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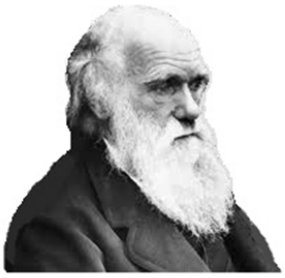

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Epidermal Microbes: Comparative study to Evaluate Bacteria Diversity on Human Skin

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ABSTRACT

Millions of microbes such as bacteria, fungus, and viruses are collectively known as skin microbiota. The epidermal microbes are found on the human skin which is a living and functioning ecosystem that is home to many microorganisms. The aim of this study was to evaluate the diversity of bacteria on human skin. Two hundred (200) volunteers with written consent were recruited for the study. This study was conducted in Akure, Ondo State, Nigeria. Skin swab samples were collected using the Z stroke swabbing method. The samples were cultured on blood and mannitol salt agar medium. The isolated bacteria were classified based on physical and staining characteristics with respect to respondents' occupation, skin colour and other variables. Overall prevalence of 62.5% was recorded for cocci shaped and 37.5% for rod shaped bacteria respectively. The males were observed with higher colony counts (52.1%) when compared to the females. However, 72% of females were found to harbour more of cocci-shaped bacteria on their skin whereas, males skin was predominant (69.3%) with the rod-shaped bacteria ($p=0.35$, $p>0.05$). In relation to occupation, the Auto-Mechanics (33.3%) had the highest colony count, followed by bikers (18.3%), Carpenters (15.2%) and Traders (15%); however, the students had only 8.3% out of the total sampled population. Meanwhile, the percentage of cocci bacteria observed on the student population was higher (51.2%) than found on others. This study further revealed that the married individuals had more epidermal microbes (59.8%) when compared to the singles (40.2%); and dark skinned individuals had more bacteria isolates (59.1%) when compared to fair-skinned counterparts. The prevalence of epidermal microbes in relation to occupation was statistically significant ($X^2 = 17.47$, $p = 0.01$, $p < 0.05$). A higher percentage of epidermal microbes were predominant on males than the females. This study further establishes the abundance of Cocci-shaped gram positive bacteria such *Staphylococcus aureus, streptococcus* on study participants. This study therefore recommends proper skin care routine practices and emphasizes the need for intense sensitization among artisans and

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particularly among the less educated ones about adequate skin care in order to avoid proliferation and spread of pathogenic skin microbes.

***Keywords*:** Skin, Epidermal microbes, Comparative, diversity, Gram-positive Cocci, Rod-shaped Bacteria

1. INTRODUCTION

The skin is an important barrier organ that protects the body's internal organs from external environmental stresses such as dryness, high temperatures, UV radiation, allergies, poisons, and microbes (Kobayashi and Imanishi, 2021); and this functioning ecosystem is home to millions of microbes including bacteria, fungus, and viruses (Bryd *et al.,* 2018). The human skin, which is the largest and most exposed organ of the body, constantly acquires a large number of transient microbe species due to contact and exposure to different people and the external environment (Oh *et al*., 2016).

The skin microorganisms play a vital role in protecting us from invading pathogens; just as those residents in our guts, and in breaking down of natural products applied to the outer skin surface (Grice, 2015). The skin health is maintained by collaboration between the microbiota, host skin cells, and the immune system (Swaney and Kalan, 2021). However, impaired skin function can arise when there is a disruption in this delicate process, altering a balanced system due to a pathogen invasion or destruction of the skin barrier (Belkaid and Segre, 2014). Some epidermal microbes that dominate the human skin include *Staphylococcus* species, *Streptococcus* sp, *Cutibacterium* (formerly *Propionibacterium*), *Corynebacterium* etc.

Cutibacterium and *Staphylococcus* species are commonly found in the sebaceous sites such as the face and torso (Scholz and Kilian, 2016). They are also commonly found in the nose, armpit and groin. *Staphylococcus aureus* is a gram positive bacteria which has been implicated in several skin and bone infections, bacteremia and food poisoning.

Corynebacterium dominates moist areas like the conjunctiva of adults and throats (Aoki *et al*., 2021). They are gram positive, anaerobic organisms which can cause serious health issues when encountered via airborne droplets and usually replicate on the skin or throat. Some strains are also found on the elbow and knee creases (Hernandez *et al*., 2020). *Streptococcus* are also gram positive bacteria responsible for many disorders such as wound and skin infections, sepsis and endocarditis. They are commonly found on the skin surface and throat and involved in mixed infection of the brain, abdomen and lungs (Brower *et al.*, 2023).

In contrast to bacterial communities, *Malassezia* fungi are present throughout the body, but they predominate in oily areas like the face, chest, back and head. (Findley *et al*., 2013). The microbial load on different skin sites varies with people, location, genetic-make up and environmental factors. Furthermore, despite the fact that the taxa of bacteria that colonize the skin of healthy people are generally identical, the skin microbiota of people with primary immunodeficiency is more ecologically permissive, with changed population patterns (Oh *et al*., 2013; Lehman, 2014). There is therefore no doubt that the skin microbiota's makeup can substantially change as disease progress (Kong *et al*., 2012).

Although it is unknown whether these changes are the cause or result of the skin condition however, particular microbe species or strains have been strongly associated with cutaneous disorders such as eczema, psoriasis, and *Acne vulgaris* (Chng *et al*., 2016; Quan *et al*., 2020).

Hence, knowledge about the makeup of the skin microbiota at distinct sites is useful for clarifying the aetiology of common skin illnesses such as eczema within the elbow and psoriasis on the outside of the elbow that have preference for specific skin sites (Kong *et al*., 2012).

This study was carried out to investigate and compare the various epidermal microbes inherent on skin of people from different location in respect to their daily activities and environment.

2. MATERIALS AND METHODS

2. 1. Description of the study area

The study was conducted at the Federal University of Technology Akure, (FUTA) and its environment. Akure is the capital and largest city of Ondo state, which covers a land area of 14,793 square kilometers within south-west of Nigeria. It lies between latitude 7°15'0''N and longitude 5°11'42''E, and has a population of 717,000. FUTA is situated between latitude 7.3070°N and longitude 5.1398°E with a population of about 20,000 students excluding the residents in the vicinity. It is located at an elevation of 15.97 metres above sea level, with district yearly temperature of 28.29 °C. Akure, has a rich tropical and forest vegetation which supports agricultural and other business activities.

2. 2. Study design

Two hundred (200) volunteers with written consent were recruited for the study. Skin swab samples were collected using the Z stroke swabbing method and the samples collected were cultured on blood and mannitol salt agar medium. The isolated bacteria were classified based on shape, colour and staining characteristics. A structured questionnaire was administered before sampling procedures, to obtain relevant information on gender, age, marital status, occupation, literacy level, bath and hygiene routine, skin colour and usage of skin care products.

2. 3. Sample collection

Using sterile cotton swab sticks, 200 samples were collected at random from volunteers within FUTA and its environment. The respondents encompass the students, auto-mechanics, food traders, Bike-riders, carpenters and hairdressers. The samples were collected using a swabbing method. The dorsal side of the hand was gently swabbed in a Z stroke manner with a sterile cotton swab stick which has initially been dipped into and saturated in 1ml of sterile water so as to soak the cotton swab for easy collection. A serial dilution factor of 0.4 was done for each samples, after which 1ml of the diluted samples was introduced into the sterilized petri dishes containing the prepared agar media. The media were left to gel and the plates were incubated at 37 °C for 24hours.

2. 4. Swab culture

Using an inoculating loop under a sterilized environment, colonies from each plates were streaked on another prepared agar plate and incubated for 24-78 hours at 37 °C. On the already streaked plates, using an inoculating loop, colonies were picked and placed on sterile glass slides on which gram staining was done.

2. 5. Counting of colonies

The counting of bacteria was done by dividing the plates into equal sections (1\4). After counting a section, the count was multiplied with the total number of sectors to estimate the whole plate count. Each media plates were counted per section to estimate the whole plate count. The total number of colonies was calculated using the formula:

$$\text{The total number of colonies} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Volumeof culture plate}} = \frac{2500 \times 0.4}{5} = 200$$

2. 6. Bacteria Identification

The Bacteria were identified using physical examination and gram staining. The method described by Bisen *et al*. (2012) was used for the identification and classification of the bacteria after obtaining a pure culture of each sample. They were classified based on colour, shape, size, and staining characteristics.

2. 7. Statistical analysis

The identified bacteria based on the method of sample collection was compared with paired t-test using Statistical Package for the Social Sciences (SPSS) version. All tables and graphs were plotted using Microsoft Excel.

3. RESULTS

Table 1. Demographic data of the participant in the study population.

Demographic variable	Subgroup	Frequency (N)	Percentage (%)
Gender	Male	58	29.0
	Female	142	71.0
Occupation	Student	100	50.0
	Trader	76	38.0
	Bike rider	11	5.5
	Mechanic	3	1.5
	Carpenter	4	2.0
	Hairdresser	6	3.0
Subdivision of study samples	FUTA Student	100	50.0
	FUTA Area Residents	100	50.0

Marital Status	Single	120	60.0
	Married	80	40.0
Skin colour	Dark	134	67.0
	Fair	66	33.0
Bathing pattern	Twice a day	200	100.0
Total		200	100.0

Bacteria growth and characteristics

Out of the 200 samples analysed, majority of the bacteria isolates were spherical/cocci (62.5%), while the cylindrical/ rod shape bacteria made up 37.5%; based on their physiological characteristics, all the bacteria isolates were gram positive, opaque and white in colour.

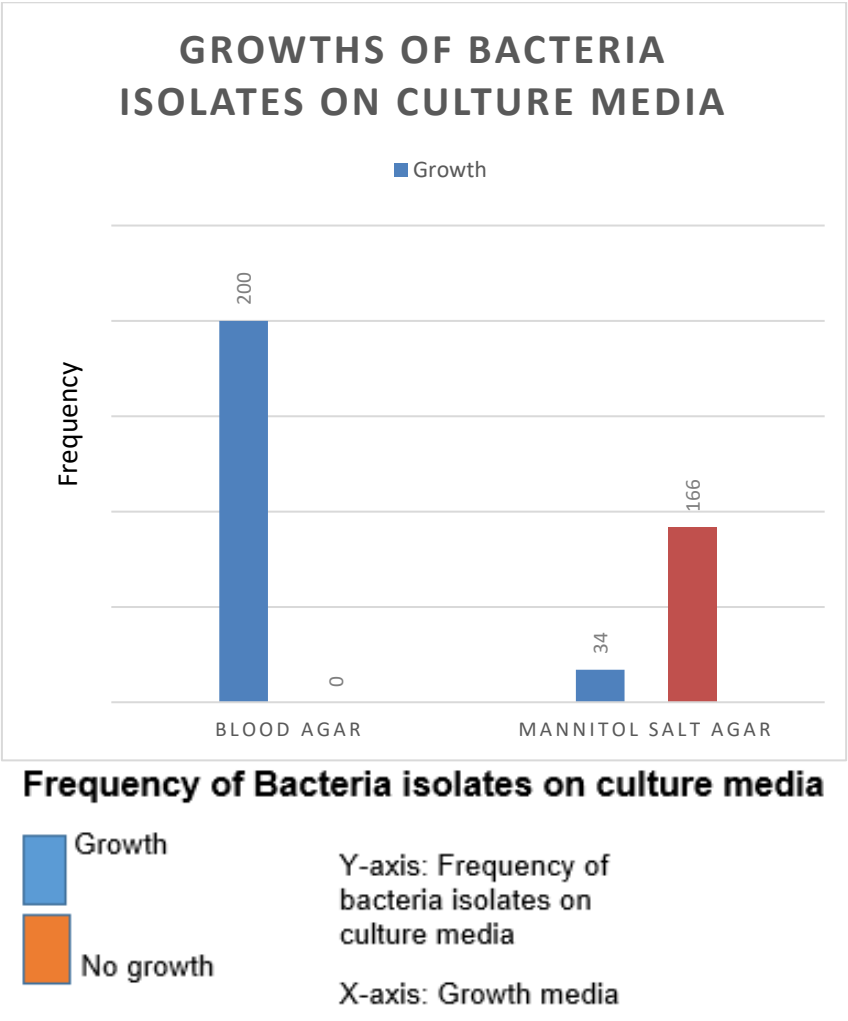


Fig. 1. Bacteria Isolates growths on culture media.

Bacteria colonies in the culture media

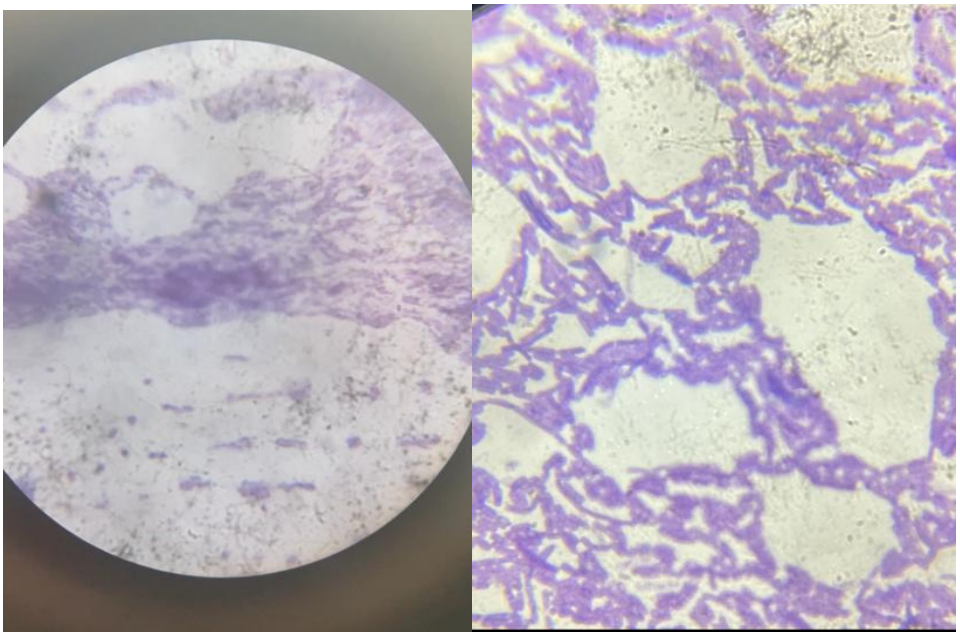


Fig. 2. Photomicrograph of bacteria isolates from study population (*cocci-left, rod-right*).

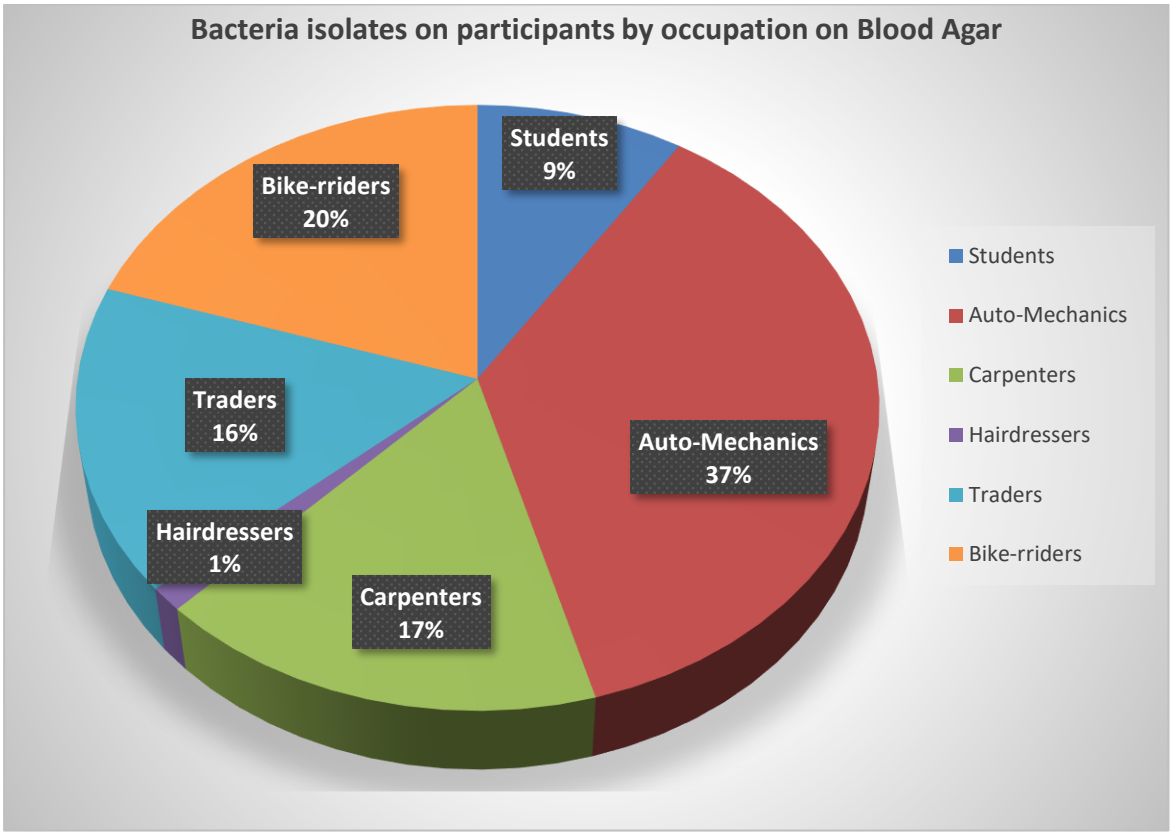
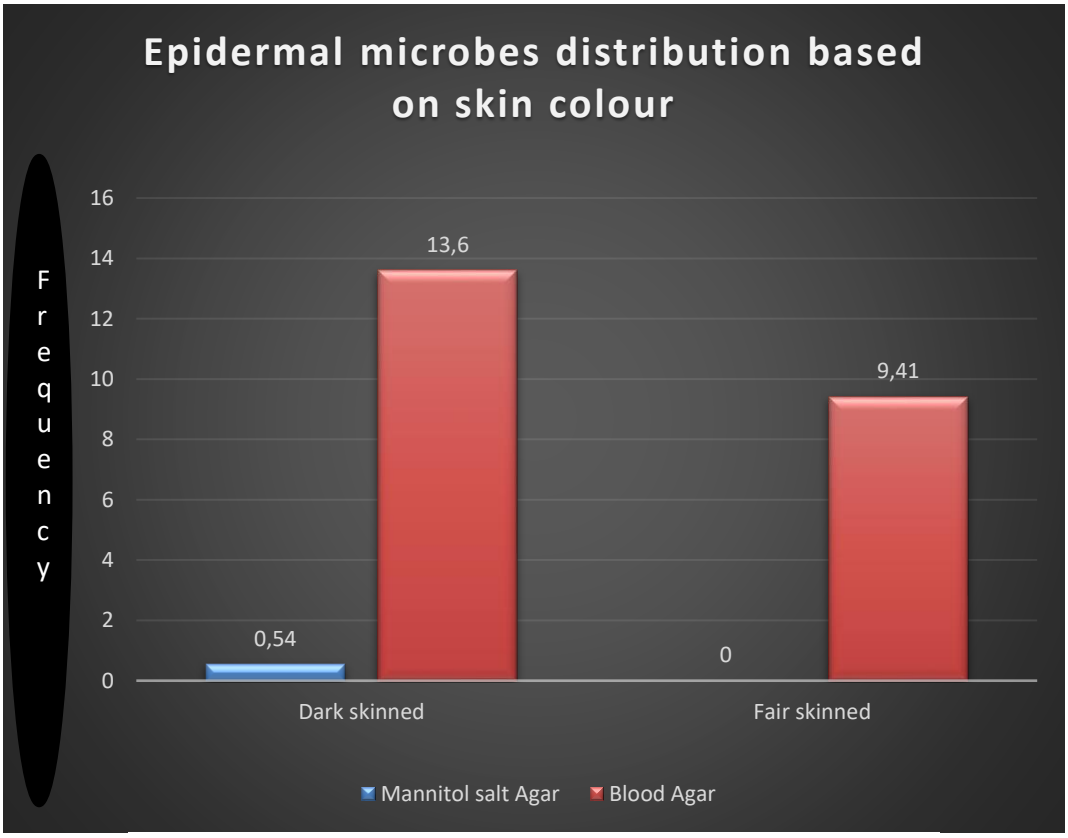


Fig. 3. Epidermal microbes distribution by occupation.

The males had higher colony count on blood agar (52.1%) when compared to the female (47.9%). However, there exists no relationship between gender and colony count ($p = 0.35$). Auto-Mechanics had the highest number of bacteria colony count (33.3%) which was followed by bike riders (18.3%) and the least colonies were recorded among students (8.4%). The comparison between the subdivision of study samples showed that FUTA students had a lower bacterial colony count on blood agar (35%), compared to FUTA area residents with (65%). Participants who were single (40.2%) had lower bacteria colony count compared to married (59.8%). Based on the skin colour, those with fair skin had a lower bacterial colony count (40.9%) compared to those with dark skin (59.1). Demographic variables of the participants based on the occupation was statistically significantly ($p < 0.05$). The overall mean bacteria colony count for blood agar in the study population was 12.21 ± 0.52.



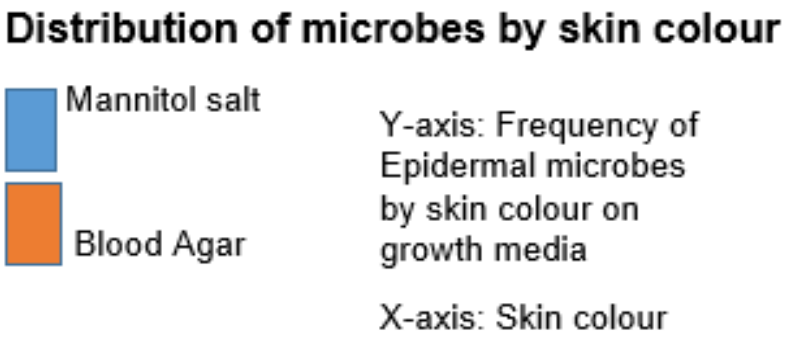


Fig. 4. Distribution of bacteria isolates by skin colour on culture media.

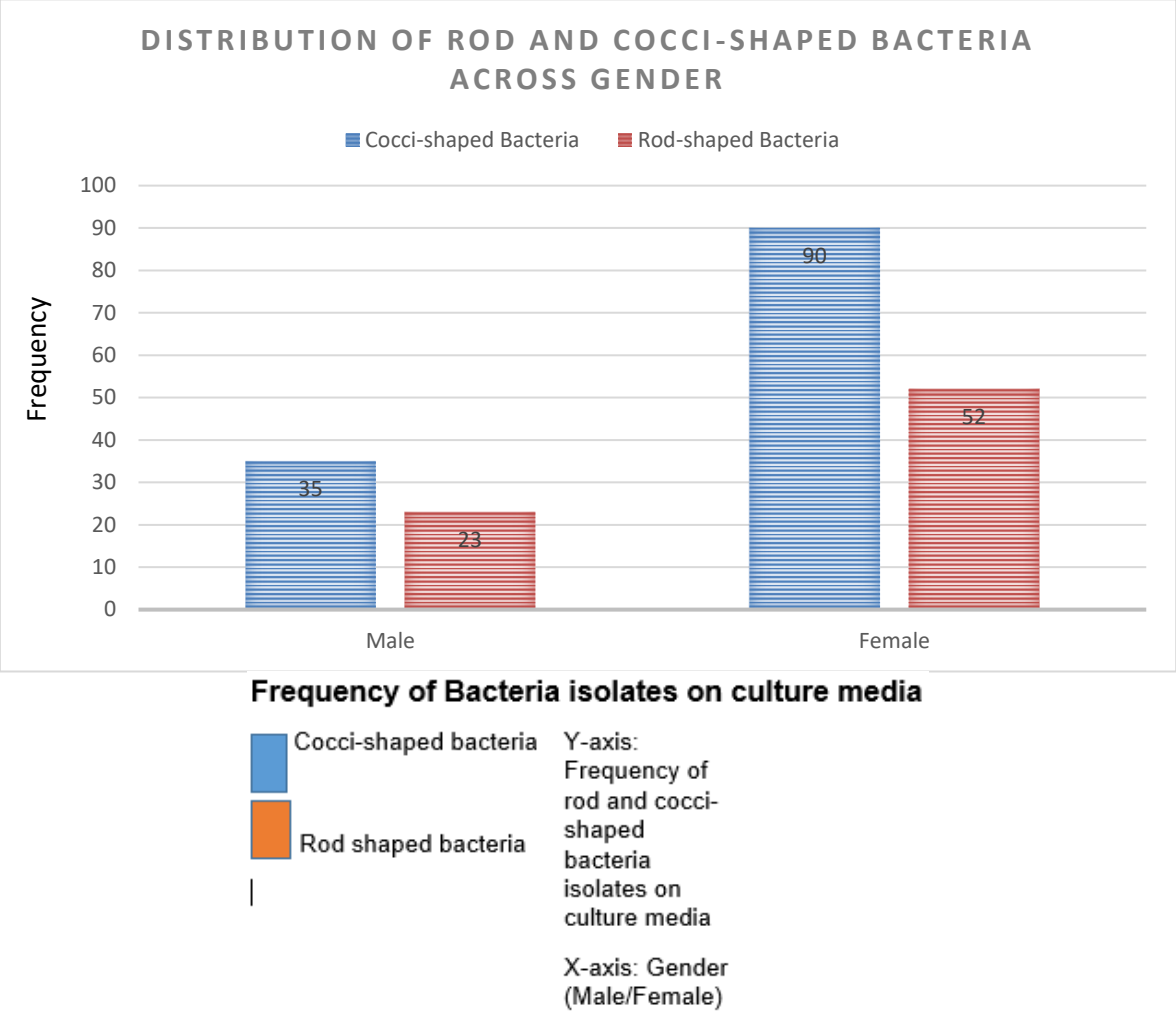


Fig. 5. Bacteria Isolate types across gender in study population.

Table 2. Prevalence of bacteria isolates on study participants.

Demographic variable	**Subgroup**	**Shape N (%)**		**χ^2 (df), p-value**
		Cocci	Rod	0.16 (1), 0.69
Gender	Male	35 (60.3)	23 (39.7)	
	Female	90 (63.4)	52 (36.6)	
Occupation	Student	64 (64.0)	36 (36.0)	17.47 (1), 0.01
	Trader	46 (60.5)	30 (39.5)	
	Bike rider	11 (100.0)	0 (0.00)	

	Mechanic	0 (0.00)	3 (100.0)	
	Carpenter	3 (75.0)	1 (25.0)	
	Hairdresser	1 (16.7)	5 (83.3)	
Subdivision	FUTA Student	64 (64.0)	36 (36.0)	0.19 (1), 0.66
	FUTA Area Residents	61 (61.0)	39 (39.0)	
Marital Status	Single	75 (62.5)	45 (37.5)	0.00 (1), 1.00
	Married	50 (62.5)	30 (37.5)	
Skin color	Dark	82 (61.2)	52 (38.8)	0.30 (1), 0.59
	Fair	43 (65.2)	23 (34.8)	
Overall		125 (62.5)	75 (37.5)	

4. DISCUSSION

The study provides the assessment of microbiological examination of skin swabs for bacteria species associated with the skin of different demographic groups in and around the Federal university of technology, Akure, Ondo State. The result of this study shows that gram-positive cocci and rods were preominant on the study participants. This further establishes the dominance of gram-positive bacteria such as *Staphylococcus*, *Micrococcus*, and *Corynebacterium* sp. as natural skin microflora (Chiller *et al*., 2001; Byrd *et al*., 2018). However, when some pathogenic species of these gram-positive cocci and rods are found on the skin, they could cause skin irritations and inflammation.

Results from this study showed that the males had a higher colony count than the females. The female had a higher prevalence of cocci bacteria while the male had a higher prevalence of rod bacteria. Physiological and anatomical differences between male and female cutaneous environments such as sweat, sebum and hormone production, also partially account for the microbial differences seen between the genders (Giacomoni *et al*., 2009; Boxberger *et al*., 2021). This study observed that mechanics and bike riders had the highest mean bacteria colony count while the least colony count was observed in students. The results obtained, showed that the rate of bacterial isolates from the skin of various occupational groups for the population under study is similar to those of several previous studies (Ogaraku and Onovo, 2007; Obiazi, 2016). For instance, in the study of Obiazi (2016) Out of the 25 samples collected from each occupation, 11(44%) were positive for students, 21(84%) were for bike riders, 12(48%) for office workers, and 20(80%) for bricklayers. Obiazi (2016) further reported that Bike riders appeared to be the occupation with the highest bacterial isolates on the skin followed by bricklayers, office workers and students. In this study, bike riders had the highest prevalence of cocci bacteria while mechanics had the highest prevalence of rod bacteria.

This study revealed that FUTA students had a lower bacterial colony count on blood agar compared to FUTA area residents. It also showed that FUTA students had a higher prevalence of cocci bacteria while the FUTA residents had a higher prevalence of rod bacteria. Participants who were single had lower bacteria colony count compared to married participants. However,

both participants who were single and married had an equal prevalence of cocci and rod bacteria. Participants who were fair-skinned had lower bacteria colony count compared to those with dark skin for both blood and mannitol salt agar isolates. The reason for this is unknown as there has not been a specific study found in the literature linking skin pigmentation and the diversity of bacteria species. Participants who were dark-skinned had a higher prevalence of rod bacteria while those who were fair-skinned had a higher prevalence of cocci bacteria. This study also shows that there is a high number of colonies among the residents compare to students due to the environment and exposure to sun. It was also observed that all human skin has microbes in respective to the environment. Proper precautions must be taken to avoid exposure of the skin to dangerous microbes. Although, it can be established from this study that FUTA residents are more prone to microbes due to exposure to constant sun and impurities from the environment.

5. CONCLUSIONS

The pH of the skin surface is naturally acidic ranging from 4-4.5 due to the lactic acid perspiration and skin bacteria. The skin's antibacterial component is more effective under acidic conditions, which may promote the development of pathogenic microorganisms. Although, temporary microorganisms like Gram-negative bacteria like *Escherichia* and *Pseudomonas* or Gram-positive bacteria like *Candida albicans* do not develop at this pH, but mutualistic flora like *Staphylococci*, *Micrococci*, and *Corynebacterium* does. It is therefore safe to conclude that several factors like occupation, skin colour, clothing choice and antibiotic usage, may impact on the microbial diversity of the skin. Thus, establishing a relationship between epidermal microbe inherent on the skin in reference to the skin colour, occupation, gender and location. Lastly, the diversity and population of bacteria on the skin is affected by the gender, occupation, skin colour and marital status of the participants. People should therefore take proper care of their skins to avoid the proliferation of pathogenic skin bacteria; and this can be done with effective personal hygiene, appropriate diet and clothing.

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