academicJournals

Vol. 9(23), pp. 1548-1557, 10 June, 2015
DOI: 10.5897/AJMR2015.7457
Article Number: 2529D1C53672
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Oral microflora of supragingival and subgingival biofilms in Algerian healthy adults

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Received 28 February, 2015; Accepted 7 May, 2015

The purpose of this investigation was to define the cultivable oral microflora in supragingival and subgingival plaques of Algerian healthy adults. Supragingival and subgingival plaque samples were collected from 65 Algerian caries-free, periodontally healthy subjects. Samples were taken from approximal surfaces and analysed for bacterial content after being inoculated on non-selective and selective media and incubated under different atmospheres; aerobic, capnophilic and anaerobic. The standard identification procedures by biochemical tests were used. Pearson's Chi-square χ2 test (P < 0.05, χ2-test) was used to assess the differences between the isolation frequencies. Gram negative anaerobic rods (*Porphyromonas assacharolytica, Porphyromonas gingivalis, Prevotella melaninogenica*, *Prevotella intermedia*, *Prevotella buccae*, *Fusobacterium mortiferum, Bacteroides ureolyticus*, *Bacteroides ovatus*, *Bacteroides eggertii*, *Capnocytophaga* sp. and *Aggregatibacter actinomycetemcomitans*) more often were detected in subgingival plaque than supragingival plaque (p<0.05, χ2-test). However, streptococci and *Actinomyces naeslundii* were isolated more frequently from supragingival plaque. (p<0.05, χ2-test). Facultative anaerobic Gram positive cocci were also isolated both from supragingival and subgingival plaques in comparable proportions (P>0.05, χ2-test) with the predominance of enterococci which were isolated even from supragingival and subgingival plaques in considerable proportions. The supragingival bacterial flora in healthy adults was composed mainly of Gram positive cocci and anaerobic Gram positive rods with the predominance of Streptococci and *Actinomyces naeslundii*, respectively; whereas, anaerobic Gram negative rods and facultative anaerobic Gram positive cocci were the predominant bacteria in subgingival plaque.

Keys words: Oral microflora, dental biofilm, supragingival plaque, subgingival plaque, cultivable bacteria, healthy adults.

INTRODUCTION

The commensal human microbiome is estimated to outnumber the amount of human body cells by a factor of 10 (Turnbaugh et al., 2009). These complex microbial communities are normal residents of the human body and carry a broad range of functions indispensable for the well being of the host. (Wilson, 2008).

The oral cavity, like other habitats in the body, is colonized by a characteristic and complex microbiota that grows as diverse oral biofilms. However, when the balance between the microbiota and the host is lost, the disease is manifested (Zaura et al., 2009). Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans. In addition, some oral bacterial species have been implicated in several systemic diseases, such as bacterial endocarditis (Berbari et al., 1997), aspiration pneumonia (Scannapieco 1999), osteomyelitis in children (Dodman et al., 2000), preterm low birth weight (Offenbacher et al., 1998; Buduneli et al., 2005) and cardiovascular disease (Beck et al., 1996; Wu et al., 2000). Despite early theories focusing on identifying a single pathogen responsible for oral diseases such as dental caries, gingivitis and chronic periodontitis, it is now generally accepted that these diseases result from the concerted actions of multispecies microbial communities of the oral biofilm (Do et al., 2013). Bacterial cultural methods were previously used as the reference method for detection of oral anaerobes, this approach is still known as gold standard to identify the major putative periodontal pathogens and a large number of oral bacteria in order to study the mechanism and nature of oral colonization, or to predict treatment outcome (Jervoe-Storm et al., 2005, Verner et al., 2006, Tomazinho and Avila-Campos 2007; Atieh 2008; Kistler et al., 2013). In contrast to an earlier view that the oral microbiome consists of large numbers of uncultivated species (Paster et al., 2001), it has been recently demonstrated that the majority of oral bacterial profiles detected by 16S pyrosequencing method could be mapped to cultivated species (Griffen et al., 2012)

The impact of the oral microbial community on shifting the balance from health to disease cannot be understood without a comprehensive view of a healthy community. Unfortunately, little attention has been paid to the human oral microbiome of the healthy oral cavity, as most studies of the human oral cavity have focused on identifying bacteria that might be associated with diseases (Becker et al., 2002; Kumar et al., 2003; Diaz et al., 2006; Kilian et al., 2006; Machado de Oliveira et al., 2007; Faveri et al., 2008). In order to diagnose and treat disease at an early and reversible stage, one needs to describe the commensal microbiome associated with health (Keijser et al., 2008). Thus, understanding changes in the oral microbiome at the early stages of periodontitis and dental caries, the most prevalent chronic oral diseases, would allow diagnosis and treatment before the appearance of periodontal pockets or dental hard tissue loss (Zaura et al., 2009). Previous studies have shown significant differences in the mean proportions of subgingival species in samples from healthy and periodontitis subjects in different countries. In fact, the microbial profiles of subgingival plaque samples differed from Swedish and American subjects who exhibited periodontal health or minimal disease (Haffajee et al., 2005). Furthermore, the microbiological profile of pooled subgingival plaque sample seemed to differ significantly between periodontal patients of Caucasian and Asian ethnic origin (Kim et al., 2009). Since there are no data on the healthy oral microbiome of adult individuals in Algeria, the present investigation aimed to estimate the detailed bacterial species richness of supragingival and subgingival microflora of the healthy adult population in the west of Algeria (Tlemcen), and more specifically to compare the cultivable bacteria of supragingival and subgingival plaques in 65 caries- free and periodontally healthy subjects.

MATERIALS AND METHODS

Subjects

Sixty five (65) subjects, representing both genders, ranging in age from 18 to 35 and with no clinical signs of oral diseases were included in the study. Subjects did not suffer from severe halitosis. They were required to have no pockets with probing depth >4 mm. Subjects did not have active white spot lesions or caries on the teeth and had a full set of natural dentition or at least 24 teeth and none of them wore any removable or fixed prosthetic appliances. They had not used antibiotics for the last three months because antibiotic therapy may change the density and composition of the normal flora and it takes weeks to return to normal. Each individual signed an informed consent document. The approval of the local Ethics Committee was obtained prior to the study.

Microbiological sampling

Sampling was performed in the morning before the participants ate breakfast; each subject was asked to refrain from eating or drinking and tooth cleaning for 12 h before sampling. Approximal supragingival plaque was selected to be sampled; this site was selected regarding its protection from cleaning and oral removal forces which enable the best development of dental biofilm at these stagnant sites.

The area to be sampled was isolated and kept dry with cotton rolls. Approximal supragingival plaque was taken with a sterile cotton swab (Citolabo, France) between the central and lateral incisor, between the premolars, and between 1st and 2nd molars. On the other side, the subgingival samples were taken from the gingival crevice in the same sites from where the approximal cotton swab samples were taken. The paper point (Revo-S^{TM}, Micro-Mega, France) was kept in place for 15 s and moved around the abutment.

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For each participant, both supragingival and subgingival plaque were collected and pooled separately into two sterile eppendorf tubes consisting each of 1 ml prereduced BHI (Brain Heart Infusion, pH 7.2 Oxoid, Basingstoke, UK) broth and processed for analysis immediately.

Cultivation

Plaque samples were processed for microbiological examination immediately after collection as follows: mixed on a Vortex shaker (IKA VibriFix, Staufen, Germany) for 30 s. Samples were diluted (10^{-1}-10^{-4}) in prereduced BHI broth and aliquots of 100 µl of each dilution and the corresponding undiluted suspension were plated onto non-selective and selective media. Columbia agar base (Oxoid, Basingstoke, UK) supplemented with 5% (v/v) laked blood, was used to isolate cultivable facultative and anaerobic bacteria. For selective isolation of anaerobic Gram negative rods, Kanamycin -Vancomycin Laked blood agar (KVLB; Oxoid, Basingstoke, UK) was used. Chocolate agar was used for the isolation of capnophiles, MacConkey agar (Oxoid, Basingstoke, UK) for Enterobacteria, and Chapman agar (Oxoid, Basingstoke, UK) for Staphylococci. Inoculated plates were incubated at 37°C for two to seven days in different atmospheres: aerobic atmosphere, anaerobic atmosphere using an anaerobic gas generating system composed of anaerobic jar (BD GasPack^{TM} EZ container, USA) with gas container generating sachets (Genbox anaer, Biomérieux, France), and capnophilic atmosphere (5% CO_2) using CO_2 generating pouch system (GENbag, Biomérieux, France).

Identification of bacterial strains

Each different colony type from positive cultures was subcultured for purity and identification. Results from Gram-staining and atmospheric growth requirements of each colony type were used to determine the additional biochemical tests required to identify the isolates. The standard identifications of bacteria with commercial kits (API 20A, API 20Strep, API 20E, API 20 NE, and API 20Staph) (Biomérieux, France) were used. Antibiotics sensitivity testing was also used as further additional tests. Bacterial identification was achieved using the API System Electronic Codebook Program.

Statistical analysis

Statistical analysis was performed on SPSS statistics software version 22 and the Pearson's Chi-square $\chi 2$ test was used to assess the differences between the isolation frequencies. Statistical significance was set at P-value < 0.05.

RESULTS

This study was carried out in 65 caries free and periodontally healthy adults, and 130 supragingival and subgingival plaque samples were treated during this study which was conducted from October 2013 to June 2014.

Isolated bacteria

Two hundred and forty three (243) species were isolated in 130 supragingival and subgingival samples, with an average of two bacterial species per sample, the bacterial respiratory types in supragingival and subgingival samples were distributed as follows: aerobic bacteria were isolated in 54.5% of supra-gingival samples and 25.8% of subgingival samples, anaerobic bacteria were detected in 21.2% of supragingival samples and 42% of subgingival ones, while association of aerobic and anaerobic bacteria was also indicated both in supra-gingival and subgingival samples in 24.2 and 32.25% of cases, respectively (Table 1).

Distribution of isolated bacteria in supragingival and subgingival plaque according to bacterial morphotype and respiratory type

Aerobic bacteria were isolated more frequently from supragingival plaque (67.7%) compared to in subgingival plaque (30.8%). Conversely, anaerobic bacteria were more frequent in subgingival plaque (62.3%) than supragingival plaque (26.2%) ($p< 0.05$) (Figure 1). Moreover, There was a significant difference between supragingival and subgingival biofilm in Gram-positive (aerobic and anaerobic) and Gram-negative bacteria (aerobic and anaerobic) ($p<0.05$), with a large predo-minance of Gram positive bacteria in supragingival plaque (71.5%) whereas, Gram negative bacteria were more abundant in subgingival plaque (41.5%) (Figure 2).

Aerobic bacteria

Table 2 presents the distribution of aerobic bacteria in supragingival and subgingival plaques, streptococci (55.4%) (*Streptococcus intermedius, Streptococcus constellatus, Streptococcus acidomonimus, Streptococcus agalactiae, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus anginosus, Streptococcus uberis* and *Streptococcus oralis*) and *Actinomyces naeslundii* (20%) were isolated more frequently from supragingival plaque ($P< 0.05$) and were the predominant bacteria of the supragingival microflora. In addition, other Gram positive cocci (*Enterococcus faecium*, *Enterococcus avium*, *Lactococcus lactis, Aerococcus viridans, Gemella haemolysans,* and *Abiotrophia defectiva)* were also isolated both from supragingival (38.5%) and subgingival (41.5%) plaques in comparable proportions ($P>0.05$) with predominance of enterococi (*E. faecium* and *E. avuim)* which were isolated even from supragingival and subgingival plaques in considerable proportions (17% and 21.5%), respectively ($p>0.05$) (Table 2).

Anaerobic bacteria

As evident from Table 3, Gram negative anaerobic rods

Table 1. Distribution of bacterial respiratory types in supragingival and subgingival samples.

Bacterial respiratory types	Aerobic bacteria	Anaerobic bacteria	Aerobic + Anaerobic Bacteria
Supragingival samples	54,5%	21.2%	24.2%
Subgingival samples	25.8%	42%	32.25%

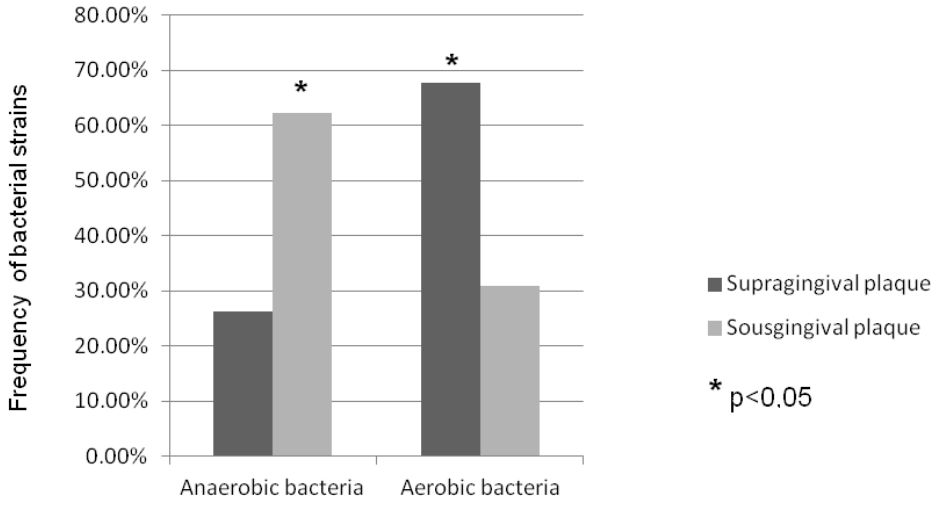

Figure 1. Anaerobi and aerobic bacteria in Supragingival and subgingival plaque of Algerian healthy adults.

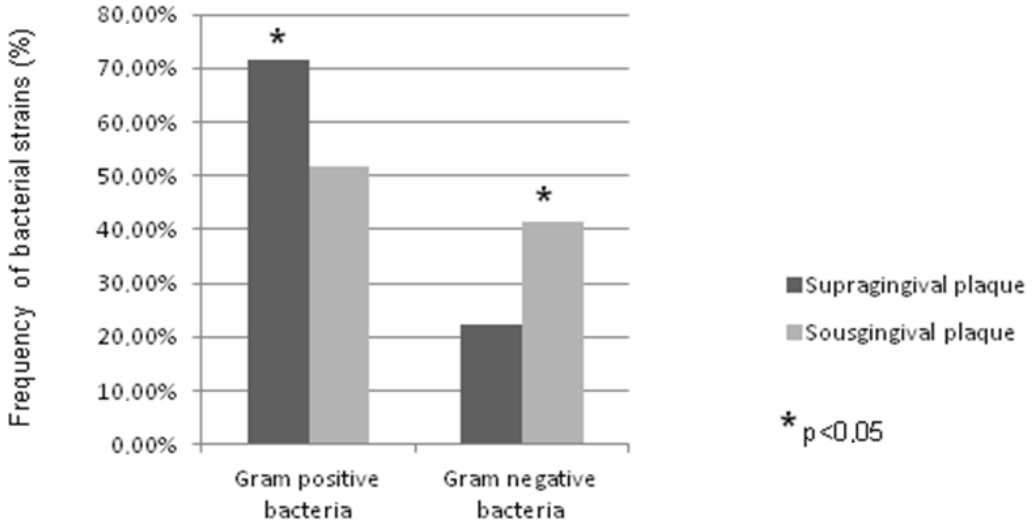

Figure 2. Gram positive and Gram negative bacteria in Supragingival and subgingival plaque of Algerian healthy.

(*Porphyromonas assacharolytica, Porphyromonas gingivalis, Prevotella melaninogenica*, *Prevotella intermedia*, *Prevotella buccae*, *Fusobacterium mortiferum, Bacteroides ureolyticus*, *Bacteroides ovatus*, *Bacteroides eggertii*, *Capnocytophaga* sp. and *Aggregatibacter actinomycetemcomitans*) were detected more often in subgingival plaque in high proportion (66.2%) ($p < 0.05$), and they were the most frequently isolated bacteria with

Table 2. Number and isolation frequencies (%) of anaerobic bacteria in supragingival and subgingival plaques of Algerian healthy subjects**.**

Bacterial species	Supragingival plaque (n= 65) Subgingival plaque (n= 65)				
	Number[a]	Frequency[b]	Number[a]	Frequency[b]	P value[c]
Anaerobic bacteria					
Peptostreptococcus sp.	0	0	2	3.07%	
Actinomyces israelii	0	0	4	6.2%	
Bfidobacterium sp.	3	4.6%	8	12.30%	p=0.009
Propionibacterium propionicum	2	3.07%	3	4.6%	
Actinomyces naeslundii	13	20%	6	9.23%	p=0.008
Lactobacillus acidophilus	2	3.07%	5	7.7%	
Lactobacillus fermentum	2	3.07%	3	4.6%	
Veillonella parvula	0	0	7	10.8%	
Anaerobic Gram negative rods	12	18.5%	43	66.2%	P=0.007
Prevotella melaninogenica	3	4.6%	3	4.6%	
Prevotella intermedia	0	0	4	6.2%	
Prevotella buccae	0	0	3	4.6%	
Porphyromonas assacharolytica	1	1.5%	3	4.6%	
Porphyromonas gingivalis	0	0	5	7.7%	
Bacteroides ureolyticus	2	3.07%	3	4.6%	
Bacteroides ovatus	0	0	2	3.07%	
Bacteroides eggertii	0	0	2	3.07%	
Fusobacterium mortiferum	0	0	2	3.07%	
Capnocytophaga sp.	6	9.23%	14	21.5%	
Aggregatibacter actinomycetemcomitans	0	0	2	3.07%	
Bacteria	34		81		

[a]Number of bacterial strains isolated from supragingival and subgingival plaques. [b]Detection frequency (%) of bacterial strains isolated from supragingival and subgingival plaques. [c]Pearson's chi-square test ($\chi 2$, $P < 0.05$) comparing the detection frequencies of bacterial strains.

predominance of *Capnocytophaga* sp. (21%) and *Prevotella* sp. (15%). *Bifidobacterium* sp. (12.3%) and *Veillonella parvula* (10.8%) were also isolated more frequently from subgingival plaque (P<0.05) (Table 3).

DISCUSSION

The findings of this study show a crucial biodiversity of the oral microflora both in supragingival and subgingival plaque of the healthy oral cavity. In supragingival samples, aerobic bacteria accounted for 54.5% of cases and 25.8% of cases in subgingival samples, while anaerobic bacteria were isolated in 21.2% of supragingival samples and 42% of subgingival ones. On the other hand, associations of aerobic and anaerobic bacteria were also observed both in supragingival (24.2%) and subgingival (32.25%) samples.

It has long been known that oral bacteria preferentially colonize different surfaces in the oral cavity as a result of specific bacterial adhesins binding to complementary specific receptors on a given oral surface (Gibbons et al., 1976; Gibbons 1989). The study of Mager et al. (2003) showed that the profiles of 40 cultivable bacterial species differed markedly on different oral environments; saliva, supragingival and subgingival plaques from healthy subjects. Such reports support the results of the present investigation that revealed a statistically significant difference in bacterial composition of supragingival and subgingival plaques of the healthy oral cavity. This difference was between aerobic bacteria which were isolated more frequently from supragingival plaque and anaerobic bacteria which were more frequent in subgingival plaque. Additionally, there was a predominance of Gram positive bacteria in supragingival plaque whereas, Gram negative bacteria were more abundant in subgingival

Table 3. Number and isolation frequencies (%) of aerobic bacteria in supragingival and subgingival plaques of Algerian healthy subjects.

Bacterial species	Supragingival plaque (n= 65)		Subgingival plaque (n= 65)		
	Number[a]	Frequency[b]	Number[a]	Frequency[b]	P value[c]
Aerobic bacteria					
Gram positive cocci except streptococci	25	38.5%	27	41.5%	P=0.089
Enterococcus sp *:*	11	17%	14	21.5%	
Enterococcus avium	1	1.5%	5	7.7%	P=0.372
Enterococcus faecium	10	15.4%	9	13.85%	
Lactococcus lactis	5	7.7%	8	12.30%	
Aerococcus viridians	5	7.7%	4	6.2%	
Gemella haemolysans	4	6.2%	0	0	
Abiotrophia defectiva	0	0	1	1.5%	
Streptococcus sp.	36	55.4%	9	13.85%	p=0.000
Streptococcus intermedius	4	6.2%	1	1.5%	
Streptococcus constellatus	2	3.07%	0	0	
Streptococcus acidomonimus	4	6.2%	3	4.6%	
Streptococcus agalactiae	5	7.7%	2	3.07%	
Streptococcus mutans	4	6.2%	1	1.5%	
Streptococcus pneumoniae	4	6.2%	0	0	
Streptococcus anginosus	5	7.7%	0	0	
Streptococcus uberis	3	4.6%	1	1.5%	
Streptococcus oralis	5	7.7%	1	1.5%	
Staphylococcus epidermidis	3	4.6%	0	0	
Staphylococcus capitis	4	6.2%	0	0	
Micrococcus sp.	3	4.6%	0	0	
Aeromonas hydrophila	2	3.07%	1	1.5%	
Moraxella sp.	2	3.07%	0	0	
Pseudomonas luteola	2	3.07%	0	0	
-Enteric rods	11	17%	3	4.6%	p=0.006
Klebsiella pneumonia	4	6.2%	1	1.5%	
Enterobacter amnigenus	3	4.6%	2	3.07%	
Serratia ficaria	4	6.2%	0	0	
Bacteria	88		40		

[a]Number of bacterial strains isolated from supragingival and subgingival plaques. [b]Detection frequency (%) of bacterial strains isolated from supragingival and subgingival plaques. [c]Pearson's Chi-square test ($\chi 2$, $P < 0.05$) comparing the detection frequencies of bacterial strains.

plaque. Rozkiewicz et al. (2006) found that Gram positive bacteria were isolated more frequently than Gram negative bacteria ($p< 0.05$) from supragingival plaques of caries free children. Oral anaerobic Gram negative rods were often defined as putative periodontal pathogens (Noiri et al., 2001). Hardly any data was given on their carriage in the healthy adults' population living in the Arab Maghreb region in particular Algeria.

However, some authors have reported the high frequency of these organisms in subgingival plaque of Algerian patients with aggressive and chronic periodontitis **(**Yacoubi et al., 2010). The present study demonstrates a high prevalence of anaerobic Gram negative rods (66.2%) in subgingival plaque of Algerian caries free and periodontally healthy adults with predominance of *Capnocytophaga sp* (21%) and *Prevotella* sp. (15%) followed by *Porphyromnas* sp. (12.3%) and *Bacteroides* sp. (10.74%). The pigmented *prevotella* species were more detected; *P. melaninogenica* (4.6%) and *P. intermedia* (6.2%). *Porphyromnas* was isolated in two species;

P. gingivalis (7.7%) and *P. assacharolytica* (4.6%). Previously, *Porphyromonas gingivalis* was not considered as belonging to the commensal oral microflora view its potential association with periodontal disease (Aas et al., 2005). Nevertheless, recent studies revealed a high prevalence of *Porphyromonas gingivalis* and other anaerobic bacteria belonging to Bacteroides phyla (*Prevotella sp, Capnocytophaga* sp., and *Bacteroides* sp.) in saliva of healthy subjects and they were less frequent in dental plaque of the same subjects (Keijser et al., 2008). The other anaerobic gram negative rods were rarely isolated in this study, *Fusobacterium mortiferum* (3.07%) and *Aggregatibacter actinomycetemcomitans* (3.07%). *Fusobacterium sp* was often detected in dental plaque of healthy subjects (Keijser et al., 2008). However, *Aggregatibacterium actinomycetemcomitans* was found associated to periodontal disease and it was very abundant in subgingival plaque of Algerian patients with aggressive periodontitis and less frequent in patients with chronic periodontitis (Yacoubi et al., 2010).

On the other hand, the results of this investigation show the importance of Gram positive cocci both in supragingival and subgingival plaque with predominance of streptococci (55.4%) in supragingival plaque. The most common species isolated were *S. oralis*, *S. anginosus*, *S. agalactiae* and they accounted each for 7.7% of cases. Several species of *Streptococcus*, including *S. sanguinis*, and *S. gordonii* were detected on the tooth surface of healthy subjects (Aas et al., 2005). In contrast, *Peptostreptococcus* sp. was rarely isolated in this study and it was detected only in subgingival plaque in low frequency (3.07%). Kumar et al. (2005) have reported the association of *Peptostreptococcus* with periodonditis due to its high carriage in these entities.

Moreover**,** our results show that *Enterococcus* sp. was very abundant in supragingival (21.5%) and subgingival plaque (17%). It has long been known that enteroccoci are the common inhabitants of the human oral cavity and they were often isolated from dental plaque (Smyth et al., 1987). However, some authors reported a high frequency of *E. faecalis* in teeth with necrotic pulp and in teeth with failing endodontic treatment (Gomes et al., 2006); this species was also associated with different forms of periradicular diseases (Rôças et al., 2004). In this study, two species of *Enterococcus* were isolated; *E. avium and E. faecium* with predominance of *E. faecium* in supragingival samples (15.4%) and subgingival ones (13.85%). Some authors' investigations aimed to inspect whether enterococci from food are able to reside in oral biofilm and showed that food-borne enterococci might not only be transient microorganisms but could also survive in the oral biofilm (Al-Ahmad et al., 2010).

Other aerobic Gram positive cocci were also detected in this study; *Lactococcus lactis* was more frequent in sub-gingival plaque (12.30%) whereas, *Gemella haemolysans* (6.2%), *Staphylococcus epidermidis* (4.6%), *Staphylococcus capitis* (6.2%)*, and Micrococcus* sp. (4.6%), were isolated only from supragingival plaque. Furthermore, *Actinomyces naeslundii* (20%) were isolated more frequently from supragingival plaque, while *Bifidobacterium* sp. (12.30%) and *Veillonella parvula* (10.8%) were more abundant in subgingival plaque. Recent findings indicated high proportions of Actinobacteria, particularly *Actinomyces* and they were higher in health and remained constant from health to periodontitis (Abusleme et al., 2013). The results of Keijser et al. (2008) showed a large abundance of streptococci and *Actinomyces* sp. in dental plaque whereas, *Veillonella parvula* was often found both in dental plaque and salivary microfolra. *Bifidobacterium* sp. and *Veillonella parvula* could frequently be isolated from subgingival and supragingival plaque and were found to be associated with periodontal disease and dental caries. (Rozkiewicz et al., 2006; Filoche et al., 2010).

Our results concurs with previous reports that showed high numbers of aerobic and facultative anaerobic Gram positive bacteria, in particular streptococci and *Actinomyces sp* with lower frequencies of anaerobes and Gram negative organisms in supragingival surfaces (Sixou et al., 2007; Do et al., 2013). In contrast, subgingival biofilm had the highest proportions of proteolytic obligate anaerobes, many of which were Gram negative anaerobes. (Sixou et al., 2007; Do et al., 2013)

Furthermore, it is interesting to note that this investigation showed a crucial biodiversity with more than 40 bacterial species of aerobic and anaerobic bacteria both in supragingival and subgingival plaques of healthy adults. Thus, enteric rods (*Klebsiella pneumonia, Enterobacter amnigenus, and Serratia ficaria)* and *Pseudomonas luteola* were also isolated in this study from supragingival plaque but in low proportion from subgingival plaque. The prevalence of oral enteric rods have been found to be in relation with oral and general health, so that an increased prevalence of oral Enterobacteriaceae carriage have been detected in patients with illnesses of varying severity compared with healthy subjects (Sedgley and Samaranayake 1994). Many authors reported that individuals in good health are able to eliminate the daily load of Gram-negative enteric rods from the oral cavity by means of innate defense mechanisms, so that bacterial counts rapidly decrease, and less than 1% of the original inoculum can be recoverable within 3-h of inoculation (Laforce et al.,1976; Mobbs et al., 1999).

In addition, other authors indicated that Gram-negative enteric rods are merely transient microorganisms within the subgingival environment both in healthy and chronic periodontitis subjects and suggested that the periodontal clinical status appeared not to be influenced by thepresence of these species (Martínez-Pabón et al., 2010). However, there was definitely a higher prevalence of Enterobacteriaceae among nail-biting individuals than

the individuals without any habit. This higher prevalence of Enterobacteriaceae among subjects with nail-biting could be due to orofecal route of transmission of Enterobacteriaceae and poor general hygiene maintenance (Baydaş et al., 2007; Reddy et al., 2013).

The crucial biodiversity of supragingival and subgingival plaques indicated in this study was confirmed by several previous reports that showed that the highest numbers and the greatest diversity of micro-organisms are found at stagnant sites within the oral cavity such as approximal surfaces which afford protection from oral removal forces (Do et al., 2013). Moreover, analysis of dental plaque in healthy adults demonstrated much more diversity than originally hypothesized (Marsh and Martin, 1999). Other authors reported that oral microbiomes of children suffering from severe dental caries are much less diverse than those of children with oral health (Kanasi et al., 2010). On the other hand, asymptomatic lesions of infected root canals displayed a higher level of biodiversity than did the symptomatic ones (Filoche et al., 2010). It has been reported also that the need for biodiversity in health may suggest that every species carries out a specific function that is required to maintain equilibrium and homeostasis within the oral cavity (Do et al., 2013). Subsequently, in health, microorganisms prevent disease progression in several ways: they can prevent the adherence of pathogens onto specific surfaces by occupying the niche preferred by a pathogen, they can actively prevent a pathogen from occupying a site, they can hinder a pathogen's abilities to multiply, and they can degrade a pathogen's virulence factors (Socransky and Haffajee, 1992).

Defining the healthy oral cavity microflora is a very important tool to understand microbial diversity and function as well as etiology of disease better, in order to diagnose diseases at an earlier and reversible stage (Zaura et al., 2009). Many studies indicated that patients with high salivary levels of potentially cariogenic bacteria such as mutants streptococci and lactobacilli, were designated as being at "high risk" for future caries, and were selected for additional clinical and therapeutic attention (Shi et al., 1998; Walsh and Tsang 2008). Such investigations share a common goal, which is to support the clinician in the diagnosis of oral diseases, providing crucial information for advanced treatment plans and therapy for "at risk" patients, and prevention strategies for healthy patients (Gibbons, 1989).

Culturing organisms remains an important tool for the detection of bacteria from dental plaque biofilm and other sites in the oral cavity in order to detect and understand pathological changes that occur within the microbial ecosystem and which may break down the ecological balance between the microbiota and the host and initiate disease within the oral cavity. This technique can detect multiple bacterial species coincidentally as it can be done by culture –independent methods, but the bacterial cultures have real advantages, that they can detect unexpected bacteria and also allow the determination of antibiotic resistance.(D'Ercole et al., 2008)

In conclusions, this investigation shows a crucial biodiversity in supragingival and subgingival plaques of the healthy oral cavity of Algerian adults. In addition, bacterial composition differed markedly in supragingival and subgingival plaques; the supragingival bacterial flora in healthy adults was composed mainly of Gram positive cocci and anaerobic Gram positive rods with the predominance of streptococci and *Actinomyces naeslundii,* respectively. Whereas, anaerobic Gram negative rods and facultative anaerobic Gram positive cocci were the predominant bacteria in subgingival plaque. Although, we confirmed previous observations of species associated with oral health, we also extend those findings, implicating additional species that will be targets for future research that could provide an important tool in understanding host–microbe interactions in health and disease. Further study of the oral microflora associated with oral health in other oral sites is also warranted and may lead to new therapeutic approaches to prevent oral diseases.

Conflict of interest

There is no conflicting interest.

ACKNOWLEDGEMENTS

This research was supported by the National Committee of Research program and assessment (Cnepru-code I02020130110) which is gratefully acknowledged.

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