

Differences of Skin Normal Microbiota in Adult Men and Elderly

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Introduction: Skin is the largest human body organ, colonized by a variety of microorganisms that are mostly harmless or even beneficial to the host. Microbiota includes bacteria, fungi, viruses, and archae that inhabit various areas of the body. Microbiota is a dynamic and will be shift its diversity with age.

Objective: To see the difference in normal skin microbiota in adult men and elderly.

Methods: This research is an observational study using a case control design to see differences in normal skin microbiota in 10 adult men and 10 elderly men. Each skin smear was taken on the forehead, axilla and forearms of the patient. Cultivation of bacteria or fungi is carried out and then isolates of bacterial or fungal are identified using Vitek-2 compact.

Results: The forehead was dominated by phylum *Firmicutes* (adult men: 74%, elderly men: 82.3%) followed by *Actinobacteria* (adult men: 25.9%, elderly men: 17.6%). Axilla was dominated by phylum *Firmicutes* (adult men: 77.2%, elderly men: 86.9%) followed by *Actinobacteria* (adult men: 22.7%, elderly men: 13%). In the forearms was dominated by phylum *Firmicutes* (adult men: 57.5%, elderly men: 77.7%) followed by *Actinobacteria* (adult men: 42.4%, elderly men: 22.2%).

Conclusion: Based on the predominant phylum, the normal microbiota of the skin on the forehead, axilla and forearms of adult men and elderly is not too different. Based on the three location, we found the forearms area had the highest diversity of microbiota and was followed by the forehead area and the least diversity was found in the axilla area.

Index Terms- Microbiota, microbiome

I. INTRODUCTION

The term "normal microbial flora" denotes the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons. The microorganisms that live inside and on humans (now referred to as the normal microbiota) are estimated to outnumber human somatic. The genomes of these microbial symbionts are collectively defined as the microbiome.¹ Human skin microbiomes refer to the entire collection of bacterial microbes, archaea, fungi, viruses, and mites that are on human skin.²

Sequencing of the skin microbiome showed that the human skin microbiota comprises around 113 phylotypes that belong to six bacterial divisions.³ Analyses of the topographical

diversity of microbes of the human skin using 16S rRNA gene phylotyping revealed that 19 phyla are known to be part of the bacterial skin microbiome. Major examples are *Actinobacteria* (51.8%), *Firmicutes* (24.4%), *Proteobacteria* (16.5%) and *Bacteroidetes* (6.3%). The majority of the identified genera are *Corynebacterium*, *Propionibacterium* and *Staphylococcus*.⁴

The skin microbiome is dynamic during a human lifespan.⁵ In utero, fetal skin is sterile, but minutes after birth, colonization begins to occur.⁶ The initial microbial colonization of the skin in infants is influenced by the route of delivery.⁷ In infants, during the first year of life, the skin bacterial communities show high interindividual variation, along with tremendous diversity in both community composition and timing of bacterial acquisition. During the transition through puberty, the skin microbiome again shifts dramatically from predominance of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* to more lipophilic *Corynebacteriaceae* and *Propionibacteriaceae*. Further alterations during later stages of life have not yet been explored.⁵

The diversity of these transient and permanent microbes on our skin is dependent on the topographical regions of the body that have distinctive characteristics (pH, moisture, salinity, and sebum content), and may also vary due to intrinsic factors (e.g. genotype, age, and sex) and extrinsic individual dependent factors such as occupation, lifestyle, geographical location, and use of antibiotics, or cosmetics.⁸ The stability of the microbial community are determined primarily by the specific physiological characteristics of the skin sites; different distribution of hair follicles, eccrine and apocrine glands, and sebaceous glands contribute to the variable cutaneous microenvironments and likely select for subsets of bacteria that can thrive in those specialized conditions. Several structural and functional changes in the skin occur intrinsically as the skin ages.⁹ Skin aging is characterized by a decrease in sweat, sebum and the immune functions thus resulting in significant alterations in skin surface physiology including pH, lipid composition and sebum secretion. These physiological changes provide potential alterations in the skin ecology that may affect the skin microbiome.¹⁰

Investigations in recent years in the human microbiome project have revealed the main impact of microbiota on the host.³ Human skin microbiota is able to control the colonization of potentially pathogenic microorganisms.⁸ Commensal skin

microbiota contributes to host health and plays a role in protection against various infections.¹¹

Most of the literature on skin microbiota concentrates on the role of pathogens from various types of microbiota on the skin, but little research has been done on the effect of skin microbiota on skin health.¹² Commensal skin microbiota is very important for the integrity of the structure and function of the skin barrier. Therefore, characterizing skin microbiota will provide a basis for understanding the role of microbiota in the skin barrier function, and the balance between health and skin disease. Microbes are thought to play a role in the pathophysiology of various skin diseases with predilection in specific skin areas.¹³ Where previous studies regarding the description of skin microbiota in adult men and elderly men are still very limited, so we interested to know about the proportion of skin microbiota in adult men and elderly men based on the location of the topography on the skin.

II. METHOD

This research was conducted from October 2019 to November 2019. It was an observational study with a case control design involved 10 adult men (18 – 59 years old) and 10 elderly men (60 – 74 years old) who came to Departement of Dermatology and Venereology Haji Adam Malik General Hospital Medan. The researcher recorded patient demographic data include the patient's identity (name, date of birth, gender, last education, occupation and address). Then anamnesis is performed including the absence of dermatology diseases in the patient. After the patient signed an informed consent then the material was taken with a skin smear using a sterile cotton swab on the forehead, armpits and forearms as long as 2 x 5 cm in the forehead, 2 x 5 cm in the armpit and 4 x 10 cm in the forearm area. The specimens were brought to the microbiology laboratory for cultivation on media nutrient agar and Saboraud's Dextrose Agar (SDA). Incubated for 24 hours at a temperature of 35 - 37° Celsius. Then examination of the bacterial species that have been cultured using Vitek-2 Compact. This research was carried out after obtaining permission from the Research Ethics Commission of the Faculty of Medicine, Universitas Sumatera Utara and a research permit from the Directorate of Human Resources and Education of the Research and Development Installation of H. Adam Malik General Hospital Medan.

III. RESULTS

A total of 20 subjects; 10 adult men and 10 elderly men, with forehead, armpits and forearms swab's sampling, the following types of microbiota were obtained:

Table 1. Proportion of normal skin microbiota species in adult men and elderly forehead

| Species | Adult | | Elderly | |
|---|-------|------|---------|------|
| | n | % | n | % |
| <i>Staphylococcus epidermidis</i> | 7 | 25.9 | 5 | 14.7 |
| <i>Staphylococcus hominis ssp hominis</i> | 6 | 22.2 | 5 | 14.7 |
| <i>Staphylococcus warneri</i> | 3 | 11.1 | 3 | 8.8 |
| <i>Micrococcus luteus</i> | 3 | 11.1 | 4 | 11.8 |
| <i>Staphylococcus aureus</i> | 2 | 7.4 | 2 | 5.9 |
| <i>Kocuria varians</i> | 2 | 7.4 | 1 | 2.9 |
| <i>Staphylococcus saprophyticus</i> | 1 | 3.7 | 2 | 5.9 |
| <i>Staphylococcus capitis</i> | 1 | 3.7 | 3 | 8.8 |
| <i>Micrococcus lylae</i> | 1 | 3.7 | 0 | 0 |
| <i>Kocuria rosea</i> | 1 | 3.7 | 1 | 2.9 |
| <i>Staphylococcus haemolyticus</i> | 0 | 0 | 2 | 5.9 |
| <i>Staphylococcus vitulinus</i> | 0 | 0 | 2 | 5.9 |
| <i>Staphylococcus sciuri</i> | 0 | 0 | 2 | 5.9 |
| <i>Granulicatella adiacens</i> | 0 | 0 | 2 | 5.9 |
| Total | 27 | 100 | 34 | 100 |

Based on table 1, we found only two varians phylum; *Firmicutes* and *Actinobacteria*, which *Firmicutes* is dominant from forehead both of adult and elderly men (adult: 74.0%, elderly: 82.3%). From genera level, we found 2 genera in adult (*Staphylococcus*: 74.0% and *Micrococcus*: 25.9%) and 3 genera in elderly (*Staphylococcus*: 76.4%, *Micrococcus*: 17.6% and *Abiotrophia*: 5.8%). *Staphylococcus epidermidis* was the dominant species in adult's forehead and in elderly men (adult: 25.9%, elderly: 14.7%).

Table 2. Proportion of normal skin microbiota species in the axilla of adult and elderly men.

| Species | Adult | | Elderly | |
|---|-------|------|---------|------|
| | n | % | n | % |
| <i>Staphylococcus epidermidis</i> | 6 | 27.2 | 4 | 17.4 |
| <i>Staphylococcus hominis ssp hominis</i> | 5 | 22.7 | 8 | 34.7 |
| <i>Staphylococcus capitis</i> | 2 | 9.1 | 0 | 0 |
| <i>Staphylococcus aureus</i> | 1 | 4.5 | 1 | 4.3 |
| <i>Staphylococcus saprophyticus</i> | 1 | 4.5 | 1 | 4.3 |
| <i>Staphylococcus warneri</i> | 1 | 4.5 | 1 | 4.3 |
| <i>Streptococcus parasanguinis</i> | 1 | 4.5 | 0 | 0 |
| <i>Micrococcus luteus</i> | 1 | 4.5 | 1 | 4.3 |
| <i>Micrococcus lylae</i> | 1 | 4.5 | 0 | 0 |
| <i>Kocuria rosea</i> | 1 | 4.5 | 1 | 4.3 |
| <i>Kocuria varians</i> | 1 | 4.5 | 0 | 0 |
| <i>Kocuria kristinae</i> | 1 | 4.5 | 1 | 4.3 |
| <i>Staphylococcus haemolyticus</i> | 0 | 0 | 2 | 8.7 |
| <i>Granulicatella adiacens</i> | 0 | 0 | 2 | 8.7 |
| <i>Aerococcus viridans</i> | 0 | 0 | 1 | 4.3 |
| Total | 22 | 100 | 23 | 100 |

Based on table 2, we found only two varians phylum; *Firmicutes* and *Actinobacteria*, which *Firmicutes* is dominant from axilla both of adult and elderly men (adult: 77.2%, elderly: 86.9%). From genera level, we found 3 genera in adult (*Staphylococcus*: 72.7%, *Micrococcus*: 22.7% and *Streptococcus*:

4.5%) and 4 genera in elderly (*Staphylococcus*: 73.9%, *Micrococcus*:13.0%, *Abiotrophia*: 8.6% and *Aerococcus*: 4.3%). *Staphylococcus epidermidis* was the dominant species in adult's axilla and *Staphylococcus hominis ssp hominis* in elderly.

Table 3. Proportion of normal skin microbiota species in the forearms of adult and elderly men.

| Species | Adult | | Elderly | |
|--|-------|------|---------|------|
| | n | % | n | % |
| <i>Micrococcus luteus</i> | 8 | 24.2 | 5 | 13.9 |
| <i>Staphylococcus hominis ssp hominis</i> | 6 | 18.2 | 5 | 13.9 |
| <i>Staphylococcus epidermidis</i> | 3 | 9.1 | 6 | 16.7 |
| <i>Staphylococcus warneri</i> | 3 | 9.1 | 2 | 5.5 |
| <i>Kocuria rosea</i> | 3 | 9.1 | 3 | 8.3 |
| <i>Staphylococcus capitis</i> | 2 | 6.1 | 1 | 2.8 |
| <i>Staphylococcus haemolyticus</i> | 2 | 6.1 | 1 | 2.8 |
| <i>Kocuria varians</i> | 2 | 6.1 | 0 | 0 |
| <i>Staphylococcus aureus</i> | 1 | 3.0 | 4 | 11.1 |
| <i>Staphylococcus saprophyticus</i> | 1 | 3.0 | 0 | 0 |
| <i>Enterococcus hirae</i> | 1 | 3.0 | 0 | 0 |
| <i>Micrococcus lylae</i> | 1 | 3.0 | 0 | 0 |
| <i>Staphylococcus vitulinus</i> | 0 | 0 | 1 | 2.8 |
| <i>Staphylococcus sciuri</i> | 0 | 0 | 1 | 2.8 |
| <i>Staphylococcus arlettae</i> | 0 | 0 | 1 | 2.8 |
| <i>Staphylococcus cohnii ssp urealyticus</i> | 0 | 0 | 1 | 2.8 |
| <i>Streptococcus mitis</i> | 0 | 0 | 1 | 2.8 |
| <i>Enterococcus cecorum</i> | 0 | 0 | 1 | 2.8 |
| <i>Enterococcus columbae</i> | 0 | 0 | 1 | 2.8 |
| <i>Aerococcus Viridans</i> | 0 | 0 | 1 | 2.8 |
| <i>Alloiococcus otitis</i> | 0 | 0 | 1 | 2.8 |
| Total | 33 | 100 | 36 | 100 |

Based on table 3, we found only two varians phylum; *Firmicutes* and *Actinobacteria*, which *Firmicutes* is dominant from forearms both of adult and elderly men (adult: 77.2%, elderly: 86.9%). From genera level, we found 3 genera in adult (*Staphylococcus*: 54.5%, *Micrococcus*: 42.4% and *Enterococcus*: 3.0%) and 6 genera in elderly (*Staphylococcus*: 63.8%, *Micrococcus*: 22.2%, *Enterococcus*: 5.5%, *Streptococcus*: 2.7%, *Aerococcus*: 4.3% and *Alloiococcus*: 2.7%). *Micrococcus luteus* was the dominant species in adult's forearm and *Staphylococcus epidermidis* in elderly.

IV. DISCUSSION

Study by Wilantho et al on the forehead of 10 adult men and 10 elderly men, found the most dominant phylum is *Proteobacteria* followed by *Firmicutes* and *Actinobacteria*. From the level of phylum *Proteobacteria*, the most dominant genera was found *Rhizobiales* (adult: 31.3%, elderly: 32.1%). From the phylum *Firmicutes*, found the most dominant genera is *Staphylococcus* (adult: 23.4%, elderly: 9.5%) and the dominant species is *Staphylococcus epidermidis* (adult: 17.4%, elderly: 31.3%). From the phylum *Actinobacteria*, found the most dominant genera is *Propionibacterium* (adult: 7%, elderly: 0.9%). In this case, there is a difference between our study with

Wilantho et al. Our study found the predominant phylum was *Firmicutes* (adult: 74.0%, elderly: 82.3%) and *Actinobacteria* (adult: 25.9%, elderly: 17.6%). This study didn't found bacteria from the phylum *Proteobacteria* and also bacteria from the genera *Propionibacterium*. There is also similarities result with study by Wilantho that we found from Phylum level, which the dominant genera from phylum *Firmicutes* is *Staphylococcus* (adult: 74.0%, elderly:76.4%) and dominant species is *Staphylococcus epidermidis* (adult: 25.9%, elderly: 14.7%).¹⁴

Study by Staudinger et al from the forehead of adult men, found 4 bacterial phylum. The most dominant phylum is *Actinobacteria*, followed by *Firmicutes*, *Proteobacteria* and *Bacteroidetes*. The dominant genera was found *Propionibacterium* (*Actinobacteria*) and *Staphylococcus* (*Firmicutes*). This result is different from the research obtained. The phylum was dominated by *Firmicutes* and followed by *Actinobacteria* and our study didn't found *Proteobacteria* and *Bacteroidetes*. *Staphylococcus* (*Firmicutes*) and *Micrococcus* (*Actinobacteria*) were the most common genera that was found.¹⁵

Study by Trocaz et al from the axilla of adult men, found the most dominant phylum are *Firmicutes* (72.2%) and *Actinobacteria* (26.8%) and the most dominant genera was found *Staphylococcus*. This is consistent with our study where the most dominant phylum in the axilla was found *Firmicutes* (77.2%) and *Actinobacteria* (22.7%) and the genera *Staphylococcus* (72.7%). Trocaz also found the genera *Streptococcus*, *Micrococcus* and *Aerococcus* in the axilla and this is the same as the results of our study.¹⁶

Study by Uzeh et al from the axilla of adult men, species dominated was found *Staphylococcus Epidermididis* (35%) . The same result was also found by Costello et al. Whereas in adult men axilla were dominated by *Staphylococcus*. This is consistent with our studies which is the most common bacteria found *Staphylococcus epidermidis* in axilla of adult men (27.2%).^{17,18}

Slightly different from the study conducted by Gao et al, from the axilla of adult men, found that microbiota in axilla was dominated by the genera *Corynebacterium*, followed by *Staphylococcus* and *Betaproteobacteria*. In our study, we found the genera that predominates in the adult's axilla area is the *Staphylococcus* followed by *Actinobacteria*. In our study, we didn't found bacteria from genera *Corynebacterium* or *Betaproteobacteria*.¹⁹

Study by Zhai et al from forearms of adult men obtained the most dominant phylum; *Firmicutes*, *Proteobacteria* and *Actinobacteria* and the most dominant genera found *Staphylococcus*. This results is the same as of our study where in the forearms of adult men the most common was the phylum *Firmicutes* (57.5%) and the genera *Staphylococcus* (54.5%). Different results were obtained in the forearm of elderly men in zhai's study which obtained the most dominant phylum; *Proteobacteria*, *Actinobacteria* and *Firmicutes* and the most dominant genera is *Moraxella*, while in our studies are dominated by the phylum *Firmicutes* (77.7%) and the genera *Staphylococcus* (63.8%).²⁰

Gao et al in his study on the forearms of adult men (6 people) found that the most dominant phylum was *Actinobacteria* (51.4%), *Firmicutes* (23.8%), and *Proteobacteria* (19.4%) and based on the genera level obtained

Propionibacterium (22%), *Corynebacterium* (19%), *Staphylococcus* (11.1%) and *Streptococcus* (5.8%). These results differ from our study which the most dominant phylum in the forearms of adult men is *Firmicutes* (57.5%) and *Actinobacteria* (42.4%). Based on the level of the most dominant genus *Staphylococcus* (54.5%) and followed by the genus *Micrococcus* (42.4%) and *Enterococcus* (3.0%).²¹

On examination of three skin locations, forehead, axilla and forearms in adult men and elderly, *Firmicutes* and *Actinobacteria* are the dominant phylum and *Staphylococcus* is the most dominant genera which found on the forehead (74%), axilla (72.7%) and forearms (54.5%). Of the three locations compared, were found in the forearms area (both in adult men and elderly) with the highest diversity of genera and species bacteria. And this is in accordance with the research by Perez et al which on the forehead, axilla and forearms of adult men obtained *Firmicutes* and *Actinobacteria* as the dominant phylum, with *Staphylococcus* is the most dominant genera, found in >80% in the forehead and axilla area and <50% in the arm. Of the three locations compared by Perez et al also found that the highest number of genera and species bacteria were found in the forearms, followed by the forehead and at least in the armpit area.²²

In this study, majority Gram positive bacteria was found on the forehead, axilla and forearms. This is the same as the study conducted by Staudinger et al who conducted microbiota examination on the forehead and forearms of adult men obtained only Gram-positive bacteria. Previous studies have also reported that Gram-positive bacteria are more common in superficial skin areas.^{15,22}

V. CONCLUSIONS

Based on the predominant phylum, the normal microbiota of the skin on the forehead, armpits and forearms of adult men and elderly is not too different. Of the three locations compared, it was found that the forearm area had the highest diversity of microbiota and was followed by the forehead area and the least diversity was found in the armpit area

VI. SUGGESTION

Conduct further research with a larger sample size for identification of skin microbiota.

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