# Comparison of Oily or Dry Dandruff scalp: Clinical, Instrumental and Targeted Metagenomic Data.

**Linked to the poster 446**

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## ABSTRACT

Oily dandruff (OD) is associated to high sebum production and inflammation, yellowish and flakes adherent to the scalp and hair, and dysbiosis microbiota. Dry dandruff (DD) are small, whitish flakes, not adherent to the scalp and can be associated with itching and dry scalp. The aim of this study was to compare DD scalps by clinical, instrumental, and targeted metagenomic data *versus* OD scalps.

33 subjects with mild to moderate OD or DD were included. For both populations, the clinical status of dandruff was assessed. Instrumental measurements of hydration, lipid index, pH and trans epidermal water loss were done. Targeted metagenomic analysis and digital droplet PCR were performed on DNA extraction from swab samples. Microbiota population was compared between the two groups.

Hydration and lipid index were significantly higher for OD than DD, while TEWL data were significantly higher in DD population.
*Cutibacterium* and *Staphylococcus genus* dominate OD and DD bacteria populations. Significant differences were observed on under-represented microorganism communities.
Regarding the fungal microbiota, the abundance of the family *Malasseziaceae* and the *genus Filobasidium* was different between OD and DD scalps.

Dehydration and low sebum production are characteristic of DD scalps while OD scalps display high sebum production and inflammation. Interestingly, the barrier function of the skin scalp seems to be more impaired in DD scalp. In addition, we have shown here that scalp microbiota of these two dandruff states are different. These results highlight the importance to address DD and OD scalps with specific scalp care products.

**Keywords:** Oily Dandruff Microbiota, Dry dandruff microbiota, Metagenomic, Clinical study

## Introduction.

Oily dandruff (OD) is associated to high sebum production and inflammation, yellowish and flakes adherent to the scalp and hair, and dysbiosis microbiota. Dry dandruff (DD) is in the form of small, whitish flakes, not adherent to the scalp and can be associated with itching and dry scalp [1]. If Oily dandruff Microbiota is well characterized, nothing is published about dry dandruff microbiota. Scalp microbiota is characterized by the presence of *Malassezia, Cutibacterium* and *Staphylococcus* genus [2].

The aim of this study was to compare Dry Dandruff scalps *versus* oily dandruff scalps by clinical, instrumental and targeted metagenomic data, in order to answer the question: Is dry dandruff microbiota dysbiosis have the same microorganism's actors than oily dandruff one? If microbiota is different between dry dandruff and oily dandruff scalp, it could justify the development of adapted product to each type of scalp dandruff.

## Materials and Methods.

**Clinical Study:** One study with 33 subjects with mild to moderate scaly state of dandruff was done (clinical score of dandruff ≥ 4 on a 10 points scale). 16 subjects had oily dandruff and 17 subjects had dry dandruff. For both population (OD and DD), clinical dandruff condition on whole scalp using clinical 10-point-scale was assessed, itching and discomfort sensations were auto-assessed by subjects using clinical 10 -point-scale and instrumentally data were measured on scalp as followed: hydration with Dermalab$^{®}$, Lipid Index (IL) with Sebumeter$^{®}$ pH with pHmeter$^{®}$ and Trans Epidermal Water loss with Aquaflux$^{®}$. Statistical analysis was done by Student's T-Test.

**Microbiota studies:** swabs were sampled on scalp from both groups according to the clinical study protocol. Sample DNAs were extracted with QIAcube instrument using QIAamp DNA Investigator kit.

Bacteria population: Taxonomic identification was based on the sequencing of the ribosomal RNA gene, which is present in all microbial genomes. V1-V3 region of 16S ribosomal RNA genes (prokaryotes) were amplified by PCR using oligonucleotides targeting conserved regions common to all bacteria.

Fungi population: Taxonomic identification was based on the sequencing of the ITS1 region of the ribosomal RNA gene, which is present in all fungi genomes. ITS1 region of the ribosomal RNA genes (Eukaryotes) were amplified by PCR using oligonucleotides targeting conserved regions common to all fungi.

The sequence of each DNA fragment thus duplicated was then carried out by high-speed sequencing (MiSeq Illumina). The analysis of the sequences obtained and their comparison with international databases allowed the phylogenetic identification of the microorganisms present in each sample in relation to already known organisms.

After a cleaning process, the sequences with 100% homology between them were grouped into unique sequences and then into OTUs (operational taxonomic units: 99% threshold) which will be identified later.

The bioinformatics analysis of the sequencing data allowed the identification of microorganisms present at different taxonomic levels (phylum to genus). The analysis allowing phylogenetic affiliation up to the genus level was carried out using bioinformatics tools for the processing of large amounts of sequence data (Mothur). The identification was carried out on the Findley (2013) DATA BASE taxonomy for fungi [4] and Greengenes DATA BASE taxonomy for bacteria.

Alpha and beta diversity were compared after normalization by rarefaction. Observed, Chao1, Shannon and invSimpson indices were compared by an ANOVA analysis for Alpha diversity evaluation. Beta diversity was evaluated with Jaccard, Bray Curtis, Unifrac and weighted Unifrac indices. The *pValue* of the comparison was carry out by PERMANOVA analysis.

Differential analysis between the two populations was carried out, after GMPR normalization and DEseq2 method to identify *genus* that were significantly more abundant in one group than the other.

At the species level, absolute quantitative evaluation of *Malassezia restricta*, *Malassezia globosa*, *Cutibacterium acnes* and *Staphylococcus epidermidis* was done.

# Results.

## Clinical and instrumental analyses

Clinical dandruff condition (dandruff state -figure 1 (A)), itching and discomfort of scalp (figures 1 (B) and (C)) were not significantly different between oily and dry scalp.

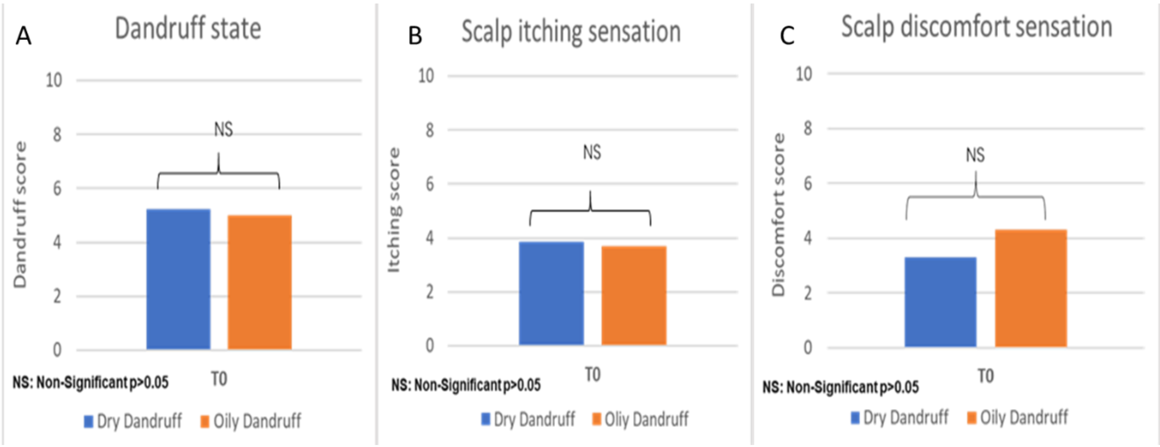

*Figure 1: Clinical dandruff evaluations (A) Dandruff state, (B) Itching, (C) Discomfort*

Instrumental evaluation of pH wasn't significantly different between oily dandruff and dry dandruff (figure 2 (A)), while sebum secretion was significantly higher in oily dandruff (Figure

2 (B)), hydration and barrier function were significantly lower in dry dandruff (Figures 2 (C) and D).

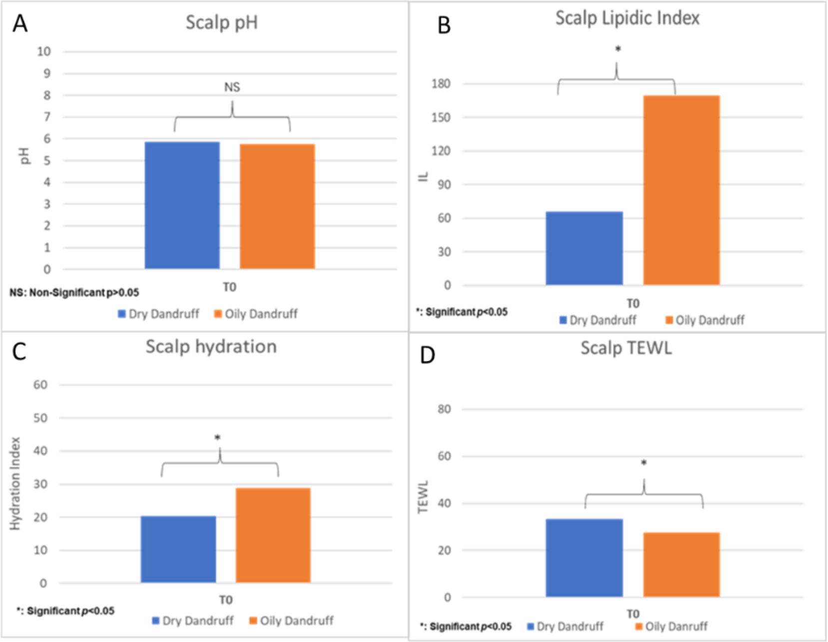

*Figure 2: Instrumental evaluation of pH (**A**), lipid index (**B**), Scalp hydration (**C**), and barrier function (**D**)*

## Microbiota analysis

### Bacteria population:

*Cutibacterium* and *Staphylococcus* are the most represented bacteria genus in scalps with oily or dry dandruff (Figure 3).

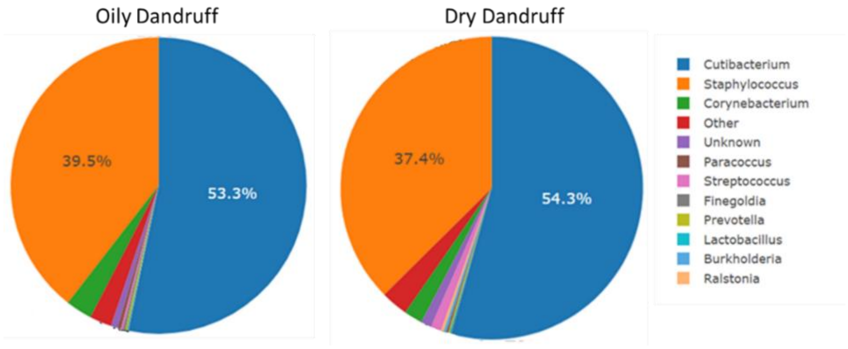

*Figure 3: Descriptive analysis: average relative abundance per group of the top ten of the most abundant bacteria at the genus level.*

The abundance of genus of *Actinobacteria* phylum was higher on dry dandruff scalp (Figure 4 (A)). This observation was exemplified by differential analysis of the metagenomic data who highlighted the more plentiful of *Actinobacteria* population genus on dry dandruff scalp than oily dandruff scalp. *Pelomonas*, *Ralstonia* and *Neisseria genus* were significantly more abundant on dry dandruff scalp while only *Brachybacterium* genus was showed significantly more abundant on oily dandruff (Figure 4 (B)).

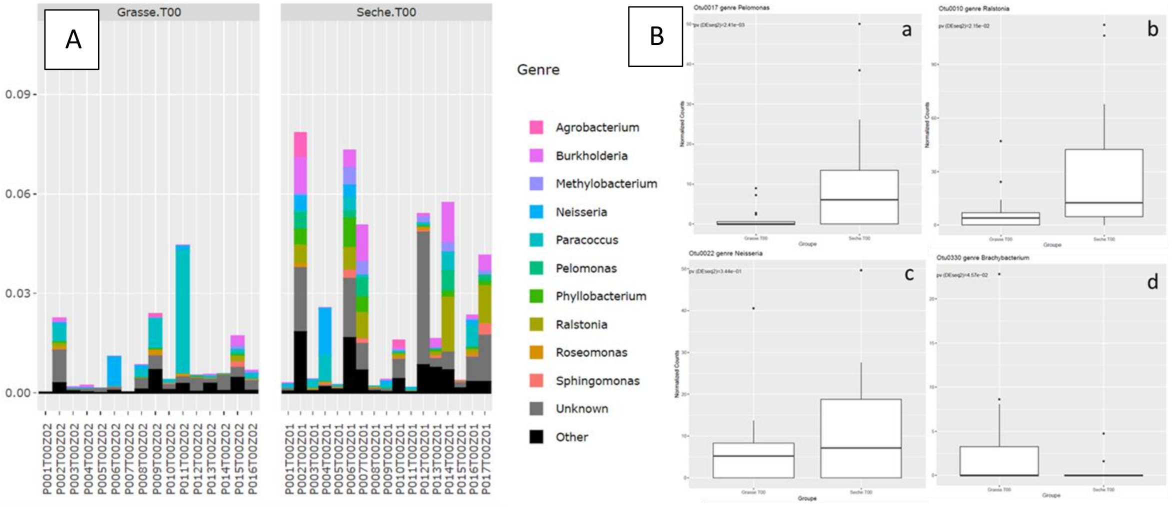

*Figure 4: **(A)** Histogramme of genus of Actinobacteria of each subject with dry or oily dandruff. **(B)**: Boxplots of genus more abundant on dry or oily dandruff after differential analysis. (a): Pelomonas, (b): Ralstonia, (c): Nesseria, (d): Brachybacterium*

For the other bacteria *Phylum*, *Capnocytophaga* and *Streptococcus genus* were significantly more abundant on dry dandruff scalp (figure 5)

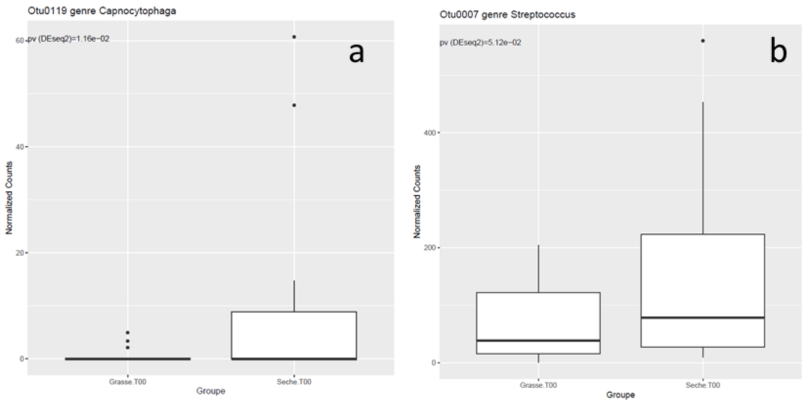

*Figure 5: Boxplots of genus more abundant on dry or oily dandruff after differential analysis. (a): Capnocytophaga, (b): Streptococcus.*

**Absolute quantification of *Cutibacterium acnes* and *Staphylococcus epidermidis*:**

Absolute quantification, by digital droplet PCR, of *Cutibacterium acnes* and *S. epidermidis* didn't show any significant differences between oily and dry dandruff.

**Fungi population:**

The descriptive analysis of fungi population underlines the importance of *Mallasseziaceae* family, Malassezia genus and unknown fungi population, on scalps with dry or oily dandruff. As expected, unidentified genus of *Malasseziaceae* family, and in particular *Malassezia restricta* species appeared to be more abundant on oily scalp dandruff (data not shown).

The differential analysis of the fungi population highlights that *Filobasidium* genus should be link with dry dandruff scalp (figure 6).

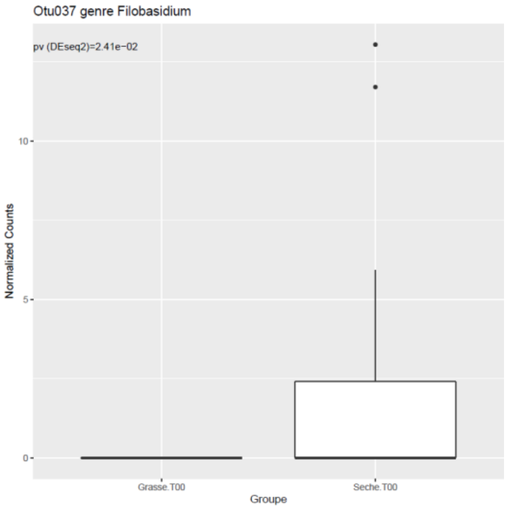

*Figure 6: Boxplots of Filobasidium genus more abundant on dry than oily dandruff.*

**Absolute quantification** by digital droplet PCR of both *Malassezia* species: *restricta* and *globosa*, showed the predominance of the *restricta* species over the *globosa* species on both oily and dry dandruff. The abundance of *Malassezia restricta* was significantly higher in subjects with oily dandruff than in subjects with dry dandruff, whereas the population of *Malassezia globosa* showed no significant difference between the two groups.

**Microbiota biodiversity**

Alpha and beta diversity analysis of bacteria and fungi population didn't show any significant differences between oily and dry dandruff.

## Discussion.

Few studies have been published on the differences between dry and oily dandruff. In 2020, Ji-Seon Yoon et al. [1] compared two populations of dandruff sufferers according to the level of sebum in their scalp. In the present study, we used clinical and instrumental measurement to characterize our two population groups before microbiota study. As published by Ji-Seon Yoon et al, [1] our results showed differences between oily and dry dandruff in terms of sebum levels and scalp hydration, while no significant differences were measured in pH, dandruff score and scalp discomfort. In contrast to the work of Ji-Seon Yoon et Al. (2020), [1] our results show significantly lower barrier function in dry dandruff than in oily dandruff. As expected, the microbiota study shows some differences in the composition of microorganisms between oily and dry dandruff. The main players in the bacterial population of the healthy scalp are bacteria of the genus *Cutibacterium* and *Staphylococcus*. Compared to healthy scalp, the dandruff microbiota shows a decrease in *Cutibacterium* and an increase *Staphylococcus*. Li Wang et al (2022) [2] suggested that the balance between the genus *Cutibacterium* and *Staphylococcus* might be correlated with the severity of dandruff. In this study, *Cutibacterium* and *Staphylococcus* populations are most abundant on the scalp of dandruff without any significant difference between dry and oily dandruff (figure 2). Absolute quantification of *Cutibacterium* population was in agreement with this result. The genus of the phylum *Actinobacteria* appear to be more abundant and represented in dry dandruff (Figure 3). To illustrate this observation, differential analysis showed that the genus *Pelomonas*, *Ralstonia*, *Nesseria*, of the *phylum Actinobacteria* were more abundant on the scalp of dry dandruff than on that of oily dandruff. Only one genus of this *phylum* is more abundant in oily dandruff : *Brachybacterium.* Differential analysis also identified *Capnocytophaga* and *Streptococcus* as more abundant in dry than in oily dandruff.

The fungal microbiota of dandruff scalp is dominated by *Malasseziaceae* family [2]. While differential analysis highlighted that they were more abundant in oily dandruff than in dry dandruff, our metagenomic study didn’t give us more information at species level. Absolut quantification of *Malassezia restricta* and *globosa* showed that *restricta* species were significantly more abundant on oily scalp dandruff. The genus *Filobasidium* was more abundant on dry dandruff scalp. This *genus* was published to be associated with dandruff by Hee Kuk Park et Al in 2012 [2].

No significant differences were demonstrated by analyzing the alpha and beta diversity of the bacterial and fungal populations. This result may be due to the small number of subjects enrolled in this study, or to the fact that we are comparing two dysbiosis.

## Conclusion.

According to published data, dehydration and low sebum production are characteristic of dry dandruff scalps whereas oily dandruff scalps display high sebum production and inflammation. Our results confirm the previous findings. Interestingly, the barrier function of the skin scalp seems to be more impaired in dry dandruff scalp. In addition, we have shown here that scalp microbiota of these two dandruff states are different. These results highlight the importance to address dry dandruff and oily dandruff scalps with specific scalp care products. This study is linked with the poster N°446: Effectiveness of Targeted Antidandruff Shampoos: Clinical, Instrumental and Metagenomic Analysis

**Conflict of Interest Statement**. NONE

## References.

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