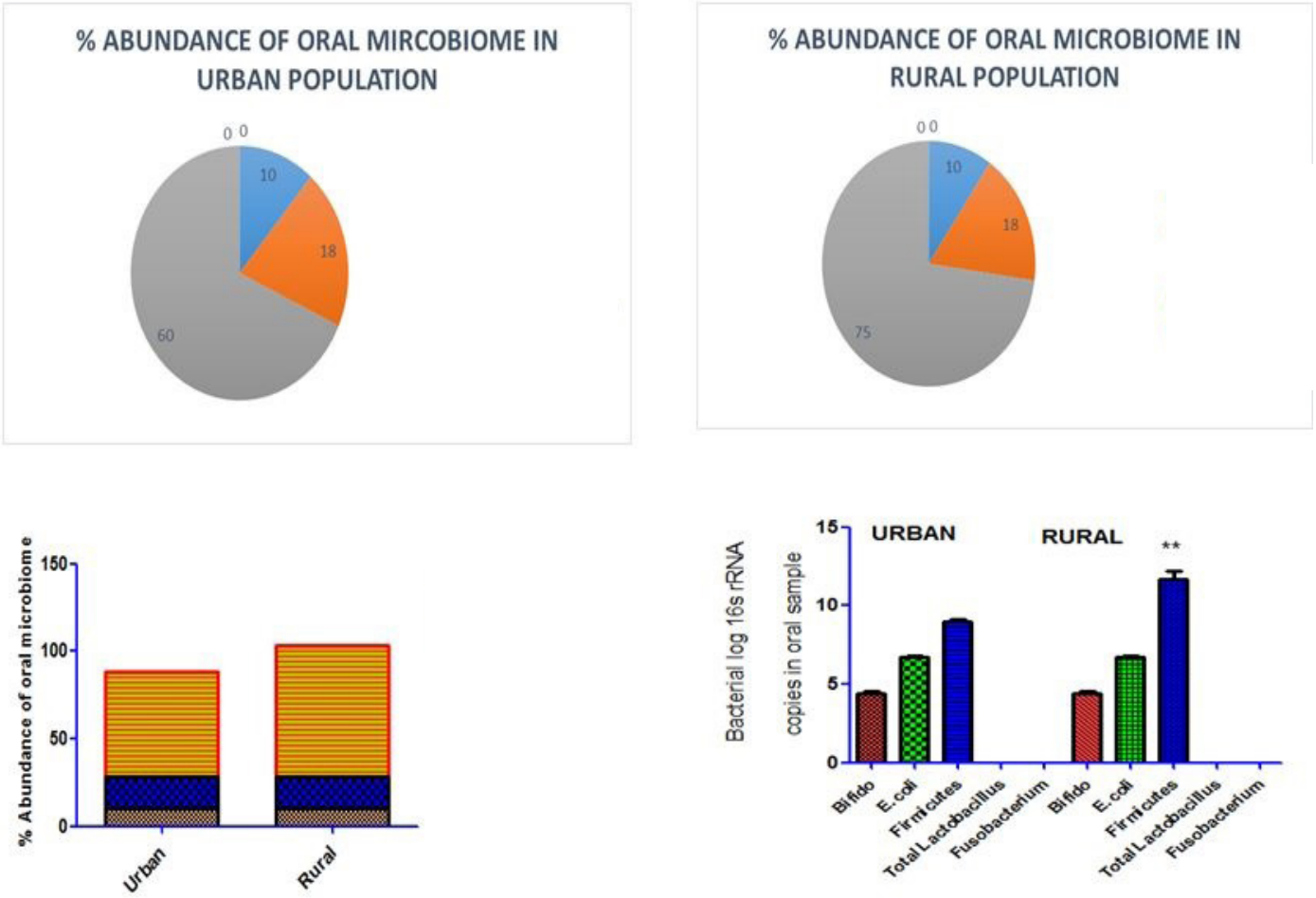

Figure 3. % abundance of oral bacterial profile in samples collected in 5000 individuals at urban and rural areas of Faisalabad. The Abundance analysis of microbiome in urban and rural individuals 16s rRNA quantification by PCR revealed a significant (P value **p<0.01) elevated abundance of *Firmicutes* in rural population however non-significant difference in abundance of total *Bifido* and *E. coli* was found. Total *Lactobacillus* and *Fusobacterium* were not detected in oral sample of both urban and rural population. The abundance of 16S rRNA genes comparison was carried out by Mann–Whitney U-test in Graph Pad Prism, version 5 Software.

Note: (■) *Bifido,* (■) *E.coil,* (■) *Firmicutes,* (■)*Total Lactobacillus,* (■) *Fusobacterium,* (■) Total *Bifido,* (■) *E. coil,* (■) *Firmicutes,* (■) *Total Lactobacillus,* (■) *Fusobacterium.* ***Urban:*** (■) *Bifido,* (■) *E. coil,* (■) *Firmicutes.* **Rural:** (■) *Bifido,* (■) *E. coil,* (■) *Firmicutes.*

Fusobacterium: The analysis showed the absence of *Fusobacterium* in both rural and urban populations. Results presented in (Figure 3) and (Supplementary file 1 and Figure 4) the % abundance and corresponding band. Clearly implies null influence of environmental changes in the bacterial population of oral microbes.

Discussion

The gut microbiome, a massive habitat of microorganisms lived in the human body, located in the gut. In human intestinal tract, there are an estimated 100 trillion microorganisms, the most of which are bacteria, but also include fungus, viruses and protozoa. Initially, it was considered ten times higher microscopic cells in our bodies than human cells [36], However, a recent discovery has led us to believe that human and microbes coexist in our bodies in a 1:1 ratio, implying that microbes and human cells are roughly equal in number [32]. We are all living in various geographic locations, such as the East or the West; as a result of such geographical variances, our lifestyles vary, which leads to differences in dietary habits, such as some eating more vegetables and relying solely on a fresh, leafy, and fibrous diet, while others rely solely on a protein diet, because they are more flesh lovers, or as modernization increases, we are now more prone towards junk food [36]. So, these variations also affect our gut microbiome in a way that increases the number of one species while reducing the other one just by fluctuations in dietary habits [36]. In the present study, the dynamics of some oral and fecal microbiota in different populations residing in various regions of district Faisalabad were evaluated in order to interpret the dysbiosis index on the basis of socioeconomic status of different areas. The effect of demographic and geographical variation on % abundance of Total *Bifido*, *Blautia spp*, *E. coli*, *Fecali bacteria*, *Firmicutes*, Total *lactobacillus*, *Ruminococcaceae*, *Gamma Protebacteria*, *Fusobacterium*, Compylobacter coli in fecal sample and Total *bifido*, *E. coli*, *Fecali bacteria*, *Firmicutes*, and *Fusobacterium* were assessed in oral samples. Results of our study showed positive expression of Total *Bifido*, *E. coli*, *Fecali bacteria* and total *Lactobacillus*, however *Blautia spp*, *Firmicutes*, *Ruminococcaceae* % abundance in the gut of rural and urban areas human population was varied as it was increased in rural populations. The pathogenic *Gamma Protebacteria*, *Fusobacterium*, *Campylobacter coli* were not detected in both demographic and geographic areas. Out of five oral detected microbial profiles total *Bifido* and *E. coli* showed positive expression in rural and urban human participants. *Firmicutes* was detected in variable % abundance and showed to increased abundance in rural population's oral cavity. Total *Lactobacillus* and *Fusobacterium* were absent in oral sample. Total *Bifido* and *Firmicutes* were detected in both oral and fecal microbiota samples of rural and urban population of Faisalabad. These are beneficial microbes that are present in the normal flora of human gut and oral cavity. The significance of these bacteria in the gut is also confirmed by other studies that stated members of the genus *Bifido*bacterium have been identified as almost ubiquitous inhabitants of the human host performing an important role in the gut at birth [7]. Other studies showed the effect of geographical changes in Total *Bifido* and *Firmicutes* for instance, American, Japanese, Korean and Chinese communities showed high abundances of *Firmicutes* [26]. At the genus levels, Japanese showed high abundances of *Bifido*bacterium [27]. In line with these finding our studies also described the variable abundance of *Firmicutes* in the stool or oral cavity of rural and urban participants. The results of our study demonstrated the *Blautia spp*, *Firmicutes*, *Ruminococcaceae* abundance in the gut and *Firmicutes* in oral cavity of rural and urban areas human population was varied. The variation in abundance of this bacterial population depicts that the variation of living standard imparts a potential effect on gut and oral cavity bacterial profile and mediate dysbiosis associated disorders. Although, many previous researches have stated *Blautia spp* presence in fecal samples but the extent may vary in different population. A previous study conducted by represented that the Salvador sewage contained 79 of the 81 *Blautia oligotypes* detected in Brazilian human fecal samples whereas the combined Milwaukee sewage contained only 61 (75.0%). The occurrence of the most common *Blautia oligotypes* in each of the human fecal samples supports the idea that sewage samples reflect microbial populations in humans [12]. Another study suggested the distinct provincial microbial structures may respond differently to diet, lifestyle and other host factors. For example, some genera varied significantly across provinces/megacities including *Blautia*, and *Fecalibacterium*. The regional variations in these genera suggested that subpopulations from different geographic locations may have variable levels of susceptibility to certain diseases [14]. *Lactobacillus* bacteria has been detected in current study in the fecal samples of both rural and urban areas of District Faisalabad and has not been detected in oral samples as it was mostly present in disease condition like dental caries in oral cavity and normally in gut microbiome. The findings of the present study are supported by previous studies as it has been reported that two genera, *Bifido*bacterium and *Lactobacillus*, both are common inhabitants of the human intestine [24]. But in oral cavity the presence of *Lactobacilli* is correlated with dental caries [6]. In previous study it was shown that the subjects colonized with enteric pathogens belonging to the family *Campylobacteraceae* and 2 subjects were detected with Helicobacter. Campylobacter is the leading global cause of foodborne disease associated with the consumption of contaminated chicken, and with the consumption of contaminated water in the developing countries [18]. In another study it has been stated that

Fusobacterium sequences were enriched in carcinomas, confirmed by quantitative PCR and 16S rRNA sequence analysis of 95 carcinoma/normal DNA pairs. 52 Fusobacteria were also visualized within colorectal tumors using Fluorescence in-situ hybridization [19]. *Compylobactor coli* and *Fusobacterium* were not detected in current study in both urban and rural population of Faisalabad as these are pathogenic bacteria for oral and gut health and their presence is associated with disease conditions. The variable abundance of *Ruminococcus* spp has been detected in the gut microbial sample of both urban and rural populations of Faisalabad. It is one of the commensal bacteria present in human gut microbiota. These findings also supported by literature i.e. it has been stated that *Ruminococcus* is one of the important member of microbial systems. For example, in humans, *Ruminococcus* spp. is found as abundant members of a —core gut microbiome [29]. In another study it has been demonstrated that *Ruminococcus* is dominated in intestinal microbiome of healthy persons from the Netherlands [5]. *Gamma protebacteria* is one of the pathogenic bacteria present in gut microbiome and was not detected in present study, both in rural and urban population of Faisalabad region. Previous studies show that these bacteria are only present in the conditions involving GI inflammation and are not essentially present in gut flora [30].

We included the detection of these bacteria in current study to evaluate whether the abundance of these bacteria are affected by the geographical and demographic changes. Our study evaluated the qualitative effect of geographic and socioeconomic variation on alteration of gut and oral cavity microbiome however, genomics, proteomics and metabolic approaches are required to be elucidated.

Conclusion

We concluded that geographical and demographic variation alters the gut and oral cavity microbiota and might contribute development of dysbiosis associated systemic disorders. Our finding demonstrated the demographic and geographical variation had potential effect on dysbiosis of *Blautia spp*, *Firmicutes*, *Ruminococcaceae* in gut and *Firmicutes* in oral cavity might cause dysbiosis associated gut and other systemic disorders.

Declarations

Ethics approval and consent to participate

The study was approved by ethical Committee on Human Research (CHR) of Koç University Institutional Review Board (IRB) (2022. 216. IRB3.122) and all methods were performed in accordance with relevant guidelines and regulations. The informed consent was obtained from all subjects and their legal guardians.

Data availability statement

The data used during the current study is presented in this article.

Author contributions

I hereby declare that the work presented in the article is my own effort, and that the article is my own composition. No part of this article has been previously published in any journal.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships and declared no conflict of interest.

Consent for publication

Not applicable.

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