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ORIGINAL ARTICLE

A pilot Study of Salivary Microbiome in Algerian Obese Adult Females: Insight into Obesity and Oral Health Relationship

[1]Manel Zeraoulia*, [2]Houria Ouled-Haddar, [3]Hassen Cheraitia, [1]Kahina Gribi, [4]Nihel Klouche-Khelil

[1]Laboratory of Applied Microbiology in Food, Biomedical and Environment (LAMAABE), Department of Biology, Faculty of Nature and Life, Earth and Universe Sciences, Abou Bekr Belkaïd University of Tlemcen, 13000 Tlemcen, Algeria

[2]Laboratory of Molecular Toxicology, Faculty of Nature and Life Sciences, University of Jijel, 18000 Jijel, Algeria

[3]Department of Mathematics, Faculty of Exact Sciences and Informatics, University of Jijel, 18000 Jijel, Algeria

[4]Laboratory of Applied Microbiology in Food, Biomedical and Environment (LAMAABE), Department of Biology, Faculty of Nature and Life, Earth and Universe Sciences, Abou Bekr Belkaïd University of Tlemcen, 13000 Tlemcen, Algeria; Laboratory of Experimental Surgery, Dental Surgery Department, Faculty of Medicine, Abou bekr Belkaïd University of Tlemcen, 13000 Tlemcen, Algeria

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Corresponding Author:
Manel Zeraoulia
Laboratory of Applied Microbiology in Food, Biomedical and Environment (LAMAABE), Department of Biology, Faculty of Nature and Life, Earth and Universe Sciences, Abou Bekr Belkaïd University of Tlemcen, 13000 Tlemcen, Algeria
manel.zeraoulia@univ-tlemcen.dz

ABSTRACT

Background: *Obesity has become a significant global health care problem. Several studies have reported the important role of microbiome in the development of obesity. Nevertheless, the composition of salivary microbiome (SM) of obese people is still inconclusive.* ***Objectives:*** *this pilot study endeavored to profile the salivary microbiome of Algerian obese adult females and to investigate the relationship between obesity and oral health status.* ***Mmethodology:*** *Sixty adult females from two cities in Algeria (Tlemcen and Jijel) were included (obese N= 30, and normal weight N= 30). Unstimulated saliva was gathered for high-throughput sequencing of V3-V4 region of the 16S rRNA gene. Statistical analyses (Student's T-test and Chi-square test) were implemented in order to analyze the connection between obesity and oral health status, socio-economic status and lifestyle.* ***Results:*** *Overall, 15 phyla, 21 classes, 39 orders, 65 families and 147 genera were characterized in the saliva samples. Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Fusobacteria were the common phyla in the Algerian female population. Alpha diversity indices suggested increased species richness and decreased diversity among the obese group. Over-abundance of Pseudomonas has been reported in the obese group from Jijel City. The obese individuals revealed significantly lower physical activity, lower socioeconomic status and higher values of oral indices.* ***Conclusion:*** *This is the first investigation reporting the diversity of the salivary microbiome in obese and normal weight Algerian adult females and suggesting the direct association of socioeconomic status and oral health with obesity.*

INTRODUCTION

Obesity has become a public health issue reaching epidemic proportions [1]. Spread of obesity is increasing worldwide and its rate in Algeria exceeds 50% of the population [2]. Obesity, frequently estimated by a body mass index (BMI) above 30 kg/m^2, is deemed a multifactorial etiology disease that appears to originate from genetics, but needs psychological, environmental and social factors to manifest, a large portion of those environmental factors concerns diet and related eating habits [3]. However, salient research indirectly advocates the assumption that oral microbiota could be linked to obesity, the utmost accurate proof results from research on animals, which reported that obesity may be related to a dysbiosis of the normal microbiota[4].

For million years, our resident microbes throughout the human body, have coexisted in a largely harmonious symbiotic relationship[5]. It is believed that the mouth harbors the second most diverse and most studied microbial community [6]. The salivary microbiome (SM) plays a significant role in safeguarding the oral cavity against various afflictions [7]. Differences in food consumption, socioeconomic status and lifestyle are considered crucial influences in the multifactorial background of oral diseases, namely dental caries and periodontal disease which are recognized to be strongly related to obesity and influence the oral bacterial profile[5, 8].

There is a large body of evidence on the relationship between obesity and gut microbiota, whereas, few interest has been drawn to the identification of the oral microbiome in obese individuals, hence those few studies vary in terms of design and sample size and have shown contradictory findings. Goodson *et al.*[7] were the pioneers to confirm the connection between obesity and SM. In the subgingival plaques of obese adolescents, a sixfold rise of *Neisseria mucosa* and *Campylobacter rectus* was detected [9]. Furtheremore, a study involving 322 Danish adults indicated that the low level of oral *Lactobacillus* might be a new marker to declare those with elevated potential for weight gain[10]. Another study conducted on 33 obese adults and 29 controls demonstrated lower bacterial diversity and richness in obese subjects [11]. Another research of Finnish children indicated significant relationship between gender and body size and the composition of the SM [12]. A recent study investigated on 30 obese subjects and 30 controls reported that the abundance of *Prevotella*, *Veillonella* and *Haemophilus* was impacted by body mass index [13]. Most recently, a study conducted on Kuwaiti adolescents illustrated different salivary core microbiome among different BMI groups [1]. The conclusions drawn from the aforementioned studies form useful databases and enlighten the direction of our research as they establish its significance.

With the pile of research conducted on the association between SM and body size, there has not been, to the researcher's current knowledge, any research investigated in Algeria applying Next-Generation Sequencing technology. Considering the significance and necessity, we carried out this pilot study to profile the SM composition of Algerian obese adult females, as well as we explored the relation of oral health status with obesity.

METHODOLOGY

Ethic statement:

This study was granted by the Ethical Committee of Tlemcen University (Algeria) (approval number: ED. Autism. 05.22 dated 12nd April 2022). After being informed about the aims and procedures of the research, each participant approved to participate by providing written informed consent. All procedures were conducted in compliance with the Declaration of Helsinki.

Study population:

The present study was conducted on sixty healthy women (N= 60) from two geographic locations in Algeria (Tlemcen city from the North West and jijel city from the North East); participants underwent anthropometric assessment, dental examination and saliva samples collection.

To be considered for the study, patients had to fulfill the following requirements: age between 18 and 45 years, absence of acute or chronic illness, no use of antibiotic treatment within the previous three months, presence of more than 20 teeth. The BMI was determined and the subjects were divided according to the BMI WHO classification into a normal weight group (BMI < 25) and an obesity group (BMI ≥ 30).

Participants answered a questionnaire that covered topics regarding their anthropometric measurements (i.e., height, weight and BMI), socio-economic status, meal frequency, medication and oral hygiene habits. Each subject received an oral examination, number of Decayed Missing and Filled Teeth (DFMT), Plaque Index (PI) and Gingival Index (GI) were the baseline clinical measurements employed.

Sampling procedure:

Between 9 and 11 a.m., collection of unstimulated saliva was performed by direct spitting. The samples were immediately aliquoted and stored at -80° C [11].

DNA Extraction and 16SrRNA Gene Sequencing:

Salivary genomic DNA extraction, 16S-rRNA sequencing and initial bioinformatics analyses were performed at the Novogene Company (Novogene Bioinformatics Technology Co., Ltd., Beijing, China). Samples were divided into obese group A (N= 30) and control group B (N= 30). Each group was further divided into subgroups based on the patient's area of living, (A1) for the obese patients of Tlemcen city (N= 15), (A2) for the obese patients of Jijel city (N= 15), (B1) for the control patients of Tlemcen city (N= 15), (B2) for the control patiens of Jijel city (N= 15).

Extraction of total genomic DNA was carried out according to the combined method of cetyltrimethyl ammonium bromide/sodium dodecyl sulfate (CTAB/SDS). Concentration and purity of DNA were examined by electrophoresis on 1% agarose gels. Using the primer set 342F/806R, the V3-V4 region of the 16S gene was amplified. PCR reactions were performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, USA).

The PCR produced materials were visualized using SYBR green on a 2% agarose gel. Amplicons ranging between 400 and 450 base pairs (bp) were selected then purified with the Qiagen Gel Extraction Kit (Qiagen, Germany). The sequencing libraries were generated using the NEBNext® Ultra TM DNA Library Prep Kit for Illumina according to the manufacturer's protocols. To evaluate the quality of the library, Qubit and Q-PCR were used. Sequencing on the Illumina NovaSeq 6000 PE250 system (Illumina, San Diego, CA, USA) was performed to generate 250 bp paired-end reads.

16S data processing and statistical analysis:

To generate raw tags, the sequences were merged using FLASH (V1.2.7) [14]. QIIME (V1.7.0) was employed to filter raw tags [15]. Clean tags obtained after filtration were compared against the reference database

(Gold) to identify chimeric sequences using a UCHIME algorithm [16]. After chimeric sequences detection and elimination, effective tags were obtained [17], the effective tags with similarity ≥ 97%, were clustered to one Operational Taxonomic Unit (OTU) using Uparse software [18]. The Mothur program and SILVA SSU rRNA database were employed to analyze each representative sequence of the OTUs [19]. To investigate the phylogenetic relationship, several sequence alignments were assessed by MUSCLE software [20].

Alpha-diversity analysis was conducted to investigate microbial diversity within a community. Using Qiime (V1.7.0), the Observed species, Chao1, ACE, Shannon, Simpson and PD_whole_tree indices were estimated and the rarefaction curve was plotted using R (V2.15.3). Variations in alpha diversity indices were assessed by T-test and Wilcoxon rank-sum test. As well, beta diversity analysis was conducted to analyze bacterial complexity beween groups. Weighted and Unweighted Unifrac distances were estimated [21], then hierarchial clustering using the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) was carried out using Qiime software (V1.7.0).

Statistical assessment of demographic and clinical parameters was carried out on SPSS (V.20.0) statistics software (SPSS Inc., Chicago, IL, USA) using Student's T-test and Chi-square test. Significance level was established at P< 0.05.

RESULTS

Characteristics of recruited participants:

The subjects' demographic and clinical characteristics are presented in the Table 1.

There were significant differences in age (36 ± 1,23 vs. 28 ± 0,98 years), weight (91,27 ± 2,44 vs. 55,57 ± 1,06 kg) and BMI (33,90 ± 0,64 vs. 20,62 ± 0,27 kg/m^2) between the obese and the healthy groups, respectively. However, no significant difference in height was observed.

The obese females demonstrated significant lower socio-economic status (P< 0.005), lower physical activity (P< 0,005) and lower tooth brushing frequency (P= 0,035). In addition, significant higher differences in meal frequency (P= 0,007), snacks intake (P< 0.005) and oral malodor have been reported in the obese group compared to controls. Clinical oral indices (DMFT, PI, GI) indicated significant higher values among the obese group (P < 0.05).

Table 1: Demographic and clinical characteristics

Variables		Obesity (N= 30) Mean (SD)	Controls (N= 30) Mean (SD)	*P*-value
Age (years)		36 (1.23)	28 (0.98)	***< 0.005*** [a]
Weight (kg)		91 (2.44)	56 (1.06)	***< 0.005*** [a]
Height (m)		1,64 (0,06)	1,64 (0,06)	0,636 [a]
BMI (Kg/m^2)		34 (0.64)	21 (0,27)	**< 0.005** [a]
Socio-economic Status	High	1	14	**< 0.005** [b]
	Intermediate	20	13	
	Low	9	3	
Meal Frequency	Twice	1	9	**0.007** [b]
	Three times	16	16	
	Four times	13	5	
Snacks intake between meals	Yes	23	4	**< 0.005** [b]
	No	7	26	
Physical Activity	Regular	0	6	**< 0,005** [b]
	Average	5	24	
	Rare	25	0	
Halitosis	Yes	17	4	**< 0.005** [b]
	No	13	26	
Tooth brushing Frequency	Once a day	10	15	**0.035** [b]
	More than once a day	10	13	
	Less than once a day	10	2	
DMFT	< 25%	10	18	**0.006** [b]
	25% - 50%	12	12	
	> 50%	8	0	
PI	<1	1	15	**< 0.005**[b]
	1- 2	17	14	
	> 2	12	1	
GI	< 1	3	22	**< 0.005**[b]
	1- 2	18	7	
	> 2	9	1	

SD: Standard Deviation. DMFT: Decayed Missed Filled Teeth. PI: Plaque Index. GI: Gingival Index. a: Student's T-test. b: Chi2-test.

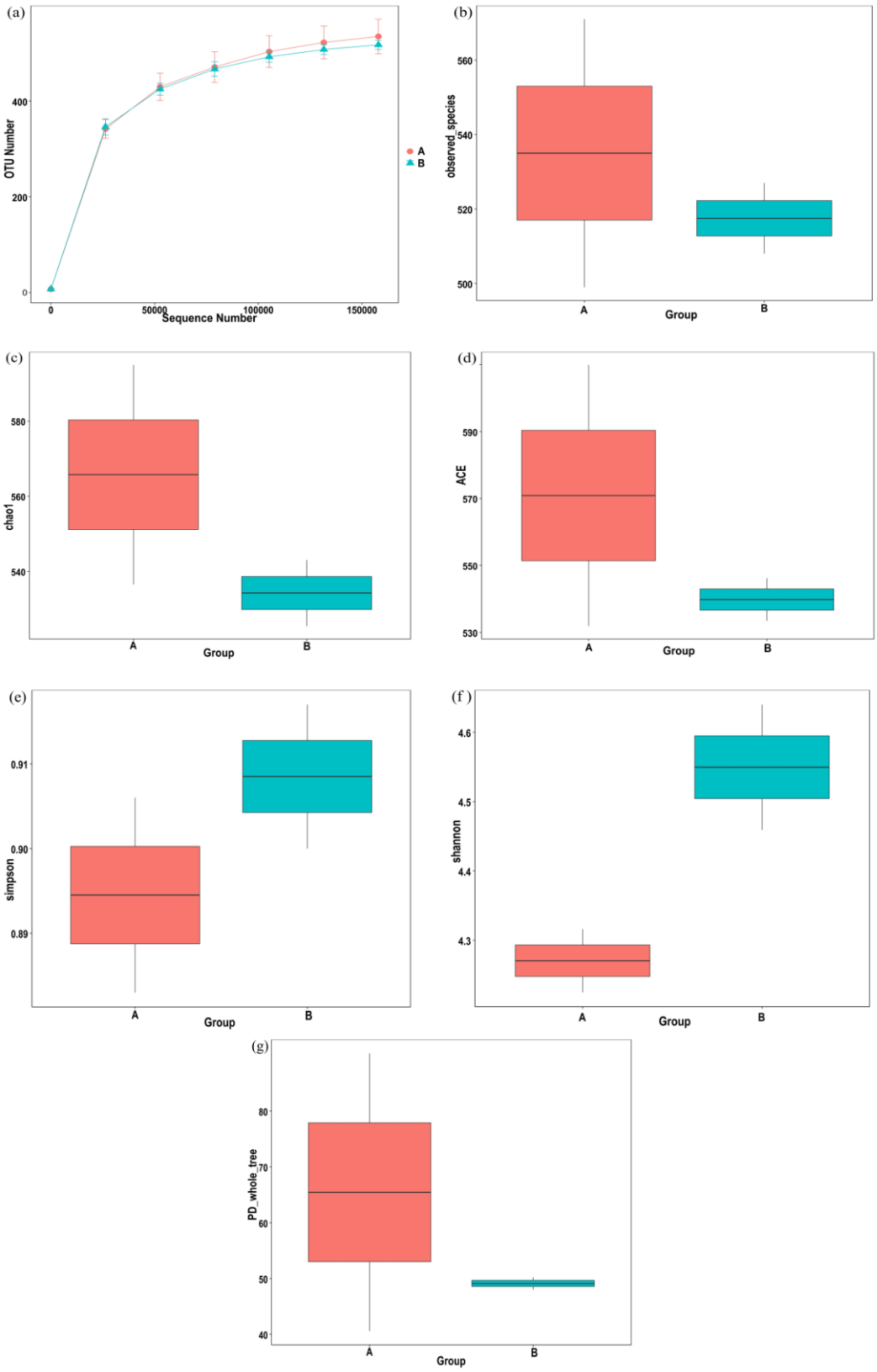

Fig. 1: Altered oral microbiota richness and diversity indices in obese patients. (a) Rarefaction curves to show the adequate depth of the sequencing. (b–d) Comparison of species richness. (e, f) α-diversity. (g) phylogenetic diversity.

Sequencing data, Taxonomic assignements:

From the 60 saliva samples, a total of 828,403 raw sequence reads were generated. Following quality filtering and data trimming, 654,533 sequence reads were obtained and the average number of reads per subgroup was 163,633. 423 bp was the average sequence length. 820 OTUs were detected, with an average of 545 OTUs per subgroup.

Alpha Diversity:

To estimate the alpha diversity, the dataset was rarefied (Fig. 1). We first plotted the rarefaction curves which tended to remain stable, suggesting that the number of extracted sequences was enough to reflect the OTUs of the sample (Fig. 1a). Next, different indices were evaluated to analyze the richness (Observed species, Chao1, ACE, Fig.'s 1b–d), the diversity (Simpson, Shannon, Fig.'s 1e, f), and the phylogenetic diversity ('PD whole tree', Fig. 1g) among obese and control group. The obtained indices indicated that the obese group showed higher species richness and phylogenetic diversity and lower diversity compared to the control group. Despite the dissimilarities noted in the alpha diversity indices, these variations were not statistically significant (P> 0.05).

Beta Diversity:

Beta diversity analysis was conducted to investigate variations of microbial communities between groups. Firstly, both Weighted and Unweighted Unifrac distance between pairwise samples were visualized as a heatmap to measure and view the dissimilarity extent, the result is represented in Fig. 2. Then, samples could be clustered following the acquired distance matrix. Using Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) a clustering tree was constructed (Fig. 3). The normal weight groups from both cities; Tlemcen and Jijel, clustered within the same branches, indicating their similarity. Further, beta analysis revealed that the diversity within the normal weight groups from both cities and the obese group from Tlemcen city was rather small; in contrast, the obese group from Jijel city recorded large differences compared to the other groups.

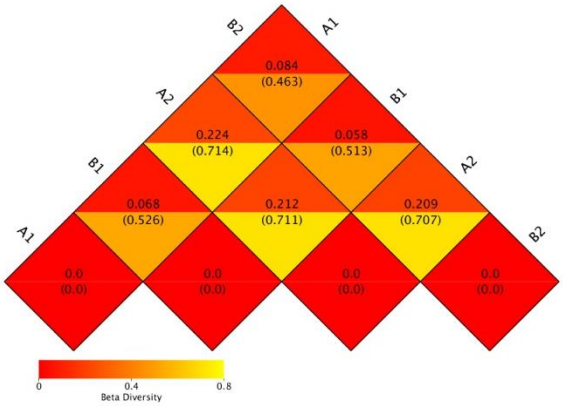

Fig. 2: Beta diversity heatmap. The differences between the samples were measured by the Weighted UniFrac distance and the Unweighted UniFrac distance. Each grid represents pairwise dissimilarity coefficient between pairwise samples, the upper and lower values represent the Weighted UniFrac distances and the Unweighted UniFrac distance, respectively. The smaller the value, the smaller the differences in microbial diversity.

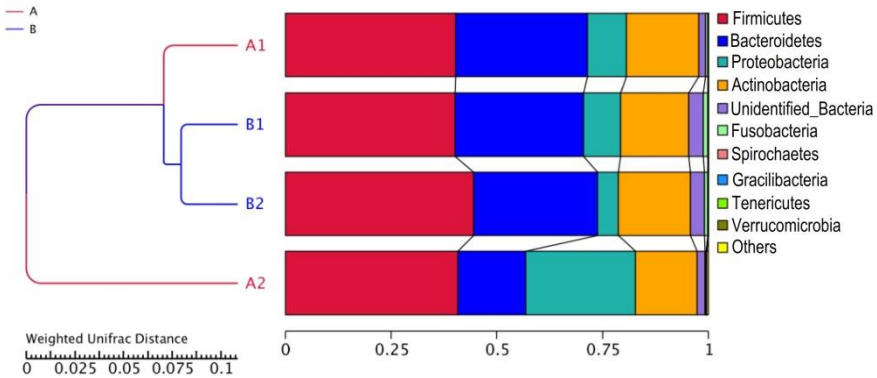

Fig. 3: UPGMA clustering tree based on the Weighted UniFrac distance matrix. On the left: the UPGMA cluster tree structure, on the right: the relative abundance distribution of the units at the phylum level. The UPGMA analysis revealed that A1 B1 B2 display little similarity with eachother. A2 revealed high dissimilarity.

Microbiome characterization:

Healthy and obese groups shared 527 out of the 820 taxa. The healthy group displayed 132 taxa lacking in the obese group while the obese group exhibited 161 taxa absent in the healthy group (Fig. 4). 15 phyla, 21 classes, 39 orders, 65 families and 147 genera were characterized. Firmicutes (41.43%), Bacteroidetes (26.74%), Actinobacteria (16.22%), Proteobacteria (12.14%) and Fusobacteria (0.74%), were the five major phyla, constituting 97.27% of salivary bacteria.

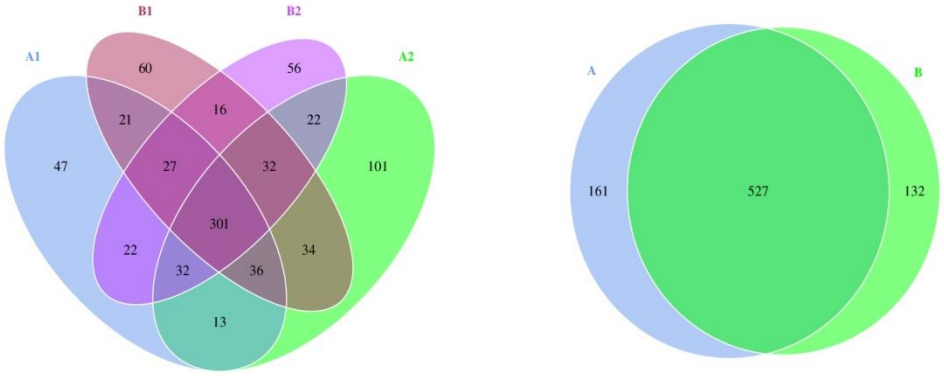

Fig. 4: Venn diagram: The salivary "core microbiome" at the OTU level. Each circle represents one group. Values in overlapping parts represent common OTUs. The others are specific OTUs in each group.

Concerning the class level, the 10 most prevalent classes accounted for ~99% of the overall abundance (Fig. 5a). Bacilli and Bacteroidia were the most frequent covering more than 60% of the total abundance, followed by Gammaproteobacteria (25.75%).

At the order level, the 10 most abundant orders accounted for ~95% of the overall abundance (Fig. 5b). Among all the most frequent bacterial orders, Lactobacillales were notably predominant (35.2%), followed by Bacteroidales (26%).

Regarding the family level, the 10 most dominant families accounted for ~89% of the overall abundance (Fig. 5c). Streptococcaceae (30%) and Prevotellaceae (19.5%) were the most frequent accounted for more than 40%.

When focusing at the genus level, an average of 84 taxa in all groups has been exhibited. The distribution pattern of the 10 utmost predominant taxa up to genera covering ~88% of the overall abundance is presented in (Fig. 5d). Most frequent genera found in the saliva of all individuals were, Streptococcus (30%), unidentified Prevotellaceae (18,5%), Rothia (11,8%), Pseudomonas (6,3%), Porphyromonas (6,3%), Granulicatella (4,7%), Haemophilus (3,7%), unidentified bacteria (2,4%) and Gemella (2,2%).

The variations in the global composition of the SM of healthy and obese subjects from both cities were analyzed. Fraction of Firmicutes in SM was relatively stable in all groups, while the obese group from Jijel city (A2) revealed sharply decreased in the fraction of Bacteroidetes (16,1%) and increased abundance of Proteobacteria fraction (25,8%). Uniformly, at the class level, the proportion of Bacteroidia was decreased in the obese group from Jijel city (A2) (16%), meanwhile, the proportion of the Gammaproteobacteria was increased (25%).

At the order level, in the obese group from Jijel city (A2), the proportions of Bacteroidales (16%), Pasteurellales (0.8%) and unidentified Gammaproteobacteria (0.4%) members were decreased; meanwhile, the proportion of Pseudomonadales was increased (24%). At the family level, Prevotellaceae revealed highest abundance in obese group from Tlemcen city (A1). Pseudomonadaceae was over-represented in obese group from Jijel City (A2). Porphyromonadaceae proportion decreased in obese groups from both cities (A1, A2), while, the control groups (B1, B2) reported its increase.

At the genus level, *Streptococcus*, *Rothia* and *Gemella* presented similar relative abundance among the different groups, whereas, an overabundance of *Pseudomonas* was found only and strictly with the obese group from Jijel city (A2).

Intriguingly, an increased abundance of *Porphyromonas* was observed in normal weight subjects from both cities, differentially occurring groups between the two cities included, *Heamophilus* which showed high relative abundance in subjects from Tlemcen city. Meanwhile, *Heamophilus* revealed higher relative abundance in both obese and normal weight subjects from Jijel city (A2).

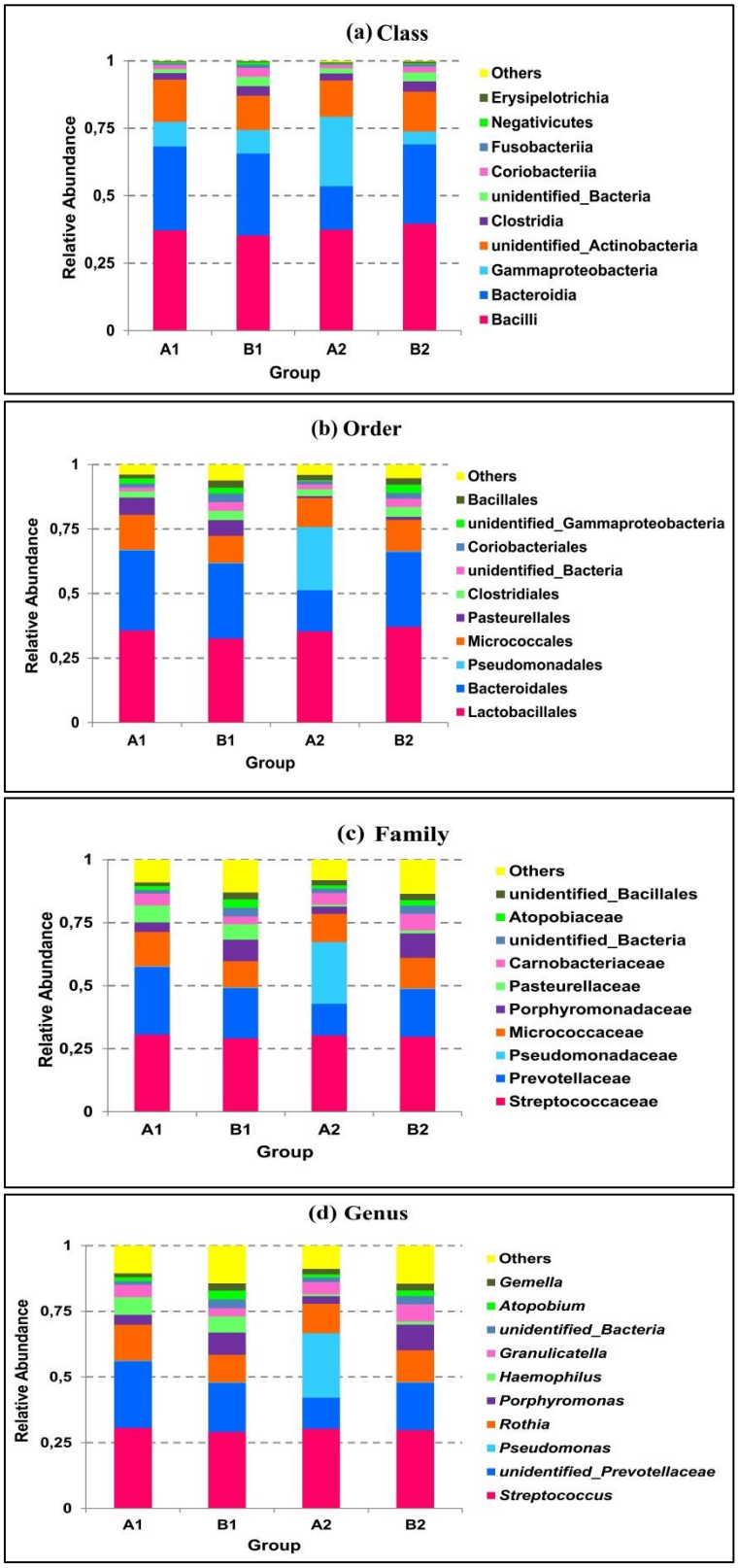

Fig. 5: Relative abundance of the 10 most-abundant taxa found at the class level (b), the family level (c) and the genus level (d) in both control and obese groups of both cities. The remaining taxa are included in the category "Others".

DISCUSSION

Due to the potential role of human microbiota in health and disease, the interest in exploring it has been escalating. Various projects in human microbiome sequencing have been accomplished or are in progress worldwide, in return, there is a concern that the microbiome diversity of African populations is still inconclusive [22].

Despite its human diversity and its geographic extended area, the knowledge about human microbiome in Algeria is relatively scarce, to the best of our knowledge, the current study is the first Next-Generation-Sequencing based study profiling the SM diversity among Algerian women. The connection among oral hygiene status, socioeconomic status, and lifestyle, and obesity was investigated by the questionnaire data.

The significant differences noted, between both obese and lean adult women, in socio-economic status, physical activity, meal frequency, snacks intake, tooth brushing frequency, and oral malodor, were in accordance with previous studies. Individuals with decreased household income and educational attainment were more prone to obesity and perceived higher halitosis as well as low tooth brushing frequency [23]. Similarly, preferences for convenient but generally unhealthy meals and snacks, combined with sedentary lifestyles were widely approved as primary factors contributing to the spread of obesity [24]. Marquezin et *al.* [5] suggested that in overweight subjects, halitosis and hyposalivation could be induced by the decreased buffer capacity and salivary flow rate, which has been explained as the evidence that pro-inflammatory cytokines produced by adipocytes and macrophages stored in adipose tissue can adversely impact salivary gland function.

Considering the fact that both dental caries and obesity are clearly connected to fermentable carbohydrates consumption and poor dietary habits, it is assumed that dental caries and obesity might strongly correlate. In fact, while some studies have revealed a positive association, most have not [25]. In our context, the obese patients revealed significantly higher DMFT index, indicating poor oral hygiene, which lines with the former hypothesis. Correspondingly, the relationship between periodontal diseases and obesity is subtle, but widely recognized. Nevertheless, until present, the research has not established a cause-and-effect connection within these conditions [5]. Periodontal diseases and obesity are chronic inflammatory health problems leading both to increased levels of inflammatory mediators' production [7]. It was established that fat stores inflammatory cytokines and that obesity may affect periodontal disease [26]. Other research, proposed the opposite by admitting the contribution of periodontal disease in the emergence of obesity [7]. These observations converge to our findings recording increased values of PI and GI in the obese patients.

Despite the fact that alpha diversity was different across obese and controls, the variations were not statistically significant. It is worth noting that the obese group demonstrated a large number of observed OTUs in comparaison with control group, that goes in line with a recent study among Kuwaiti adolescent [1]. In contrast, findings in earlier salivary microbiome studies suggested reduced alpha diversity in obese individuals [11,12 and 13]. Obesity and alpha diversity relationship varied in different populations. For instance, while higher alpha diversity in the gut microbiome of obese subjects with Hispanic and African ancestry was suggested, lower alpha diversity was reported in White obese subjects [27].

The SM distribution at the phylum level revealed Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria as predominant phyla. Same abundant phyla were observed in other different populations, regarding Sweden adults [28], Qatari adult [29], Portuguese adults [30] and southern Africa population [31].

The analysis of core microbiome composition among Algerian adult females showed *Streptococcus*, *unidentified Prevotellaceae*, *Pseudomonas*, *Rothia*, *Porphyromonas*, *haemophilus*, *Granulicatella*, *unidentified bacteria*, *Atopobium* and *Gemella* as common genera. Those are typical inhabitants of the human oral microbiome [32]. An earlier study among Angolan populations indicated lightly different core SM, counting *Neisseria*, *Streptococcus*, *Prevotella*, *Porphyromonas* and *Rothia* [31]. Similarly, another Pakistani study reported a slight diverse common core SM which consisted of *Streptococcus*, *Neisseria*, *Haemophilus*, *Veillonella*, *Prevotella*, and *Gemella* [33]. The fact that different studies do not consistently consider the same genera indicates that larger-scale high-throughput approaches are required in order to precisely define the universal core [28].

Comparative analyses of SM diversity revealed differences among obese and control groups of both cities. Overall, the obese subjects manifested a larger number of taxa (688) than the controls (659), a trend that aligns with a recent study [1]. However, other studies on saliva and gut microbiota disclosed the opposite [11, 34]. These observations indicated that obesity correlates with the depletion of certain salivary bacteria and microbial shifts. In the current study, fraction of Firmicutes in saliva was stable in all groups, while the obese group of Jijel city (A2) manifested an interesting diminution in the fraction of bacteroidetes and proteobacteria**.** In global, Firmicutes and Bacteroidetes were the two utmost frequent bacterial phyla in the SM and are linked to energy homeostasis [35]. Nonetheless, research on the

connection between obesity and Firmicutes/Bacteroidetes (F/B) ratio suggested conflicting findings. Previous study declared higher (F/B) ratio in obese individuals[35], however, subsequent studies failed to replicate these results [12, 36], and some, even reported opposite associations [1]. Magne *et al.*[37] discussed the connection between obesity and the (F/B) ratio and contended the challenges in its acceptance as the hallmark of obesity.

Regarding the taxonomic frequency distribution at the genus level, it is intriguing to note that *Streptococcus* was the upmost represented taxa responsible for approximately 30% of the total bacterial saliva, this observation conforms to the SM's typical pattern, in which few taxa recruit the majority of sequences [38]. We should also point out the similar percentages of *Streptococcus* and *Rothia* among all groups, however, de Andrade *et al.*[13] reported the same percentage of *Streptococcus* and higher relative abundance of *Rothia* in obese females. It has to be emphasized that in the SM, *Streptococcus* is one of the most bacterial communities commonly found [38].

In our study, one of the most reliable findings supported by both core microbiome composition and beta analysis was the over-representation of genus *Pseudomonas* in the obese group of Jijel city (A2). This finding aligns with a recent study reporting a slight higher abundance of *Pseudomonas* among Brazilian obese individuals [13]. Owing to their strong aerobic character, *Pseudomonas* species are believed to belong to the transient oral bacteria which rarely colonize the oral cavity, furthermore, they were found in the lower respiratory tract and connected with nosocomial infections [39].

Consistent with previous researches, we noted some variations in the occurrence of a particular bacterial population, Salivary Gram negative *Heamophilus* was enriched among obese and control individuals from Tlemcen city, which is on opposition with previous studies reporting its higher relative abundance in normal weight individuals [1, 11 and 13]. Moreover**,** Genus *Granulicatella*, identified in all individuals and defined as a core-microbiome, is recognized as commensal element in the human oral ecosystem, whereas, *Granulicatella* species, have been also associated with endodontic infection and implicated in pancreatic cancer, providing evidence for the hypothesis that specific oral bacteria could be involved in systemic diseases [1]. Previous studies recorded its higher relative abundance among obese participants [11, 36]. Similarly, one of the key periodontal pathogens, *Porphyromonas* has been reported to be linked to obesity [1], this fact does not correlate with the current research outcomes, revealing enriched *Porphyromonas* species among normal weight females. These controversies in observations could be attributed to several factors, including, study design and targeted age, prior research indicated that age could have an effect on the salivary microbiome [40]. Interestingly, it has been suggested that geographical location could have a potential effect on the SM composition. Environment, local cuisines and climate changes vary between countries and areas, leading to different diets and lifestyle behaviors which impact significantly the composition of human microbiomes.

This is a pilot study, thus one of its limitations is the small sample size wich might lead to the lack of strong correlation. Another potential weakness is that, due to cost limitations, samples of each group were pooled and analyzed simultaneously which did not allow the characterization of single sample salivary composition. However, as far as we know, this study is the first to explore the composition of SM composition of Algerian population using 16S rRNA sequencing technique. Saliva samples; non-invasive and easily collectable biological materials, were used. Only female subjects were enrolled so as to prevent gender-related variations already reported in saliva microbiota diversity. The potential impact of confounding external factors, namely drugs, antibiotics and pathological conditions was avoided by the implementation of strong exclusion criteria.

CONCLUSION

This preliminary research contributes to the first taxonomic characterization of the SM of Algerian women using metagenomic approach. Our data indicated altered bacterial composition in terms of BMI and area of living. We reported the impact of oral health status, socio-economic status and lifestyle on the prevalence of obesity. The scope of this pilot study was to provide a snapshot of the Algerian salivary microbiome and its changes related to various factors. Future investigations from larger cohorts using deeper metagenomic sequencing are recommended to provide an improved knowledge on the SM's function in health and disease.

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Conflict of Interest:
The authors declare that there is no conflict of interest.

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Ethical approval:
This study was reviewed and granted by the Ethical Committee of Tlemcen University (Algeria) (approval number: ED. Autism. 05.22 dated 12nd April 2022).

Author contributions:

- Conception and design of the study: Nihel Klouche-Khelil, Houria Ouled-Haddar and Hassen Cheraitia.
- Acquisition of data: Manel Zeraoulia and Kahina Gribi.
- Writing the original draft: Manel Zeraoulia.
- All authors have contributed to the Analysis and interpretation of data. All authors have read, critically analyzed and approved the final version of the manuscript.

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