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Aerobic Bacterial Microbiota of the Upper Respiratory Tract (Oral and Nasal Cavities) in Saudi Adults

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ABSTRACT

The collective micro-community residing in the human body is called the microbiota. Studying and comparing its composition from different body sites is now a promising approach for microbiologists. The bacterial composition of the upper respiratory tract URT flora has been extensively studied, considering many factors, including health state, age, and gender. In our study, we identified the bacterial microbiota of two specific parts of the URT: mouth and nose. We isolated and identified bacteria from 40 males and 40 females using the VITEK biochemical identification method, followed by molecular identification using 16s rRNA primers. Fifteen bacterial genera were identified, making up 29 species, of which 11 were common in both genders. The two most identified genera are *Staphylococcus*, dominant in the nose but absent in the mouth, and *Streptococcus*, dominant in the mouth (p-value < 0.05). The *Staphylococcus* genus has a significantly higher "overall" number of culturable isolates (40% ($p<0.05$)), and the most abundant species of the *Staphylococcus* are *S. epidermidis* and *S. aureus* (41% and 39% of total *Staphylococcus* species). Age significantly affects the abundance and diversity of oral flora, with higher diversity in younger participants and higher abundance in the elderly, specifically oral *Streptococcus* (F=9.09, p-value= 0.003) young > old. Gender affects the abundance, where females have significantly higher oral bacterial density than males but no significant effect on the nasal system. Based on the unique microbial signatures retrieved from this study in each test group, elderly groups of both genders show more gram-negative pathogenic species than commensals, specifically the older females with a high abundance of *Enterobacter* cloacae complex

KEYWORDS: Microbiota, Oral and Nasal Cavities, Biochemical, Molecular, Bacteria

INTRODUCTION

The human body is a habitat of a complex micro-community of bacteria, viruses, and fungi that outweigh the number of our body cells. The composition, proportions, and diversity of microorganisms in our body are known as the microbiota. The most populated human body sites are the skin, gut, upper respiratory tract URT, and genitourinary tract. Dissecting and analyzing the makeup of the human microbiota is essential in microbiology due to its impact on health (Proctor and Relman 2017). Many studies compare factors that affect microfloral diversity, such as gender, age, health state, habitat, lifestyle, and diet (Cuesta-Zuluaga et al., 2019; Durack and Lynch 2019; Zhong et al., 2019).

Computerized data from identified site-specific microbiomes can be used in health risk assessments and age and gender predictions (Huang et al., 2020). We chose to study the aerobic bacterial microbiota of URT, concentrating specifically on the oral and nasal sites. The oral and nasal cavities are important sites of the URT, harboring numerous commensal and pathogenic bacteria strains, which have been highly correlated with many health issues and diseases (Kumpitsch et al., 2019). Dissecting and identifying patterns in healthy individuals in these two sites will aid us in understanding and comparing them with individuals with related illnesses. For instance, the co-colonization of certain bacterial species and their interaction is essential in outgrowing pathogenic species. One example is the *S. epidermidis* biofilm formation in the nasal cavity, which inhibits the colonization of the pathogenic *S. aureus*. Generally, research on human microbiota in Saudi Arabia is lacking, specifically the effects of gender and age. The majority of the Saudi population-based microflora studies focus more on the impact of chronic diseases, i.e., diabetes (Al-Obaida et al., 2020; Amr et al., 2018), host habits such as smoking (Al Moaleem et al., 2020; Hattan et al., 2018), as well as habitat and lifestyle of the individuals such as fuel station workers (Alwakeel 2017). The only study found to compare the effect of age and gender is on skin microbiota published in 2019 by a research team at Princess Nourah bint Abdulrahman University (Shami et al., 2019). Dissecting the bacterial microflora of URT is essential to a better understanding the typical healthy diversity and abundance. The goal of our study was to identify and compare culturable URT aerobic bacterial microbiota of healthy Saudi Adults, taking into consideration age and gender.

MATERIALS AND METHODS

Ethics approval and consent to participate

The protocol of this study was approved by Princess Nourah bint Abdulrahman University IRB with IRB Registration Number KACS'I, KSA: H-01-R-059, and written informed consent from all the participants in this study was obtained.

Sample Source

Eighty Saudi adults from Riyadh were enrolled in this study and were divided into four groups according to their gender and age (older males, older females, young males, and young females). The average age in each group was 51, 62, 21, and 20, respectively. The study was conducted at Princess Nourah bint Abdulrahman University and King Saud University Research Centers in Riyadh.

Sample Collection

Sterile cotton swabs were used for sample collection. The same swab for each individual was passed against 5 oral sites (tongue, palate, buccal mucosa, gingiva, and mouth floor) for the oral cavity. A different swab was inserted into the nostrils along the nasal passage for the nasal cavity. The samples were stored at 4 °C for less than 2 hours (Shami et al., 2019).

Biochemical Identification

Swaps were inoculated and incubated overnight at 37 °C on MacConkey, blood, and tryptic Soy agar. Biomérieux VITEK® 2 system was used for the biochemical identification of grown bacterial colonies.

Molecular identification

According to the manufacturer's instructions, a QIAGEN DNeasy Blood & Tissue kit was used to extract DNA from grown bacterial colonies. PCR products using 16s rRNA universal primers (27F 5′ AGAGTTTGATCMTGGCTCAG 3′ - 1492R (5′ TACGGYTACCTTGTTACGACTT 3′) were purified using ExoSAP-IT (Usb. Affymetrix, Inc.), then 16s rRNA sequencing was conducted (MOLECULE-ON, New Zealand).

Data analysis

The data were analyzed using R software. Descriptive statistics were performed for quantitative variables. We used one-way ANOVA to test the difference among the four groups (older males, older females, young males, and young females).

RESULTS

Forty male and forty female subjects from Saudi Arabia, each divided into two age groups (elderly and young), participated in this study. Samples were collected from each individual's oral and nasal cavity to identify the composition of aerobic bacterial flora of the upper respiratory tract, URT. Biochemical and molecular analyses were performed to identify cultivable/ culturable strains. A total of 29 bacterial species belonging to 15 different genera were identified in this study, of which only 11 species were common in both genders, as shown in Table 1. The exact number of isolates for each of the 11 common species, isolated from each of the 4 test groups, are illustrated in Table 1. *Staphylococcus haemolyticus* was only found in elder groups, and *Streptococcus pneumonia* was only found in young groups, highlighted in grey. The distribution and details of uniquely isolated bacteria species for each group are illustrated in Figure 1. 20.6 % (6 out of 29) and 17.2% (5 out of 29) of the 29 identified bacterial species belong to the *Staphylococcus* and *Streptococcus* genus, respectively (Table 2). Although both genera are equally common in terms of the number of species, shown by the black bar in Figure 2, the *Staphylococcus* genus has a significantly higher "overall" number of culturable isolates (40% ($p<0.05$)), shown by the red bar in Figure 2. Site-specific comparisons (nose vs mouth) of *Staphylococcus* and *Streptococcus* bacteria numbers were carried out using one-way ANOVA. The number of *Staphylococcus* isolates from the nasal flora is significantly higher than oral flora ($F=27.8$, p-value= 4.4 E-7). The most abundant species of the *Staphylococcus* genus are *S. epidermidis* and *S. aureus*, 41% (19 out of 46) and 39% (18 out of 46) respectively (Table 1).

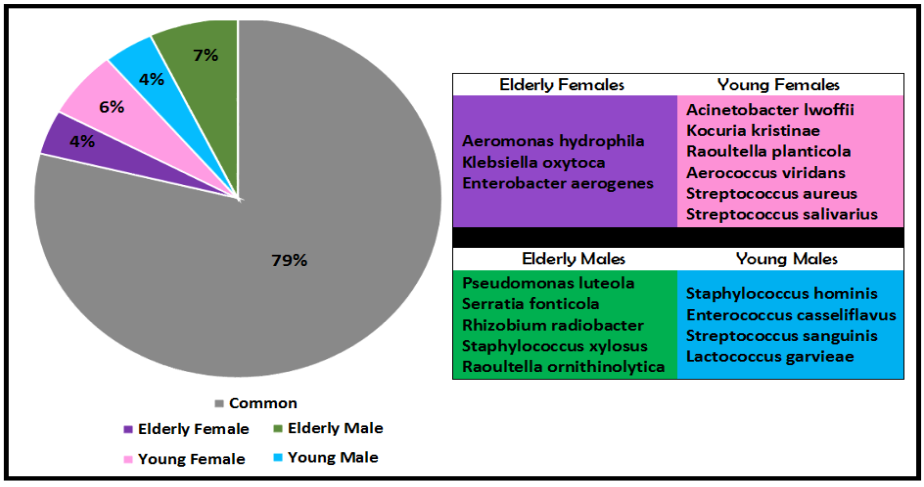

Figure 1. Distribution of Isolated Bacteria from URT Microflora. The pie chart illustrates the percentages of uniquely isolated bacteria from each test group. The table on the right presents more details of bacterial species isolated from each test group. The pie chart mentions the percentages of the number of isolates out of the 114 isolates.

Table 1. Detailed numbers of bacteria isolated from the 11 common species found in both genders' oral and nasal cavities. The four groups based on gender and age are shown separately in this table. The highlighted rows indicate species isolated from different age groups: *S. haemolyticus* (elder only) and *S. pneumonia* (young only).

Total Number of The Common Isolated Bacterial Species of Oral and Nasal Cavities					
Bacterial Specie Isolate	Females		Males		Total
	Elderly	Young	Elderly	Young	
Staphylococcus epidermidis	9	3	3	4	19
Staphylococcus aureus	2	7	5	4	18
Staphylococcus pseudintermedius	1	2	-	1	4
Staphylococcus haemolyticus	1	-	2	-	3
Streptococcus parasanguinis	2	4	2	2	10
Enterococcus faecalis	1	1	-	3	5
Acinetobacter baumannii complex	3	1	1	1	6
Escherichia coli	-	1	1	-	2
klebsiella pneumoniae	2	-	-	2	4
Enterobacter cloacae complex	10	-	3	1	14
Streptococcus pneumoniae	-	2	-	3	5

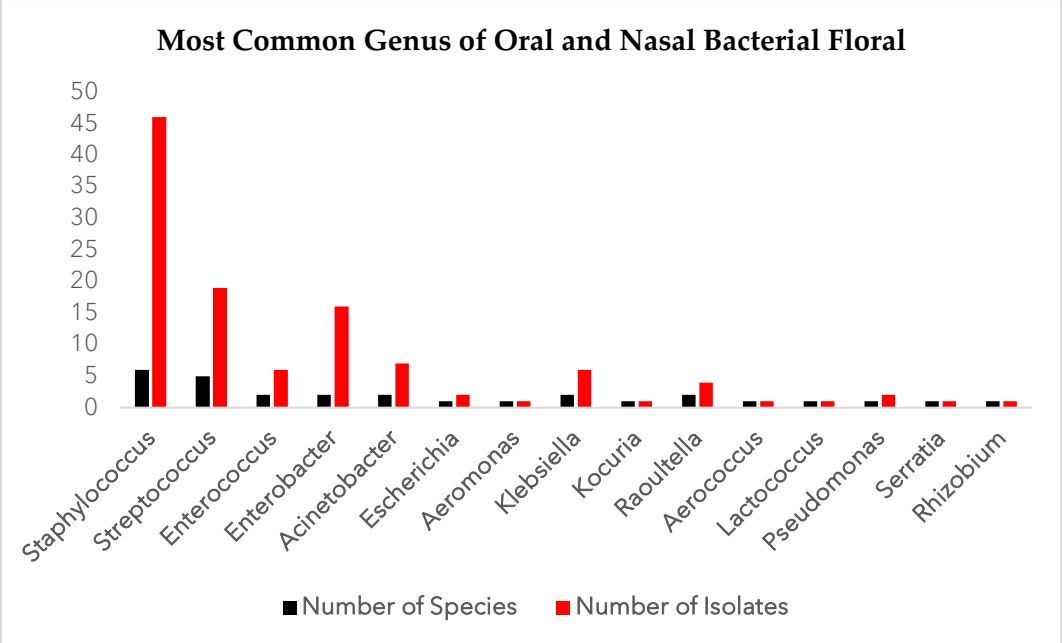

Figure 2. The number of species, black, and number of bacteria, red, for each genus isolated from the oral and nasal flora in this study.

Table 2. Number of Species and Isolates in Each Bacterial Genus Cultured from Oral and Nasal Flora

Number of Species and Isolates in Each Bacterial Genus Cultured from Oral and Nasal Flora		
Genus	Number of Species	Number of Isolates
Staphylococcus	6	46
Streptococcus	5	19
Enterococcus	2	6
Enterobacter	2	16
Acinetobacter	2	7
Escherichia	1	2
Aeromonas	1	1
Klebsiella	2	6
Kocuria	1	1
Raoultella	2	4
Aerococcus	1	1
Lactococcus	1	1
Pseudomonas	1	2
Serratia	1	1
Rhizobium	1	1
Total		
15	29	114

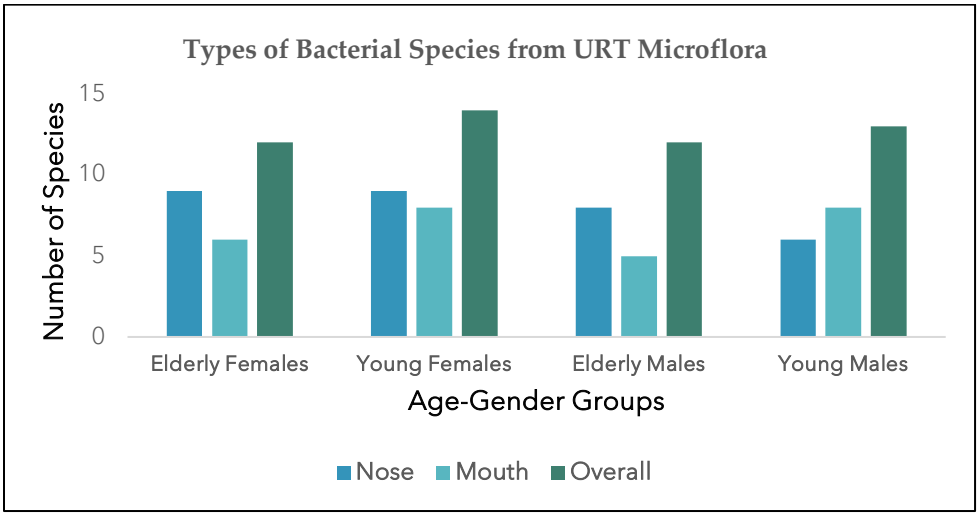

Figure 3. Site-specific demonstration of the number of types of bacterial species in each test group.

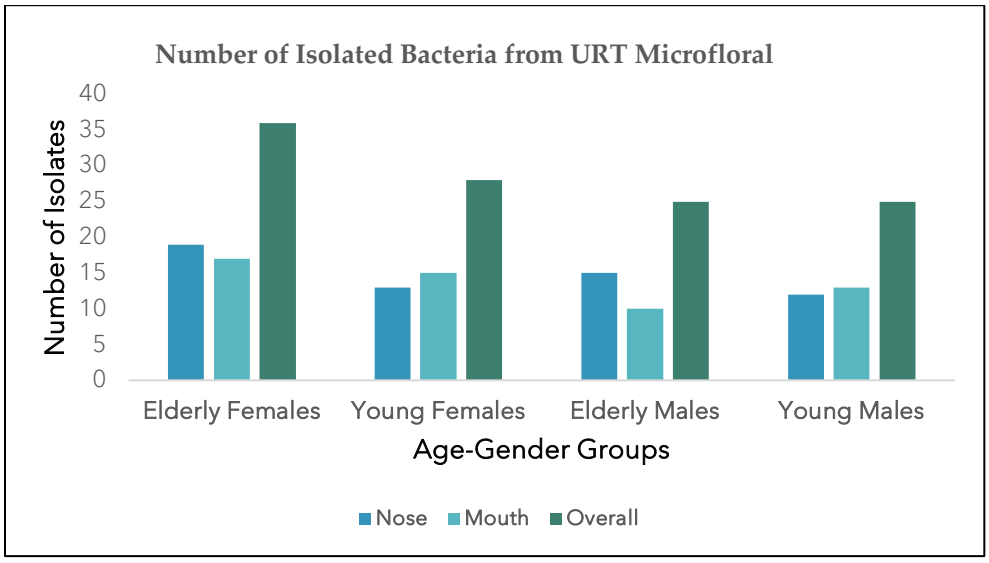

Figure 4. Site-specific demonstration of the numbers of isolated bacteria in each test group.

On the other hand, *Streptococcus* is highly abundant in the oral flora and absent in the nasal flora (F=24.6, p-value= 1.8E-6). Microbial composition is influenced by various factors, such as the host's immune response, moisture, temperature, and available nutrients, contributing to the observed differences between oral and nasal floras**.** Age significantly affects the number of oral *Streptococcus*, which is higher in young participants (F=9.09, p-value= 0.003). Comparisons of the diversity between oral and nasal sites amongst the 4 different groups are illustrated in Figure 3. Our results show that the nasal flora is slightly more diverse than the oral flora within each group except the young male group, but the difference is insignificant. The diversity of the oral microflora from our data is slightly higher in younger groups than in the elderly (Figure 3). The total number of isolates in each site for each group has also been compared, as illustrated in Figure 4. The number of isolates is higher in the nasal than the oral cavity amongst the elderly groups, while the younger groups showed more oral isolates than the nasal. Elderly females have the highest number of URT bacterial flora abundancy (36 isolates) shown in Figure 4, of which 53% are species of *Staphylococcus epidermidis* (9 out of 36 isolates) and *Enterobacter cloacae* complex (10 out of 36 isolates) shown in Table 1.

DISCUSSION

The distribution of aerobic oral and nasal bacterial microbiota is examined in 80 Saudi adults divided into 4 age and gender groups: elder females, young females, older males, and young males. Findings from the current study indicated *Staphylococcus* and *Streptococcus* as the two most abundant genera with significant site-specific patterns, nose and mouth, respectively. *Staphylococcus* was more dominant in the nose, replicating findings by Chen et al. (2019) indicated that *Staphylococcus* makes up 9.61 % of the nasal micro-community. The two most common *Staphylococcus* species in our data are *S. epidermidis* and *S. aureus*, similar to findings from several studies on the nasal microbiota (Chen et al., 2019; Chen et al., 2016). A study conducted by Frank et al. (2010) showed a significant negative correlation between the nasal density of *S. aureus* and *S. epidermidis* (Frank et al., 2010). Although not statistically significant, a negative pattern is observed between the two species from the nose of each test group. Our results show that the *Streptococcus* genus was more dominant in the oral cavity. A recent study indicated that the *Streptococcus* genus was highly

appendant in the oral cavity (Abranches et al., 2018). In addition, the absence of *the Streptococcus* genus in the nasal cavity might be due to specific conditions related to the studied individual, such as the host's immune response, moisture, temperature, and available nutrients, contributing to the observed differences between oral and nasal floras**.** Age has always impacted the diversity and the density of microbiomes in general (Sampaio-Maia and Monteiro-Silva 2014). It has been shown that the diversity decreases with host aging, but the overall density or microbial overload increases (Sampaio-Maia and Monteiro-Silva 2014). Our results match previous studies in terms of the effect of age on microfloral diversity and abundance (Sampaio-Maia and Monteiro-Silva 2014; Wu et al., 2016). In our study, the combined bacteria isolated from both sites are more diverse in younger individuals and less abundant in the elderly, specifically the oral microflora (Zawadzki et al., 2017). However, looking closely at the oral *Streptococcus* genus, even though it is more diverse in younger participants, it is also more abundant and does not follow the expected pattern. That could be a possible consequence of the dental conditions of our older test subjects; perhaps they have a higher rate of age-related tooth extraction and less bacterial growth, as previously shown by a periodontal microflora study in 2011 (Quirynen and Van Assche 2011).

The host aging effect on the nasal flora abundance from our study is, as expected, higher in the elderly and more diverse than in young individuals, which was not expected based on the reviewed literature (Prector and Relam 2017). Although not significant, we have highlighted in Table 1 that *Streptococcus pneumonia* was only present in the younger oral cavity and absent from the elderly oral cavity. However, *Streptococcus parasanguinis* was present, as was found in a 2009 Norwegian study conducted on elderly oral microflora (Preza et al., 2009). Based on the unique microbial signature for each test group (Figure 1), we can see the age-related effect on the ratio of pathogenic to commensal bacterial composition of the oral-nasal cavities. Species isolated exclusively from elderly males are mainly pathogenic, and some come from soil, contaminated meats, and water: *Pseudomonas luteola, Serratia fonticola, Rhizobium radiobacter, Staphylococcus xylosus, Raoultella ornithinolytica* (Turner et al., 2018; Cantas et al., 2012; Aljorayid et al., 2016; Mantadakis et al., 2015; Lia et al., 2004; Ferrocino et al., 2018; Hajjar et al., 2018). The same is observed in elderly women but with less diversity: *Aeromonas hydrophila, Klebsiella oxytoca, and Enterobacter aerogenes* (Citterio and Francesca 2015; Baker et al., 2019; Darby et al., 2014; Davin-Regli and Pages 2015). That pattern of mainly gram-negative bacillus species in the oral-nasal cavity of aged individuals corresponds with other findings in scientific literature. In contrast, species isolated only from younger groups are mostly gram-positive (Bodineau et al., 2009; Aleman and Valenzano 2019; Nagpal et al., 2018). Gender affects microflora composition, but from the data of our study, no significant gender-specific pattern was detected other than the higher abundance of *Enterobacter cloacae* complex in elderly females. This observation is not necessarily gender-related but could be age-related since the age factor has been well-documented in the literature on increased levels of some gram-negative bacilli, including *Enterobacter*, in older people (Bodineau et al., 2009). The other gender-associated pattern seen from our data is that a greater number of *Streptococcus* species were identified in younger males than females. Females in our study show significantly higher numbers of oral bacteria than males, which matches the findings of Ma and Li in their 2019 study comparing gender microbiomes from six body samples, including nares, saliva, and tonsils (Ma and Li 2019). However, in terms of nasal bacterial diversity in their study, it was significantly higher in males than females, whereas no significant differences were found between sexes in our study.

We know that the oral-nasal microbiota is much more diverse than what has been retrieved from this study. The automated biochemical identification of bacteria species using the VITEK 2 system has limitations. It can only identify strains cultivable in VITEK 2 recommended media: tryptic soy agar, MacConkey agar, and blood agar. Many oral and nasal fastidious commensals will not grow in such media (Kilian et al., 2016). Therefore, a vast majority of the bacterial microflora cannot be detected, reducing the accuracy of microbiome identification. As for the molecular approach used in this study, the oral sample collection by saliva would have retrieved more identifiable species by directly extracting DNA from the sample rather than pre-culturing samples from swaps.

Therefore, if this study were to be repeated, a more accurate molecular approach such as next-generation sequencing (NGS) methods would yield more representable results, allowing us to accurately measure alpha and beta diversity, the abundance of the microbiota, and the effects of age and gender on its composition. In conclusion, our findings indicated that the oral and the nasal cavities have more in common regarding the composition of the bacterial microflora. Age has a general effect of decreased bacterial diversity that consists mainly of pathogenic gram-negative species posing a threat to their health by increasing their risk of developing fatal infections.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Data availability

All datasets generated or analyzed during this study are included in the manuscript.

Ethics approval and consent to participate

National regulations for the protection of human subjects, rules and regulations of the Government of Saudi Arabia, the Princess Nourah bint Abdulrahman Institutional Review Board Policies and procedures, and the ICH Good Clinical Practice guidelines governed the techniques used in the study. Princess Nourah bint Abdulrahman University IRB approved the protocol of this study with IRB Registration Number with KACS'I, KSA: H-01-R-059, and written informed consent from all the participants in this study was obtained.

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