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Comparative Study of Skin Microbiome of Male and Female subjects in Obong University and it's Environs

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ABSTRACT
The skin is the largest organ interfacing with the external environment. The human skin though sterile prenatally, becomes besieged by microorganisms such as bacteria, fungi, protozoa and viruses after birth. The aim of the study was to compare skin microbiome of the following sites: Oily(glabella and alar crease), Moist(interdigital web space and antecubitalfossa)and Dry(hypothenar palm) of male and female subjects in Obong University and its environs. The objectives of the study were to isolates, characterize and identify bacteria populations from these study sites of the human skin. A total of 54 skin swabs, 30 from male and 24 from female were collected from eighteen (18) subjects, 10 male and 8 female subjects, and were analysed using standard microbiological methods. Five (5) bacterial isolates, *Staphylococcus aureus, Staphylococcus epidermidis, Klebsella pneumonia, Pseudomonas aeruginosa and Cutibacterium acnes* were isolated from the representative skin sites. *S. aureus* and *S. epidermidis* dominated the moist skin at 31% and 29% respectively, and also dominated the dry skin sites at 37% and 36% respectively. However,*C. acnes* was the dominant isolate on the sebaceous sites with 57% occurrence. Based on gender differences, the isolates obtained from the female subjectsfrom the three study sites showed *S. aureus, S. epidermidis. C. acnes*, *P. aeruginosa and K. pneumonia*,occurring at the percentage prevalence of 35%, 36%, 39%, 39% and 36% respectively for the females and with respective prevalence of 65%, 64%, 61%, 61% and 64% for the male subjects. Based on age differences, the isolates, *S. aureus, S. epidermidis*, *C. acnes, P. aeruginosa*, and *K. pneumonia* were prevalent at 20%, 17%, 21%, 22%, and 20% respectively, for the females between the ages of 12-19 years, and 22%, 17%, 23%, 20% and 18% respectively for the male subjects between the ages of 10-19years. The higher prevalence of*Staphylococcus aureus* in males than females in this study is attributed to the fact that men have more sweat glands than women (Kawahata, 1960).

INTRODUCTION

The skin harbors millions of microbes that compose the skin microbiota. Like those in the gut, skin microorganisms have essential roles in the protection against invading pathogens maintaining homeostasis and in the breakdown of natural products (Scharscmidt and Fischbach, 2013; Belkaid and Segre, 2014; Grice, 2015). As the largest organ of the human body, skin is colonised by beneficial microorganism and serves as a physical barrier to prevent the invasion of pathogens. In circumstances where the barrier is broken when the balance between commensal and pathogens is disturbed, skin disease or even systemic disease can result. Human skin sites can be recognized by their physiological characteristics, that is whether they are sebaceous (oily), moist or dry. Studying the composition of microbiota at different sites is valuable for elucidating the etiology of skin disorders which often prefer specific skin sites, such as eczema inside the elbow (Kong *et al*., 2012) and many regional differences overlap in the skin topography. For example, temperature and humidity are higher at vaulted sites such as the groin or armpit (approaching 37ºC, the body's core temperature) and lower at the body extremities (fingers and toes approximately 30ºC) (Schmidt-Wendtner and Korting, 2006). Sebaceous

gland density is an important variable factor involved in the secretion of many lipid compounds including fatty acids that contribute to the acidification of the skin. These characteristics induce many possibilities for creating different ecological riches housing numerous commensal bacteria (Bryd *et al*., 2018). With the recent advent of molecular biologyand next generation sequencing (NGS) as tools for microbiological identification, knowledge about the skin microbiota has grown exponentially. However, culture methods remain as essential tool for studying the characteristics of the microorganism *in-vitro* (Oh *et al*., 2016).

According to both classical and culture-based studies as well as recent metagenomic investigation, *Cutibacterium acnes* dominate in sebaceous sites and *Staphylococcus* and *Corynebacteria* in moist areas (Grice and Segre, 2011).Gene metagenomic sequencing involving 16sVRNA analyses revealed a great variety of bacteria colonizing the sebaceous site.However, bacterial colonization to these greases is overall lower than of the moist sites.

Staphylococcus is widely regarded as the most important colonizers of the human skin, both in terms of frequently and sources of infection(Kloo and Schlefer, 1986).The specific coagulase-negative *Staphyloccus* species colonizing various areas of the skin are *Staphylococcusepidermidis* and *Staphylococcusaureus*. The vulnerability of the skin microbiome lies in the many intrinsic and extrinsic factors that affect it. Studies have revealed that the composition of the human skin microbiota is strongly influenced by the host, specifically by their age, genes, immune status, concomitant health condition of the skin site being evaluated, intervention between microorganism diet and stress levels (Egret *et al*., 2017) and by environmental factors such as lifestyle, domestic and personal hygiene, cohabitation, geographical location, sunlight and occupation (Barnard and Li, 2017).

MATERIALS AND METHODS

Glassware/Equipment

Glassware used include petri dishes, testtubes, conical flasks, glass slides, pasture pipette, beakers, sterile bottles, measuring cylinder. The equipment used include autoclave, microscope, incubator, and other laboratory tools, such as bursen burner, weighing balance, colony counting chamber, spatula, test tube rack, wire loop, foilpaper, forceps and cotton wool.

Media and reagent

These include MacConkey agar, blood agar, methyl red-voges prauskauerbroth, crystalviolet, lugol odine, safranin, ethyl alcohol, hydrogen peroxide, kovac's reagent.

Sample Collection

Eighteenth (18) samples were collected from students and staff of Obong University, as well as, from subjects in Obong Ntak village where the university is located. The samples consisted of nine (9) males and nine (9) female subjects. The samples collected were skin swabs from the moist site (antecubitalfossa and inter digital web space), oily (glabella and alar crease), dry (hypothenarpalm). From the dry site the samples were collected using sterile swabs dipped in sterile water. All samples were labeled according to date, time, gender, age and representative skin sites of the subjects. Samples were transported in sterile laboratory box to the Obong University Microbiology laboratory for analysis within an hour of collection.

Media preparation and sterilization

The laboratory media were prepared according to the manufacturer's instruction and were sterilized using autoclave at 121^{o}C for 15minutes.

Microbiological Analyses

Aseptic protocols were carried out in the microbiology laboratory and the sterile swabs used to collect the skin samples were streaked on plates of blood agar, nutrient agar, MacConkeyagar, and Mannitol salt agar (MSH), labeled according to the respective skin sites. All culture plates were incubated at 37^{o}C aerobically for 24- 48 hours. Culture plates were examined for development of colonies after incubation period.

Identifications of Bacterial Isolates

Presumptive identification of bacterial isolates was carried out using colonial morphology and biochemically using the following test; Catalase test, Coagulase test, Citrate test, Indole test, and Methyl-Red Voges-Praskauertest. Representative bacteria were identified using Bergeys manual of systemic bacteriology (Holt *et al*., 1994).

RESULTS AND DISCUSSIONS

Table1: Cultural, morphological and biochemical characteristic of bacterial isolates of Skin Samples

Cultural characteristics		Morphological characteristics	Biochemical Characteristics									Triple sugar test	
Shape	Pigment	Morphology	Gram staining	IN	CAT	OX	MR	VP	COG	CIT	S	L	G
Presumption organisms													
Circular	Yellow on MSA	Cocci	+	-	+	-	+	+	+	+	+	+	+
S. *aureus*													
Smooth	Pink on MacConkey	Mucoid	-	-	+	-	-	+	+	+	+	+	+
K. *pneumoniae*													
Circular	Negative	Cocci	+	-	+	-	-	+	-	-	+	+	+
S. *epidermidis*													
Rod	Blue/Green	Cocci/rod	-	-	+	+	-	-	-	+	-	-	-
P. *aeruginosa*													
Rod		Branched/Unbranched	+	+	+	+	+	-	+	+	+	+	-
C. *acnes*													

Key: IN: Indole; CAT: Catalase; OX: Oxidase; MR: Methyl red; VP: VogesProskauer; COG: Coagulase, CIT: Citrate; S: Sucrose, L: Lactose; G: Glucose.

In this study, a total of 18 samples were collected from subjects in Obong University and its surrounding environment. Figure1: shows results of the bacteria (5 isolates) isolated and characterized from the specific skin sites (moist, sebaceous, and dry). The representative genera were *Staphylococcus, Pseudomonas, Cutibacterium and Kiebsiella. Staphylococcus aureus* are non-motile, non-sporing, Gram positive cocci that occur in clusters. They are aerobic and facultative anaerobic, catalase and coagulase positive and oxidase and indole negative. They are methyl red and Voges Proskaeur positive and can ferment sucrose, glucose and lactose. They appear as round colonies with yellow zones on Mamnitol salt agar plate. *Staphylococcus epidermidis* are non-motile, non-sporing, Gram positive cocci arranged in grape like clusters. They are facultative anaerobes, catalase and VogesProskaeur positive, coagulase, oxidase and methyl red negative and can ferment glucose, sucrose and lactose. They appear as colorless or red colonies with pink colonies on Mannitol salt agar. *Pseudomonas aeruginosa* is a motile, rod-shaped, aerobic Gram-negative bacterium that forms large, opaque, and flat colonies with irregular margins and fruity odor on Nutrient agar and round, flat and colorless colonies on MacConkey agar. It also forms green colonies due to the presence of the Pyoverdin pigment. Cetrimide agar was used for the selective isolation of *Pseudomonas aeruginosa*. Result from biochemical test shows that*Pseudomonas aeruginosa* is catalase, oxidase and citrate positive, and coagulase, indole, and Methyl red-VogesProskaeur negative. It ferments starch (glucose), with the exception of lactose and sucrose.

Klebsiella pneumonia is a non-motile, rod-shaped, lactose fermenting, Gram negative facultative anaerobe that forms large, pink mucoid colonies on MacConkey agar. It is indole, oxidase, coagulase,c and methyl-red negative, and Voges-Proskaeur, citrate and catalase positive. It causes fermentation of sucrose and glucose. *Cutibacterium acnes* are rod-shaped, anaerobic, Gram positive bacteria that form convex, semi-opaque and glistening colonies on blood agar. Itiscatalase, methyl red, citrate, oxidase, and indole positive, and VogesProskaeur negative. It causes fermentation of glucose, sucrose and lactose.

Staphylococcus species (Firmicutes) occurred at a prevalence of 40% (2/5), *Klebsiellapneumoniae* and *Pseudomonas aeruginosa* (Proteobacteria) at 40% (2/5), and *C. acnes* (Actinobacteria) at 20% (1/5). With respect to the various skin sites, *Staphylococcus aureus* and *S. epidermidis* were dominant on moist areas (interdigital web space and antecubitalfossa) at 31% and 29% respectively, and on dry areas (hypothenar palm) at 37% and 36% respectively. Furthermore, *S. aureus* and *S. epidermidis* occurred at a prevalence of 16% and 15% on sebaceous areas respectively. *Pseudomonas aeruginosa* occurred at 16%, 9%, and 6% on the moist, oily and dry areas of the skin sites respectively. *Cutibacterium acnes* occurred at percentage frequencies of 9%, 10%, and 57% on moist, dry and sebaceous areas respectively while *Klebsiella pneumonia* was isolated from the representative skin sites (moist, dry and oily) at 32%, 8% and 8% respectively.

From the 8 (eight) female subjects, *S. aureus*, *S, epidermis, C. acnes, P. aeruginosa* and *K. pneumoniae* occurred at 35%, 36%, 39%, 39%, and 36% respectively and at 65%, 64%, 61%, 61%, and 64% subsequently for the male subjects. Table 3: shows the percentage occurrence of the bacterial isolates among the age range male and female subjects.

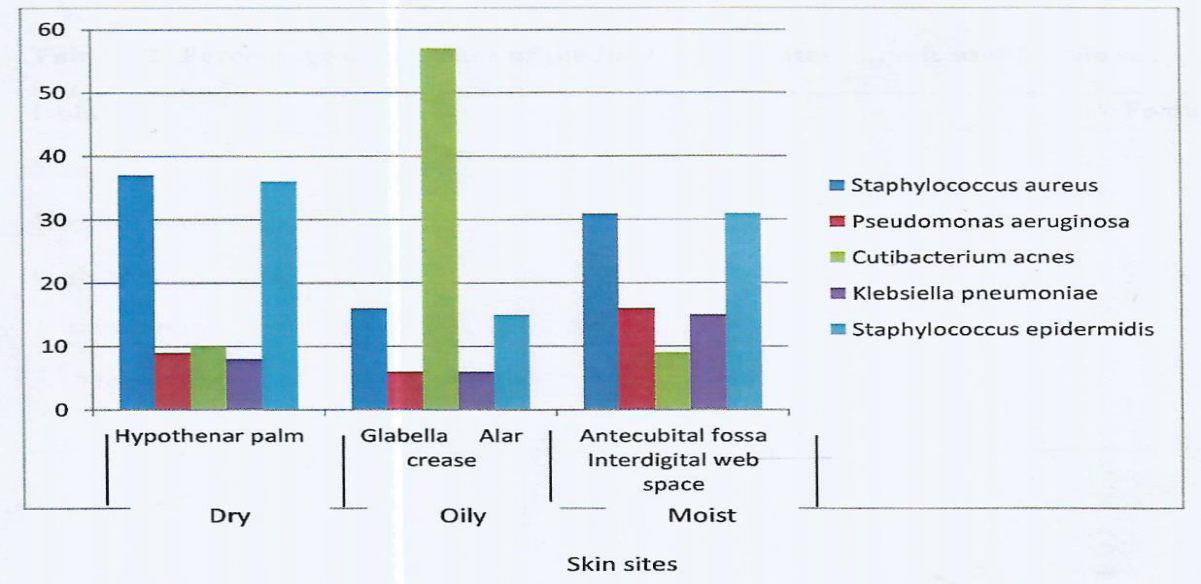

Figure 1: Percentage occurrence of total bacteria isolates from the representative skin sites

Table 2: Percentage occurrence of the bacterial isolates in male and female subjects

Isolates	Male (%)		Female (%)	
S. aureus	6	5	3	5
S. epidermidis	6	4	3	6
C. acnes	6	1	3	9
P. aeruginosa	6	1	3	9
K. pneumonia	6	4	3	6

Table3: Percentage occurrence of the bacterial isolates among the age range of male and female subjects

Isolates	Male %						Female %					
	(10-19)		(25-42)		(58-66)		(12-19)		(21-48)		(51-64)	
S. aureus	2	2	2	3	2	6	2	0	2	1	2	7
S. epidermidis	1	7	2	4	2	2	1	7	2	1	2	6
C. acnes	2	3	1	8	1	3	2	1	2	3	1	4
P. aeruginosa	2	0	1	8	9		2	2	2	0	1	8
K. pneumoniae	1	8	1	7	2	0	2	0	1	5	1	5

Key: (10-19), (25-42), (58-66), (12-19), (21-48), (51-64) = age range

The skin harbors millions of microbes, a vast majority being the bacterial populations that are distributed amongst the topographical areas of the skin. These microbes present on the skin, however, differ in composition based on influence by age and gender. In this study, experiments were conducted to isolate the various bacterial populations on the represented skin sites namely sebaceous (oily), moist and dry and to compare composition of the bacterial skin microbiome based on age and gender.

From the statistical report obtained from this study, the isolated and characterized bacteria were classified into three phyla: Actinobacteria, Firmicutes and Proteobacteria. Firmicutes (*Staphylococcus* species) occurred on the skin at 40%, followed by Proteobacteria (*Kiebsiella pneumonia* and *Pseudomonas aeruginosa*) at 40%, and finally, Actinobacteria (*C. acnes*) at 20%. This is in disparity with findings by Bryd et al. (2018) where the dominant phyla, Actinobacteria, occurred at percentage frequencies of 36-51%, Firmicutes at 24- 34%, and Proteobacteria at 11- 16%. While Bacteroidetes was relatively insignificant in the present study, Bryd et al. (2018) reported its occurrence as low as 6- 9%.In the present study, *Staphylococcus* species was the dominant isolate in moist areas at percentage of 30% and this agrees with reports by Grice and Segre (2011), whose finding revealed that *Staphylococcus* species occurred at 30-37%. This is due to the fact that *Staphylococcus* species are distinguished by an exceptionally high capacity to withstand considerable

changes in osmolarity, salt concentration and pH value (Otto, 2009). However, the results does not agree with findings by Grice and Segre, 2011 revealing *Corynebacterium* species as thedominant isolates from moist skin sites. This disparity in findings can be attributed to the culture methods utilized in this study. Furthermore, the Gram-negative bacteria, *Pseudomonasaeruginosa* and *Kiebsiella pneumonia* occurred at 15.5% on the moist sites. This report agrees with reports by Myles *et al.,* (2016), where the respective Gram negative bacteria isolated from the inner elbow of subjects occurred between 12-18%. This is due to the fact that Gram negative bacteria can survive in humid environments and thus can equally be isolated from the respective skin sites. From the present study, *Cutibacterium acnes* occurred at 9% on the moist sites, this is relatively due to the fact that it is a lipophilic microbe.

The Gram-positive bacterium *Cutibacterium acnes* showed dominance on the sebaceous areas of the skin occurring at a percentage of 57%. This data agrees with a study conducted by Leeming*et al.,* (1984) where the dominance of *Cutibacterium acnes* on oily sites was explained to be as a result of the lipid-rich substance called sebum secreted by the sebaceous glands. *Staphylococcus* species appeared sparse on the sebaceous sites of the skin occurring at 15.5%. This finding, however, is in disparity with a study by Grice *et al.* (2011), showing *Staphylococcus* species as lipophilic microorganisms and thus can be isolated from sebaceous sites like the *Cutibacterium acnes*. *Kiebsiellapneumonia* and *Pseudomonas aeruginosa* were almost insignificant at 6%, as they are non-lipophilic and do not have the capacity to survive in the sebaceous environments (Lowbury, 1969).

In the present study, *Staphylococcusaureus*, and the coagulase-negative *Staphylococcus epidermidis* were isolated from the hypothenar palm and dominated the representative skin site at 35%. According to Wilson (2008), this is attributable to the fact that *Staphylococcus* species are xerophilic, hence can survive dry conditions or environments. While *Cutibacterium acnes* occurred at 10% due to its low tolerance of dry skin environments (Bruggerman *et al*., 2004), *Pseudomonas aeruginosa* and *Klebsiellapneumoniae* occurred at 9% and 8% respectively.

Cutibacterium acnes was isolated from the female subjects aged (12-19), (21-48) and (51-64) with percentage occurrences of 21%, 23% and 14% respectively and at 23%, 18%, and 13% for the male subjects between the ages of (10-19), (25-42), and (58-66) respectively in this study. These differences may be attributed to an increased level of sebum production in adolescents during puberty, and the less abundance of *C. acnes* as a result of low sebum production as the age gradually increases (Ying *et al.,* 2015). Relatively, amongst the males, especially during adolescence, there is greater abundance of *C. acnes* due to the presence of more hair follicles on their skin as opposed to the females (Ying *et al*., 2015).

Staphylococcus aureus was observed at 20%, 21%, and27% from female subjects aged between(12-19), (21-48), and (51-64) respectively and 22%, 23%, and 26% from the male subjects aged between (10-19), (25-42), and (58-66) correspondingly. The increase in *Staphylococcus aureus* in men than women is attributed to the fact that men have more sweat glands than women (Kawahata, 1960), hence producing a more humid environment for the species to thrive. Furthermore, the disparity in the result of *Staphylococcus aureus* associated with the younger females and males compared to the older females and males is attributed to increasing sexual maturity, as the diversity of microbes occur in mature subjects (Oh *et al.,* 2012). These findings are equally applicable to the coagulase negative *Staphylococcus epidermidis* isolated from the female subjects between ages (12-19), (21-48), and (51-64) at17%, 21%, and 26% respectively, and at 17%, 24%, and 22% from male subjects aged between (10-19), (25-42), and (58-66) subsequently.

Oh *et al.*(2016) placed emphasis on the maturity of the gender that leads to a more stable and diverse microbial environment, which relatively includes Gram negative bacteria isolated from the skin of subjects varying across ages. This is seen among the female subjects in the age groups (12-19), (21-48), and (51-64).*Klebsiellapneumoniae* was observed at 20%, 15%, and 15% in the male subjects in the age groups (10-19), (25-42), and (58-56). Its occurrence was placed at 18%, 17% and 20% respectively. *Pseudomonas aeruginosa* occurred at 22%, 20%, and 18% for the female subjects subsequently aged (12-19), (2 1-48), and (5 1-64), and at 20%, 18%, and 19% for male subjects aged (10-19), (25-42), and (58-66) respectively.

CONCLUSION

In conclusion, the human skin is an ecological community, with an abundance of microbes flourishing on various sites. These microbes, contribute to the stability and sustainability of the skin and the host in general with their interactions with each other and the host. Further evidence obtained from this study reveals these microbes are greatly influenced by age and gender, and are inhabitants of specific sites of the skin.

Recommendations

Based on the findings of this study, the following recommendations should be considered.

i. Avoid synthetic and artificial colors in skin care products, as they can increase the risk of skin sensitivity and irritation. These chemicals can throw the beneficial bacteria in your skin microbiome off balance and close your pores, making acne flares up more common.

ii. Good and healthy lifestyles should be encouraged to help modulate the skin microbiome.

Infants, aged, and immune compromised people are likely to be infected with diseases from microbiome imbalance. Thus, good hygienic practices and care should be employed for the prevention of such situations. Also, administration of medications can be useful to boost the immune system against these infections.

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