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Bacterial Microbiota Analysis Present in the Nose and Pharynx of a Mexican Young Population

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A B S T R A C T

Culture-independent microbiota is relatively unexplored in Mexican population. The aim of this study was to characterize the microbiota of Mexican young healthy adults by means of traditional culture and metagenomic analysis in order to provide novel insights and have a better understanding about the healthy baselines from which to detect differences associated with diseases. The bacterial microbiota of the nose and pharynx from 75 healthy nonsmoking Mexican young adults was examined by conventional cultures and culture-independent methods. The hypervariable region (V6-V8) of the16S rRNA gene was PCR amplified from isolated DNA and DGGE analyzed, bands excised were sequenced and phylogenetic analysis was done. The study showed that the bacterial microbiota of the pharynx was richer than that of the nose. Using conventional culture methods results showed that gram positive *Staphylococcus aureus* and *S. epidermidis* were found in both niches and gram negative bacteria such as *Escherichia coli, Klebsiella sp*. and *Moraxella* sp. were the most abundant genera in the nose and *Enterobacter* sp. in the pharynx. *Firmicutes* and *Proteobacteria* phyla accounted for the majority of the bacteria detected in both niches. To our knowledge this is the first report describing partially the composition of and the variability within the nasopharyngeal microbiota of a Mexican young adult population and therefore we will be able to investigate other different cities and compared them to find out where are the highest microbial contaminated zones and take some hygienic measures in accordance to the normativity reports.

Keywords

Bacterial Microbiota, Nose and Pharynx, metagenomic analysis.

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Introduction

The nostrils or anterior nares are the outermost segment of the nose and is considered a transition zone from the skin to the nasal cavity. Like skin, the nostrils contain sebaceous glands, sweat glands, and hairs and are lined by a keratinized, stratified squamous epithelium more similar to that of skin than to the mucus-producing,

ciliated, columnar epithelium of the nasal cavity (Lemon, 2010; Wilson, 2005).

The nostrils help filter inhaled air, which contains low numbers of extremely diverse microbes (Lemon, 2010; Brodie *et al.,* 2007; Fierer *et al.,* 2008). The pharynx is constantly exposed to both inhaled and ingested microbes. This microbiota is influenced by different factors like age, immunological conditions, and the environment that in this particular case is highly polluted in Mexico City (Rosas *et al.,* 2006; Secretaria de Medio Ambiente, 2012). The nostril and oropharynx are considered distinct habitats. While the pathogen *Staphylococcus aureus* colonizes both sites (Lemon, 2010, Widmer *et al.,* 2008, Mertz, 2007, Wilson, 2005).

The knowledge on nose and pharynx microbiota has focused on pathogen carriage using traditional cultivation methods, however currently molecular approaches including DGGE 16S gene analysis, pyrosequencing, 16S rDNA PCR-RFLP, analysis of phylochip, High-Throughput Sequencing (Chakraborty *et al.,* 2014; Yi *et al.,* 2014, Ling *et al.,* 2013; Aguirre *et al.,* 2012; Brugger *et al.,* 2012; Lemon *et al.,* 2010). These methods have most used to study the relation between the dynamic equilibrium of human health and disease, being the pathogenic microorganisms the best studied. In a variety of environmental samples the study of the microbial diversity is accomplished by metagenomic DNA studies and also by traditional culture methods. The later poorly predicts resident microbiota (Hauser *et al.,* 2014). Cloning and sequencing of 16S genes amplified directly from different human sites such as the oral cavity for example, demonstrated that microbial diversity is by far more extensive compared to culture-based studies (Chakraborty *et al.,* 2014).

The nostrils are known to harbor bacteria from the genera *Corynebacterium*, *Propionibacterium*, and *Staphylococcus* (Firmicutes and Proteobacteria phyla), including the important pathogen *Staphylococcus aureus* found by molecular methods (Lemon, 2010; Wilson, 2005). The nasal cavity appears dominated by resident aerobic microbiota as *Corynebacterium* spp. and *Staphylococcus* spp. detected by cultivation (Rasmussen *et al.,* 2000). In the oropharynx species from the genera *Streptococcus*, *Haemophilus*, *Neisseria* are present, and to a lesser extent *Staphylococcus* and various anaerobic bacteria (Lemon, 2010; Wilson, 2005). Oropharynx is the site of carriage of many important human pathogens, including *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, and *Staphylococcus aureus* (Bogaert *et al.,* 2011; Lemon, 2010; Mertz *et al.,* 2007; Widmer *et al.,* 2008; Wilson 2005).

The aim of this study was to investigate the microbiota of Mexican young healthy adults by means of traditional culture and metagenomic analysis in order to have a better understanding about the healthy baselines from which to detect differences associated with diseases. Our approach was chosen because this population has been scarcely studied in regard with children and older adults.

Material and Methods

Ethic Statement and Ethical Approval

Samples were collected in accordance with relevance guidelines for ethical research design, confidentiality and protection human subjects. Protocol was reviewed and approved by the ethics and biosafety committees of the Universidad Autónoma

Metropolitana-Xochimilco (Hamdan *et al.,* 2013). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (World Medical Association Declaration of Helsinki Ethical Principles for Medical Research, 1964).

Subjects and Sample Collection

As in previous studies (Hamdan *et al.,* 2013) informed consent was obtained from all individual participants for screening enrollment and specimen collection. Great care was taken to ensure that all people understood that they would participate as volunteers; no academic or economic incentives were offered.

Seventy five (75) healthy adult volunteers, whose ages between 18-25 years old were included between January and March of 2012. Criteria for participation were: no clinical signs of illness, no antibiotic therapy within 3 months before entering the study, no pregnancy or breast feeding and nonsmokers.

Separate swabs were used to collect the samples, one for the nostrils and the other for the posterior wall of the pharynx of each participant and were placed into enrichment media (tripticase soy broth) and non enrichment media (saline buffer solution). The pharynx posterior wall was swabbed without touching the tonsils, uvula, tongue, or other oral structures.

Microbial Cultivation

Nasal and pharynx swabs were first enriched by a 24 hours cultured at 37°C in tripticase soy broth and then plated onto a 5% sheep blood agar, chocolate agar, mannitol salt agar and Mac Conkey agar plates and then were incubated at 37°C for 24 h; chocolate agar plates with a 5% CO_2 atmosphere. Bacteria identification was based on colony morphology and bacteriological biochemical conventional tests (Holtz, 1993; Winn *et al.,* 2006).

Nucleic Acid Isolation

Genomic DNA was extracted from the swabs collected in saline buffer solution and from the enrichment media from nose and pharynx using a commercial kit Fast ID Genomic DNA Extraction (Genetic ID NA Inc, USA) following the manufacturer's instructions including some modifications: ten microliters of proteinase K solution (10µg/µl), 5 µL of lysostaphin solution (0.5 µg/µl) and 15 µL of mutanolysin solution (1U/µl) were added for cellular lyses.

PCR Amplification

Hypervariable region V6-V8 of the 16S rRNA gene was amplified from the isolated genomic DNA using universal primers 968f 5'-AAC GCG AAG AAC CTT ACC-3' and 1401r 5'–GCG TGT GTA CAA GAC CC-3' (Ramírez-Saad *et al.,* 2000). The gc968f forward primer contains additional 40 nucleotide GC-rich sequence the GC clamp for DGGE technic. Amplification was performed with a Whatman Biometra TProfessional Basic 96 gradient thermal cycler (Goettingen, Germany). Each mixture (25 µl final volume) contained 1 µl DNA template, 0.5 mM primer concentration, 200 mM dNTPs, 1X PCR buffer and Q solution and 2.5 U of Taq polymerase (Qiagen TaqPCR Core kit Cat. no. 201225, Qiagen GmbH, D-407224 Hilden). DNA template was denatured for 5 min at 94°C. To increase the amplification specificity and to reduce the formation of spurious byproducts, a "touchdown" PCR was performed. The

initial annealing temperature used was 10°C above the expected annealing temperature (65°C), and the temperature was decreased by 1°C every second cycle until the touchdown temperature, 55°C, was reached; then 10 additional cycles were carried out at 55°C. Primer extension was performed at 72°C for 3 min. The tubes were then incubated for 10 min at 72°C (final extension). Aliquots (2 µl) of the amplification products were first analyzed by 1.5% agarose gels electrophoresis.

Metagenome PCR-DGGE Analysis

Profiling bacteria communities from the nose and pharynx samples was assayed by PCR-DGGE. PCR products were analyzed by Denatured Gradient Gel Electrophoresis (DDGE) by using a DCode mutation detection system (Bio-Rad Laboratories, Hercules, CA, USA). According to Muyzer (1993) cited by Morales (2009) 8% acrylamide gels (37:1 acrilamide-bisacrilamide) were formed with a linear gradient between 30% and 60% denaturant; 100% denaturant is defined as 7 M urea and 40% (v/v) of formamide.

Gels were run at 85 V for 16 h in 1x TAE buffer (40 mM Tris, 20 mM acetate, 1 mM EDTA). DNA bands were visualized as previously described using the silver stain procedure (Sanguinetti *et al.,* 1994).

Sequencing of Excised DGGE Bands

Prominent bands of the DGGE profiles were excised and reamplified for sequencing purposes, following a previously described procedure by Ramírez *et al.,* (2000) and Aguirre-Garrido *et al.,* (2012). Briefly, with a sterile blade, each band was eluted in 30 µl of sterile water overnight at 4°C.

Four microliter of the eluted DNA from each DGGE band was PCR reamplified using the conditions described above. The amplicons were size-checked for 500pb size and purified with a commercial kit EZ10 SPIN COLUM (BIO BASIC INC, Ontario Canada) following the manufacturer's instructions. Purified PCR products were sequenced by Macrogen Inc. (Seoul, Korea). When no significant sequence was obtained from a specific band, or more than one band was present, the cloning strategy was used in order to obtain a single amplicon for sequence.

Cloning Procedures

Size-checked purified 16S rRNA gene amplicons were cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Transformation was performed using competent *E. coli* TOP 10 cells provided by the manufacturer. Transformed cells were plated onto Luria-Bertani agar plates supplemented with kanamycin (50ug/mL) and incubated overnight at 37°C. For the screening, five colonies were picked from each plate and plasmid extraction was performed using the Gene JET^{TM} Plasmid Miniprep Kit (Fermentas Life Science, USA). The sequence was done by Macrogen Inc. (Seoul, Korea) using the universal M13 primers as recommended.

Phylogenetic Analysis

According to Aguirre-Garrido *et al.,* (2012) all obtained 16S rRNA sequences were checked for chimeric structures using the Bellerophon program (http://comp-bio.anu.edu.au/bellerophon/bellerophon.pl) (Huber *et al.,* 2004; Domaizon *et al.,* 2013) those identified as potential chimeras were discarded. All the sequences were assigned to species level by using the Identify tool of the Ribosomal database project (Cole *et al.,* 2014), which also provides global sequence similarities. These sequences were compared using the Mega BLAST tool of the NCBI server (Table 2).

Phylogenetic trees were built with partial 16S rRNA sequences (V6 – V8 regions), the trees included the obtained sequences and those of their close relatives, as selected from MegaBLAST tool of the NCBI server (Morgulis *et al.,* 2008; Zhang *et al.,* 2000). CLUSTAL X was used for sequence alignment (Larkin *et al.,* 2007) and MEGA program Neighbor-joining phylogenies were constructed with the Jukes-Cantor distance model and the Kimura two-parameter correction. Resulting dendrograms were tested with a Bootstrap analysis of 1000 replicates, as implemented in the MEGA software package (Tamura *et al.,* 2013). Sequences of this study were deposited in the GeneBank database under the accession numbers: KR065528 through KR065575 (Table 2).

Results and Discussion

Cultured Bacteria

Eleven genera of cultured bacteria were identified from the nose and pharynx. Results showed that *Staphylococcus epidermidis* had the highest occurrence (60 %) in the nose of the tested population while *Staphylococcus aureus* (48%) in the pharynx. Almost the same genera were cultured from both niches, except for *E. coli,* that only was present in the nose (Table 1).

Microbial Communities

Profiles were generated by PCR DGGE of the V6-V8 regions of the 16S rRNA gene. Metagenomic DNA from each sample of enrichment and non enrichment media was used as a target for amplifications. In general DGGE band patterns of enrichment media from the nose consisted in four or six strong bands and several less intense, while from non enrichment media only three or four intense bands were observed. Respect to the pharynx samples from enrichment media and non enrichment media, band patterns were similar and more complex compared with those from the nose samples and they consist of eight predominant bands and a number of bands with diverse intensities (Fig. 1).

Forty four bands from the nose and pharynx samples of enrichment and non enrichment media, and four additional bands of the isolated *Staphylococcus* strains were sequenced. Two phyla *Firmicutes* and *Proteobacteria* were found in these samples. Phylogenetic affiliations from sequences of DGGE bands are shown in Fig. 2 and were assigned to four major bacterial lineages. The most representative classes found in Firmicutes were *Bacilli* and *Clostridia* and *Beta* and *Gamma-Proteobacteria* for Proteobacteria phylum.

In this study the phylogenetic composition of the nares of a young Mexican population showed that the phylum *Firmicutes* with the genera *Staphylococcus*, *Dolosigranulum* y *Clostridium* and the phylum *Proteobacteria* with the genera *Haemophilus*, *Klebsiella*, *Enterobacter* and *Citrobacter* were found. In the pharynx the phylum *Firmicutes* with the genera *Staphylococcus*, *Peptostreptococcus, Dolosigranulum* and *Bacillus* and the phylum *Proteobacteria* with the genera *Haemophilus*, *Neisseria* and *Kingella* were also found (Table 2).

Analyses of the bacterial communities present in the nose and the pharynx were performed by culture-dependent and molecular-based methods extending our knowledge on the bacterial diversity of Mexican young adults.

In this study using culture-dependent techniques *S. aureus* which is part of the normal and asymptomatic microbial community was identified in the nose and the pharynx. Several reports (Kaspar *et al.,*

2015, Wos-Oxley *et al.,* 2010 and Pettigrew *et al.,* 2008) had pointed that the nose is by far the most common habitat for *S. aureus,* but our results show that the pharynx can be also a common habitat as well. It is important to address that in a previous reported study done by Hamdan *et al.,* (2010) with a different healthy Mexican population and standard microbiological technics *S. aureus* was the most prevalent microorganism found in the pharynx with a higher occurrence compared with the nose. The results of this assay showed the same tendency confirming the high prevalence of *S. aureus* in the pharynx. Clinical implications of these findings are that healthy carriers could be spreading these bacteria among other compromised population.

Stearns *et al.,* (2015) reported the genera *Streptococcus*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, *Dolosigranulum* and *Propionibacterium* using also cultured based methods. In our study the main genera found were, *Streptococcus, Pseudomonas, Enterobacter, Aerococcus, Klebsiella, Moraxella* and *E. coli.*

The present suggest that the presence of *E. coli* and other Enterobacteriaceae members in nose is probably due to fecal pollution suspended in the environment as described by Rosas *et al.,* (2006). This author published that different *E. coli* pathotypes were mainly found in the air tested in Mexico City. As reported by Brodie *et al.,* (2007) environmental conditions have a relationship with bacterial aerial dispersal because of the climate change and could alter the composition of microbial communities in some areas. Another possibility to explain the presence of Enterobacteriaceae is by hand to nose contamination (Wos-Oxley *et al.,* 2010).

Table.1 Genera from identified cultured bacterial in the nose and pharynx

Genera	Nose		Pharynx	
	Number of samples	% of occurrence	Number of samples	% of occurrence
Staphylococcus aureus	21	28	36	48
Staphylococcus epidermidis	45	60	21	28
Staphylococcus chromogenes	5	6.7	5	6.7
Streptococcus mitis	17	22.7	24	32
Aerococcus viridians	7	9.3	12	16
Enterobacter aerogenes	19	25.3	14	18.7
Enterobacter cloacae	3	4	1	1.3
Klebsiella sp.	11	14.7	6	8
Moraxella sp.	1	1.3	1	1.3
Escherichia coli	1	1.3	-	-
Pseudomonas spp.	1	1.3	2	2.7

Table.2 Taxonomic filiations of the bands obtained from the nose and pharynx samples.

Band (accession number)	Closest GenBank sequence (accession number)	Identity %	Phyla	Class
1 EN[a] (KR065528)	*Staphylococcus aureus* (NR_115606.1)	98%	Firmicutes	Bacilli
2 EN (KR065529)	*Clostridium sordelli* (JN048957.1)	98%	Firmicutes	Clostridia
3 EN (KR065530)	*Clostridium perfringens* (JX267089.1)	89%	Firmicutes	Clostridia
4 EN (KR065531)	*Staphylococcus epidermidis* (D83363.1)	96%	Firmicutes	Bacilli
5 EN (KR065532)	*Clostridium perfringens* (JX267089.1)	95%	Firmicutes	Clostridia
6 EN (KR065533)	*Kleibsiella pneumoniae* (KJ806418.1)	99%	Proteobacteria	γ-Proteobacteria
7 EN (KR065534)	*Clostridium sordelli* (JN048957.1)	99%	Firmicutes	Clostridia
8 EN (KR065535)	*Kleibsiella oxytoca* (JX412287.1)	98%	Proteobacteria	γ-Proteobacteria
9 EN (KR065536)	*Enterobacter aerogenes* (HQ407223.1)	96%	Proteobacteria	γ-Proteobacteria
10 EN (KR065537)	*Staphylococcus aureus* (NR_115606.1)	96%	Firmicutes	Bacilli
11 EN (KR065538)	*Clostridium sordelli* (JN048957.1)	93%	Firmicutes	Clostridia
12 EN (KR065539)	*Citrobacter* sp (KF374707.1)	98%	Proteobacteria	γ-Proteobacteria
13 EN (KR065540)	Uncultured bacterium clone (KF073687.1)	98%	Undefined	Undefined
14 EN (KR065541)	*Kleibsiella pneumoniae* (EU086097.1)	98%	Proteobacteria	γ-Proteobacteria
15 EN (KR065542)	*Kleibsiella* sp. (KF914263.1)	88%	Proteobacteria	γ-Proteobacteria
16 EN (KR065543)	*Kleibsiella singaporensis* (AF250286.1)	99%	Proteobacteria	γ-Proteobacteria
17 EN (KR065544)	*Enterobacter asburiae* (EU239106.1)	91%	Proteobacteria	γ-Proteobacteria
18 EN (KR065545)	Uncultured bacterium clone (EU777050.1)	91%	Undefined	Undefined
19 EN (KR065546)	*Methylocaldum marinum* (NR_126189.1)	92%	Proteobacteria	γ-Proteobacteria
20 EP[b] (KR065547)	Uncultured *Haemophilus* (KC348749.1)	92%	Proteobacteria	γ-Proteobacteria
21 EP (KR065548)	*Neisseria* sp (KF33704.1)	89%	Proteobacteria	β- Proteobacteria
22 EP (KR065549)	*Neisseria cinerea* (KJ585681.1)	99%	Proteobacteria	β- Proteobacteria
23 EP (KR065550)	*Neisseria* sp (KC178546.1)	99%	Proteobacteria	β- Proteobacteria
24 EP (KR065551)	*Neisseria cinerea* (KJ585681.1)	100%	Proteobacteria	β- Proteobacteria
25 EP (KR065552)	*Neisseria* sp (KC178546.1)	94%	Proteobacteria	β- Proteobacteria
26 EP (KR065553)	Uncultured bacterium clone (JF238960.1)	99%	Undefined	Undefined
27 EP (KR065554)	*Neisseria cinerea* (KJ585681.1)	97%	Proteobacteria	β- Proteobacteria
28 EP (KR065555)	*Staphylococcus* sp (AB845199.1)	91%	Firmicutes	Bacilli
29 EP (KR065556)	Uncultured *Haemophilus* (KC348749.1)	97%	Proteobacteria	γ-Proteobacteria
30 EP (KR065557)	*Peptostreptococcus stomatis* (NR_043589.1)	99%	Firmicutes	Clostridia
31 EP (KR065558)	Uncultured *Haemophilus* (KC348749.1)	99%	Proteobacteria	γ-Proteobacteria
32 NEN[c] (KR065559)	*Haemophilus parainfluenzae* (JF506651.1)	95%	Proteobacteria	γ-Proteobacteria
33 NEN (KR065560)	*Clostridium sordelli* (JN048957.1)	96%	Firmicutes	Clostridia
34 NEN (KR065561)	*Staphylococcus chromogenes* (KJ83397.1)	93%	Firmicutes	Bacilli
35 NEN (KR065562)	*Staphylococcus epidermidis* (D83363.1)	93%	Firmicutes	Bacilli
36 NEN (KR065563)	*Dolosigranulum pigrum* (NR_113774.1)	98%	Firmicutes	Bacilli
37 NEN (KR065564)	*Dolosigranulum pigrum* (NR_113774.1)	95%	Firmicutes	Bacilli
38 NEN (KR065565)	*Staphylococcus aureus* (NR_115606.1)	98%	Firmicutes	Bacilli
39 NEP[d] (KR065566)	*Staphylococcus aureus* (KJ643929.1)	100%	Firmicutes	Bacilli
40 NEP (KR065567)	*Dolosigranulum pigrum* (NR_113774.1)	99%	Firmicutes	Bacilli
41 NEP (KR065568)	*Peptostreptococcus anaerobius* (AB640692.1)	99%	Firmicutes	Clostridia
42 NEP (KR065569)	*Kingella* sp (LK985395.1)	93%	Proteobacteria	β- Proteobacteria
43 NEP (KR065570)	*Kingella* sp (LK985395.1)	93%	Proteobacteria	β- Proteobacteria
44 NEP (KR065571)	*Bacillus* sp. (KJ528251.1)	92%	Firmicutes	Bacilli
45 Sa[e] (KR065572)	*Staphylococcus aureus* (KJ643929.1)	100%	Firmicutes	Bacilli
46 Sa (KR065573)	*Staphylococcus aureus* (KJ643929.1)	100%	Firmicutes	Bacilli
47 Se[f] (KR065574)	*Staphylococcus epidermidis* (D83363.1)	98%	Firmicutes	Bacilli
48 Se (KR065575)	*Staphylococcus epidermidis* (D83363.1)	97%	Firmicutes	Bacilli

[a](EN) enrichment media inoculated with nose samples, [b](EP) enrichment media inoculated with pharynx samples, [c](NEN) non enrichment media inoculated with nose samples and [d](NEP) non enrichment media inoculated with pharynx samples, [e](Sa) *Staphylococcus aureus* isolated control and [f](Se) *Staphylococcus epidermidis* isolated control.

Fig.1 DGGE profiles of V6-V8 regions of 16S rRNA gene. a) PCR products on lanes 1-4 were obtained from genomic DNA of nose enrichment media samples, lanes 5-8 shown PCR products obtained from genomic DNA of pharynx enrichment media samples. b) PCR products on lanes 1-6 were obtained from genomic DNA of nose non enrichment media samples, lanes 7-12 shown PCR products obtained from genomic DNA of pharynx non enrichment media samples. Lanes 13-15 correspond to Staphylococcus aureus genomic DNA obtained from isolated bacteria (lane 13) and S. epidermidis (lanes 14 and 15).

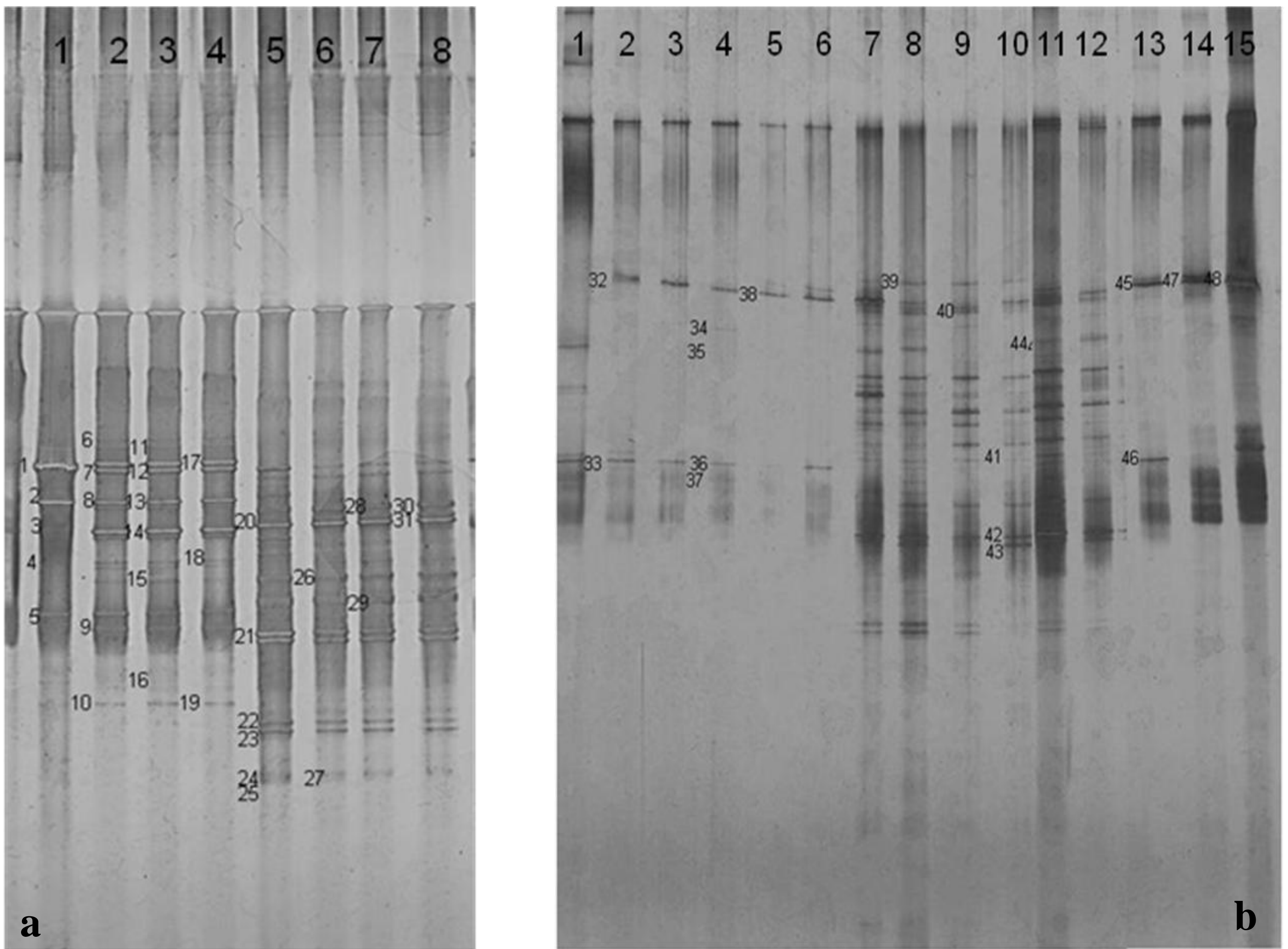
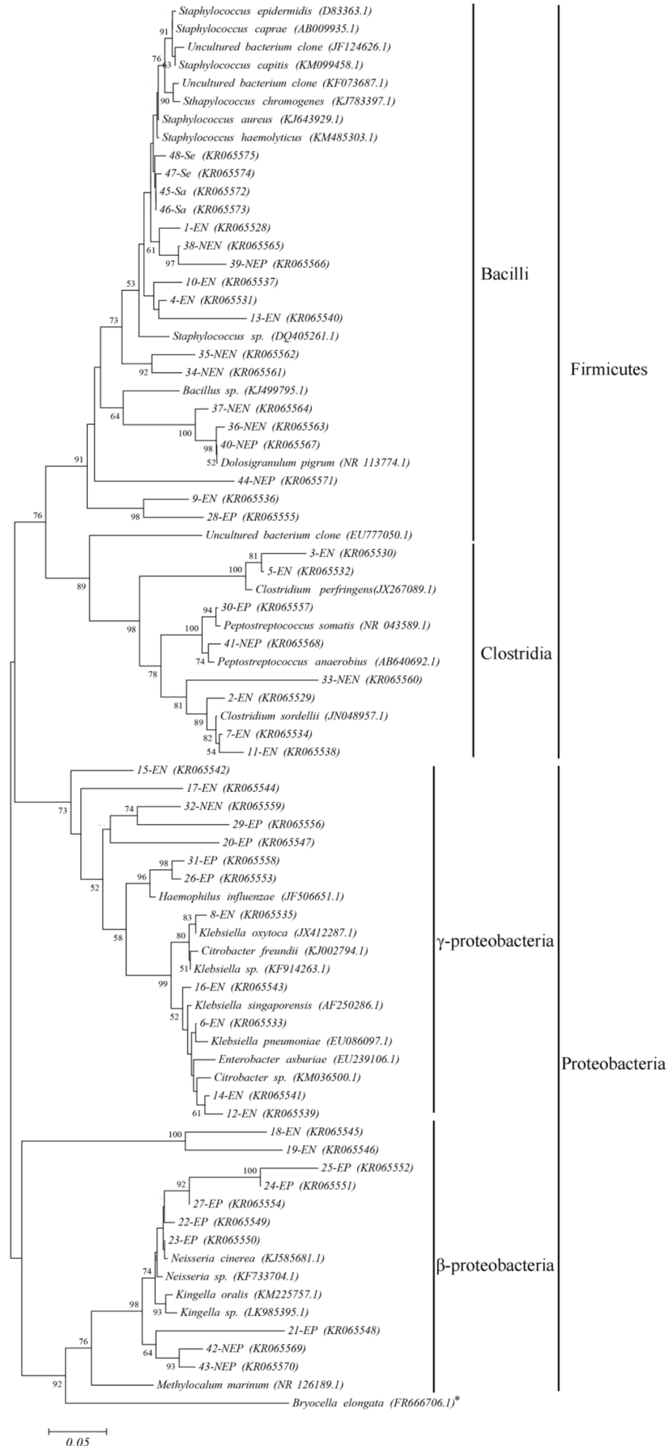


Fig.2 Sequencing of excised DGGE bands and related GeneBank sequences were used to create the neighbor-joining tree. Used sequences (±440 nucleotides) included the V6-V8 regions of the 16S rRNA gene. To identify the codes of excised bands, the letters correspond to the sample type: enrichment nose (EN), enrichment pharynx (EP), non enrichment nose (NEN) and non enrichment pharynx (NEP), (*) correspond to out-group sequence. The NCBI GenBank accession number for each sequence is shown in the parentheses.



The present study findings in healthy young people matched with others authors, Yi *et al.,* (2014) reported the genera *Streptococcus*, *Moraxella*, *Haemophilus, Neisseria, Klebsiella,* and *Staphylococcus*. Lemon *et al.,* (2010) also describe the Staphylococcaceae family. Other culture independent studies described the ecology of the healthy nose microbiota, the majority of the rRNA sequences obtained from this part of the body belonged to different bacterial phyla such as: *Actinobacteria* (Gram positive organisms for example *Corynebacteria*), *Firmicutes* (Gram positive organisms commonly *Staphylococcus* and *Streptococcus*) (Yi *et al.,* 2014; Chaban *et al.,* 2013, Bogaert *et al.,* 2011; Charlson *et al.,* 2011; Frank *et al.,* 2010). At the genus level, *Corynebacterium* spp., *Propionibacterium* spp. and *Staphylococcus* spp. *Streptococcus*, *Haemophilus*, *Moraxella* are prominent members of the upper respiratory tract microbiome (Yi *et al.,* 2014; Costello *et al.,* 2009; Frank *et al.,* 2010; Grice *et al.,* 2009; Lemon *et al.,* 2010).

This study had some methodological limitations, the 16S rRNA V6-V8 region was the only one studied, but despite of this, it includes a great diversity of microorganisms some of them were uncultured ones.

However this type of study may be a reference to characterize an important part of the microbiota found in the nose and pharynx of healthy young people and compare the pathogens in healthy carriers with the natural microbiota in order to predict potential risk of infections (Johannessen, *et al.,* 2012). To our knowledge this is the first report describing partially the composition of and the variability within the nasopharyngeal microbiota of a Mexican young adult population and therefore we will be able to investigate other different cities and compared them to find out where are the highest microbial contaminated zones and therefore take some hygienic measures in accordance to the normativity reports, besides the clinical implications that healthy carriers could spread these bacteria among other susceptible compromised population.

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