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Oral and skin microbiome as potential tools in forensic field

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ABSTRACT

The interest in the analysis of the human microbiome for personal identification purposes is based on the microbial diversity amongst individuals. The oral cavity hosts one of the most diverse and abundant microbial communities in the human body; the skin instead is a complex living ecosystem with unique microbial niches at different sites. Both skin and oral microbiomes are highly individual and relatively stable over time. As saliva and skin debris are often found at crime scenes, the analysis of their microbiome may represent a potential tool for personal identification. However, there are some gaps in knowledge on how factors such as age, sex, geographic origin, diet and pathologies can affect the composition of the microbiome. The aim of this study is to improve the existing knowledge by examining oral and skin microbiomes from the same individuals and evaluating the variability between anatomical sites and donors. For this study, 50 individuals living in Italy donated oral swab samples and provided information regarding their diet, lifestyle, health status, antibiotic use, and other demographic data. Skin swabs from 11 of the 50 individuals were also analysed and compared to the oral swabs from the same donors. All analyses were done through metabarcoding of the 16S rRNA region of DNA extracted from the samples. This research outlines the potential use of oral and skin microbiome signatures as added evidence in personal identification, providing useful investigative clues for future forensic caseworks.

1. Introduction

The oral microbiota hosts one of the most diverse and abundant microbial communities in the human body [1]. Like the oral cavity, the skin is a complex living ecosystem with unique niches that host several microbial communities at different sites [2]. Hence, both oral and skin microbiome can have an important role in personal identification because they vary among individuals, are relatively stable over time and are easy to find from saliva and skin traces on the objects' surface of crime scenes [1,2]. For these reasons, the analysis of the microbiome found on the crime scene may represent a potential tool for human identification, also providing investigative information about a person of interest [3,4]. However, there are gaps in knowledge concerning factors (such as age, sex, geographical provenience, diet, etc.) affecting the oral and skin microbiome composition.

Thus, the aim of this study is to improve existing knowledge by examining the oral and skin microbiome composition, investigating any biomarkers that could be indicators of habits or lifestyles of the subjects.

2. Material studied, methods, techniques

For this study, 50 individuals of different sex living in Italy and aged between 20 and 70 years donated oral swab samples and provided information regarding their diet, lifestyle, health status, antibiotic use, and other demographic data. Skin swabs from 11 of the 50 individuals were also analysed and compared to the oral swabs from the same donors (Fig. 1).

Microbial DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (QIAGEN), DNA quantification has been performed with NanoDrop One Microvolume UV-Vis Spectrophotometer (ThermoFisher Scientific), amplification and sequencing of the hypervariable region V4 of the 16S ribosomal RNA gene was achieved using the Illumina Miseq

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		Demographic Factor								
		Age	Residence Region *	Smoking	Alcohol use	Sport	Drugs – Medical use	Antibiotic use (3 months)	Medical Condition	Transport mode (to Work)
Level	*Lombardy*	-	20	-	-	-	-	-	-	-
	Piedmont	-	22	-	-	-	-	-	-	-
	20-30 (years)	10	-	-	-	-	-	-	-	-
	31-40 (years)	10	-	-	-	-	-	-	-	-
	41-50 (years)	10	-	-	-	-	-	-	-	-
	51-60 (years)	11	-	-	-	-	-	-	-	-
	61-70 (years)	9	-	-	-	-	-	-	-	-
	Yes	-	-	10	31	24	19	6	20	-
	No	-	-	25	19	26	31	44	30	-
	Past (Former)	-	-	15	-	-	-	-	-	-
	Private Vehicle	-	-	-	-	-	-	-	-	34
	Public Transport	-	-	-	-	-	-	-	-	8
	Foot or Bike	-	-	-	-	-	-	-	-	8
Total number of participants		**50**	**42***	**50**	**50**	**50**	**50**	**50**	**50**	**50**

Fig. 1. Demographic data of study participants. * alpha and beta diversity analysis excluded 8 samples from other residence regions as those regions were under sampled.

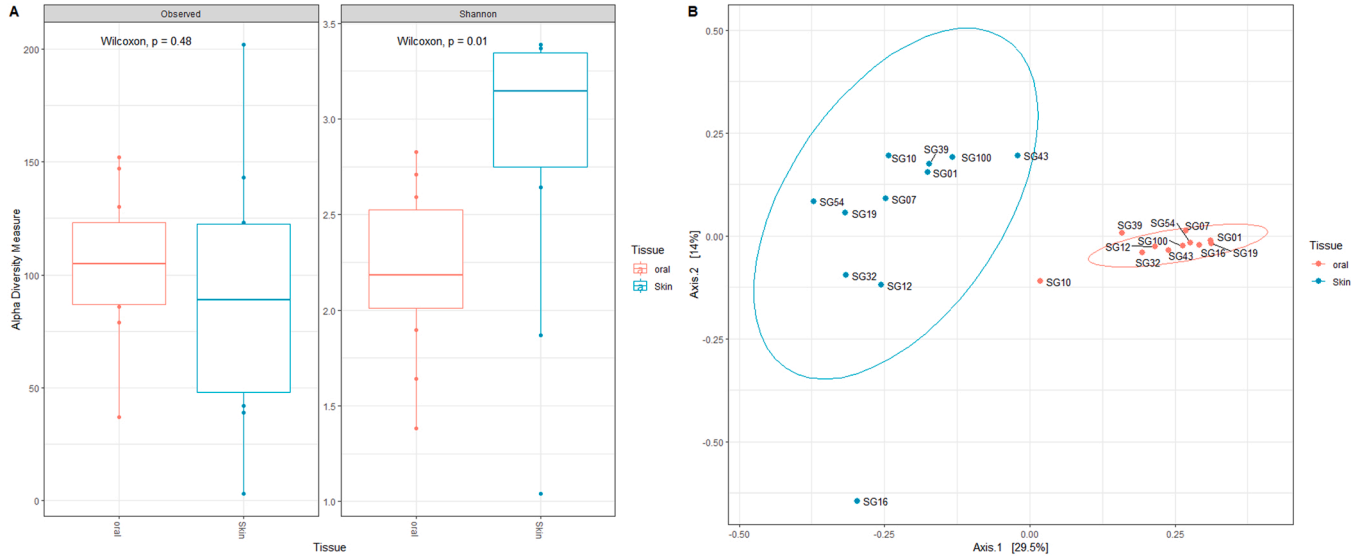

Fig. 2. Diversity analysis comparing oral and skin microbiome. **A)** Boxplot showing alpha diversity metrics; **B)** Principal component analysis plot of beta diversity.

Next Generation Sequencer (Illumina Inc.). Data analysis was carried out with QIIME 2 and R (ver 4.1.2).

3. Results

The oral microbiome of the 50 participants in this study consisted of 20 Phyla which was dominated by *Firmicutes* (almost 45 %) and *Proteobacteria* (almost 36 %). Overall, there was no significant difference in the alpha diversity of the oral samples among the groups in lifestyle habits assessed (e.g., Sex, Age, Birth Region, Residence Region, Smoking Habits, Alcohol-use, Antibiotic-Use). However, we observed trends in bacterial taxa abundances associated with Smoking Habits and Age.

As for the comparison between oral and skin microbiome, *Firmicutes* and *Proteobacteria* phyla represented almost 80 % of the total bacterial population. Finally, diversity analysis comparing oral and skin microbiome of 11 participants showed a higher species richness and alpha diversity for the skin microbiome; Principal Coordinates Analysis confirmed the distinction between oral and skin bacterial microbiomes (Fig. 2).

4. Discussion

We already proved the presence of a skin core microbiome [4], defined as taxa that was present in all samples. Our new results showed the presence of an oral core microbiome and the existence of microbial signatures associated with certain grouping conditions (Smoking Habits and Age). This has significant implications for forensic investigations for which the ability to differentiate amongst individuals is essential.

Moreover, the skin microbiome higher species richness and alpha diversity suggest that the skin signature may be more suited than oral one for human identification purposes.

5. Conclusion

This research outlines the potential use of oral microbiome signatures as additional evidence in forensic human identification, providing investigative information about the host donor. However, further research is necessary to deeply investigate the observed trend in our results (e.g., expand the study with more samples and study how other external factors can affect the composition of oral and skin microbiome).

Conflict of interest statement

The authors report no conflicts of interest.

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