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# Evaluation of the Impact of Different Footwear Brands on the Human Toe Web Microbiome of Selected Students in Awka, Nigeria

**Stephanie Ifunanya Nkamigbo*, Ugochukwu Chukwuma Okafor, Onyekachukwu Izuchukwu Udemezue**

Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

*Correspondence: stephanienkamigbo@gmail.com

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**ABSTRACT:** The synergy between microbes on human-associated objects and the environment we inhabited was of great relevance to human health and disease transmission. The type of footwear a person wore could influence the toe microbiome, which was a unique and often overlooked microenvironment. In this study, the effects of different footwear brands were investigated in relation to the toe web microbiome. Thirty (30) specimens were sampled from the toe webs of individuals who wore various kinds of footwear. They were grouped as free-toed shoes, breathable shoes, and non-breathable shoes. The assessment of these samples took place in the Microbiology Laboratory of Nnamdi Azikiwe University, Awka. The media used for the analysis such as nutrient agar (NA) and sabouraud dextrose agar (SDA)—were prepared according to the manufacturer's specifications. Identification and characterization of the isolates were based on morphology, staining reactions, microscopic examination, and biochemical tests. Four bacterial isolates were found in the toe webs of the participants: *Neisseria* species from free-toe samples, *Lactobacillus* species from breathable samples, and *Streptococcus* species isolated from both free-toe and non-breathable samples. Five fungal isolates were also found in the toe webs of the participants: *Saccharomyces* species and *Aspergillus* species from breathable samples, *Candida* species from non-breathable samples, and *Malassezia* species isolated from both free-toe and breathable samples. The results indicated that wearing shoes, especially non-breathable ones, could influence the skin microbiome in several ways. By understanding these microbial dynamics, public health guidelines and consumer choices could be better informed, potentially reducing the incidence of skin infections.

**KEYWORDS:** Microbiome; toe web; footwear; human health; fungal isolates

## 1. Introduction

The microbiome described a complete habitat. It included the microorganisms, their genes, and the environmental conditions surrounding the habitat. This definition was derived from the word "biome," which referred to the biotic and abiotic factors within a particular environment.

However, other scientists in the field limited this definition to the collection of genes and genomes present in a given microbiota [1].

The human body housed a limitless ecosystem of microorganisms collectively known as the microbiome. These microbes inhabited various niches, including the skin [2]. The skin, being the largest organ, served as a bridge between the body's internal environment and the external world. It was a dynamic ecosystem with trillions of microorganisms coexisting in complex communities that played important roles in maintaining skin health. Among the many regions of the skin were the toe web spaces, which represented a unique and often overlooked microenvironment where the interplay between biological and external factors, including footwear, had profound implications [3].

The toe web interspace was a small yet important part of the human body. Its microbiome, comprising bacteria, fungi, and other microorganisms, was an integral component of the skin's ecosystem, and these microorganisms existed in a delicate balance. They contributed to the maintenance of skin integrity, supported immune function, and provided protection against potential pathogens. The practical relationship between the microbiome and human health had sparked significant interest in recent years and had led to extensive research into the factors that shaped and influenced these microbial communities [2].

One such factor that attracted attention was footwear. Footwear had become an essential aspect of daily life because of its role in protecting the human foot. However, while providing protection, it also caused heat discomfort and hygienic issues due to the enclosed environment. Like garments, footwear restricted the free transfer of body heat and vapor to the surrounding atmosphere, thereby creating a confined habitat with high temperature and humidity [4].

The toe web was a relatively warm, moist, and nutrient-rich environment, making it an ideal habitat for microorganisms. When humidity inside footwear reached 96–100%, it significantly promoted bacterial growth and the spread of yeast-like fungi. Bacterial growth could trigger common bacterial infections such as erythrasma and lead to foot odor caused by the degradation of leucine in sweat by *Staphylococcus epidermidis*, whereas yeast-like fungi could cause diseases such as dermatophytosis [5]. Most microorganisms, including pathogens naturally residing inside or on the surface of the human body, were mesophiles and thrived optimally at around 37 °C, which corresponded to the peak value of the recorded in-shoe temperature (27–37 °C) under consistent moderate weather conditions [6].

In addition, the toe web was subjected to continuous mechanical stress and friction, further influencing its microbial inhabitants. The type of footwear a person wore could affect the moisture levels on the skin [5]. Prolonged moisture, often caused by non-breathable shoes, created an environment where certain microorganisms thrived, leading to skin infections or odors. Foot odor was often associated with microbial activity. Understanding how footwear influenced the toe microbiome could lead to better strategies for preventing or managing foot odor. By examining the relationship between footwear and the toe microbiome, individuals could make more informed choices about the types of shoes they wore to promote better overall foot health [7].

This research aimed to investigate the effects of different footwear brands on the human toe web microbiome. Previous studies on the human foot microbiome had focused mainly on the overall skin flora of the foot, often under controlled footwear conditions. However, limited attention had been given to the toe web microenvironment. This study was distinctive in focusing on the toe web, one of the most enclosed and microbe-dense regions of the foot.

Unlike previous studies, for example [2, 6], which examined the effects of footwear under controlled conditions or with only a single type of shoe, this study compared three footwear categories commonly worn in a tropical Nigerian context.

## 2. Materials and Methods

### *2.1. Study area.*

The study was carried out in the General Microbiology Laboratory of Nnamdi Azikiwe University, Awka, located in Awka South Local Government Area, Anambra State, Nigeria. The university was situated in the southeastern part of Nigeria, at latitude 6.19560° N and longitude 7.07090° E, with a land area of approximately 4.99 km² (equivalent to 499 hectares). It accommodated about 40,000 students, including both undergraduate and postgraduate populations.

### *2.2. Sample collection.*

Thirty (30) volunteer students (18 males and 12 females; aged 17–28 years, with an average age of 22.5 years) participated in the study. Ethical approval to conduct the research was obtained from the University's Research Ethics Committee, and all participants provided informed consent after being briefed on the study objectives. Individuals exhibiting visible foot infections or with a history of antibiotic or antifungal medication use within two weeks prior to sampling were excluded from participation. The participants were grouped according to the type of footwear they typically wore: (1) free-toe (sandals/slippers), (2) breathable (perforated fabric shoes), and (3) non-breathable (enclosed leather shoes). A control group consisting of five volunteers remained without footwear for at least two hours before sampling. Samples were collected during midday hours from various parts of the toe webs. All samples were properly labeled and transported in a pre-cooled insulated container with ice packs to the Microbiology Laboratory for analysis..

### *2.3. Isolation of microorganisms.*

Under aseptic conditions, the swab samples were inoculated onto pre-prepared Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) plates to assess the growth and identification of bacteria and fungi, respectively. After inoculation, the culture plates were incubated at 37 °C for 24 hours to allow microbial growth. Subsequently, suspected colonies were subcultured onto fresh agar plates to obtain pure isolates.

#### *2.3.1. Quantification of microbial load.*

After incubation, the colonies were counted using a digital colony counter. The total viable counts were expressed as colony-forming units per millilitre (CFU/mL) by multiplying the mean colony number by the dilution factor. The mean bacterial and fungal loads were then determined from two replicate plates for each sample.

### *2.4. Pure culture maintenance.*

The isolates were purified using the repeated subculture technique. The streak plate method was employed for this purpose, using Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA)

as culture media. Once a plate produced a single distinct colony, the culture was considered pure.

*2.5. Identification and characterization of the isolates.*

The isolates were identified and characterized based on their morphological and biochemical characteristics and were further classified by referencing *Bergey's Manual of Systematic Bacteriology*. Microbial identification involved several procedures, including Lactophenol Blue staining, Gram staining, motility testing, observation of colony color and pigmentation on culture plates, and odor assessment. In addition, biochemical tests such as the catalase test, citrate utilization test, sugar fermentation test, and hemolysis test were also performed.

*2.5.1. Lactophenol blue staining.*

The fungal isolates were characterized based on their cultural and microscopic appearances as described by [8]. Lactophenol Blue stain served as both a mounting medium and a staining agent in the preparation of slides for microscopic observation of fungi. A drop of Lactophenol Blue was placed on a clean microscope slide. Using a sterile inoculating loop, a small portion of growth from between the colony center and edge was carefully removed and placed on the slide containing the Lactophenol Blue stain. The sample was gently teased apart to moderately spread the fungal structures. A cover slip was then placed over the preparation, and excess stain was blotted using filter paper. The slide was examined under a light microscope at ×40 magnification. The observed characteristics were compared with the colonial and microscopic morphologies of identified fungi described in a standard fungal atlas to aid in the identification of the pure fungal isolates.

*2.5.2. Gram Staining.*

Clean microscope slides were smeared with diluted bacterial suspensions, air-dried, and heat-fixed by passing them through a flame two to three times. The fixed smears were sequentially treated with crystal violet solution for 60 seconds, rinsed with water, and then flooded with Gram's iodine for another 60 seconds. After a second rinse with water, the slides were decolorized with 95% ethanol applied dropwise for approximately 5 seconds. Following another rinse with water, the slides were counterstained with safranin for about 30 seconds and rinsed again. The slides were then air-dried and examined microscopically under oil immersion using the ×100 objective lens with a daylight filter. Gram-positive cells appeared purple, whereas Gram-negative cells appeared pink to red [9].

*2.6. Biochemical tests.*

The following biochemical tests were performed to identify Gram-positive and Gram-negative isolates by assessing differences in their biochemical and metabolic activities.

*2.6.1. Citrate utilization test.*

This procedure was carried out as described by [10] to determine whether an organism could utilize citrate as its sole carbon source. Using a sterile inoculating loop, a colony from a 24-hour culture was inoculated and gently streaked onto the citrate agar, then incubated at 37 °C for 24 hours. A positive result was indicated by the development of a blue color, whereas the

persistence of the original green color indicated a negative result. Control tests were also read and recorded.

*2.6.2. Catalase test.*

Catalase testing was performed to determine the ability of bacterial isolates to produce the enzyme catalase, which decomposes hydrogen peroxide ($H_2O_2$) into water ($H_2O$) and oxygen ($O_2$). Using a sterile loop, a small amount of colony growth was transferred onto a clean, dry, grease-free glass slide to prepare a smear. A drop of 3% hydrogen peroxide was placed on the smear, and the slide was observed for immediate bubbling, which indicated a positive reaction, whereas the absence of bubbling indicated a negative reaction [11].

*2.6.3. Sugar fermentation test.*

The test was performed using eight sugars at 1% concentration each, namely: glucose, fructose, lactose, maltose, galactose, dextrose, sorbitol, and mannitol. Using a sterile syringe, 1 mL of a 24-hour-old broth culture for bacterial isolates and a 72-hour-old broth culture for fungal isolates was inoculated into the different sugar media and incubated at 37 °C for 24 hours. Acid production was indicated by a yellow color change in the pH indicator, while gas accumulation in the inverted Durham tubes indicated gas formation, providing evidence of carbohydrate fermentation [12].

*2.6.4. Motility test.*

This technique was used to determine the motility of microorganisms. The test organisms were introduced into the culture media using the stab inoculation method, followed by incubation at 37 °C for 24 hours before observations were recorded. Diffuse growth away from the stab line indicated a motile organism, whereas growth restricted to the stab line indicated a non-motile organism [13].

*2.6.5. Hemolysis test.*

This test was performed to identify and characterize various bacterial species, particularly those belonging to the *Streptococcus* genus. It determined the ability of these organisms to lyse red blood cells. Blood agar plates were inoculated with the test organisms by streaking. A greenish discoloration around bacterial colonies indicated alpha (partial) hemolysis, a clear zone indicated beta (complete) hemolysis, and the absence of any hemolysis around the colonies indicated gamma (no) hemolysis [14].

## 3. Results and Discussion

The findings from the analysis of the toe web microbiome revealed significant variations in microbial communities associated with different types of footwear. Shoes with lower breathability tended to harbor a higher abundance of certain bacteria and fungi, whereas free-toe shoes were associated with a more diverse microbiome. This increased diversity could be attributed to greater exposure to environmental microorganisms. Table 1 presents the morphological characteristics of bacterial and yeast isolates from the different types of footwear, while Table 2 shows the morphological characteristics of the fungal isolates.

**Table 1.** Morphological characterization of the bacterial and yeast isolates.

| Isolate | Isolate Type | Form | Elevation/Shape | Opacity | Pigmentation | Margin | Texture | Probable Organism |
|---|---|---|---|---|---|---|---|---|
| 1st FTS | Bacteria | Irregular | Raised | Opaque | White | Undulate | Smooth | Streptococcus spp |
| 2nd FTS | Bacteria | Circular | Convex | Opaque | Creamy | Entire | Smooth | Neisseria spp |
| 1st BS | Bacteria | Irregular | Flat | Translucent | White | Curled | Smooth | Lactobacillus spp |
| 1st NBS | Bacteria | Irregular | Raised | Opaque | White | Undulate | Smooth | Streptococcus spp |
| 1st NBS | Yeast | Circular | Cocci | Opaque | Creamy | Undulate | Smooth | Malassezia spp |
| 2nd NBS | Yeast | Circular | Cocci | Opaque | Creamy | Entire | Smooth | Candida spp |
| 1st BS | Yeast | Circular | Cocci | Opaque | Creamy | Raised | Smooth | Saccharomyces spp |
| 1st FTS | Yeast | Circular | Cocci | Opaque | Creamy | Undulate | Smooth | Malassezia spp |

**Key:** FTS = Free Toe Shoe; BS = Breathable Shoe; NBS = Non-Breathable Shoe

**Table 2.** Colonial morphology and microscopy of fungal isolates.

| Isolates (BS) | Macroscopic Morphology | Texture | Microscopic Morphology | Probable Microorganism |
|---|---|---|---|---|
| A | Yellowish-green with white borders. Appears reddish-gold on the reverse. | Powdery | The hyphae are septate and hyaline. Conidiophores appear rough and colourless. | *Aspergillus spp* |
| B | Initial colour of white to yellow then black. The underside appears pale yellow. | Cottony | Non septate hyphae long and smooth conidiophores head. | *Aspergillus spp* |

Key: BS = Breathable Shoe

The results of the quantitative colony counts showed clear differences in microbial abundance among the footwear types (Table 3). Mean bacterial loads were highest in non-breathable footwear ($76 \times 10^3$ CFU/ml), followed by breathable footwear ($57.5 \times 10^3$ CFU/ml) and free-toe footwear ($33 \times 10^3$ CFU/ml). Fungal loads exhibited a similar trend, ranging from $22.5 \times 10^3$ CFU/ml in non-breathable footwear to $11 \times 10^3$ CFU/ml in free-toe footwear. The plate counts from the barefoot control were consistent with the previously established range ($10^4$–$10^6$ CFU/ml) of microbial load in the human foot [15].

**Table 3.** Colony Counts of Bacterial and Fungal Isolates.

| Sample | Bacterial Load (×10³ CFU/mL) Duplicates | | Mean (×10³ CFU/mL) | Fungal Load (×10³ CFU/mL) Duplicates | | Mean (×10³ CFU/mL) |
|---|---|---|---|---|---|---|
| **FTS** | 31 | 36 | 33 | 10 | 12 | 11 |
| **BS** | 56 | 59 | 57.5 | 16 | 19 | 17.5 |
| **NBS** | 72 | 81 | 76 | 20 | 25 | 22.5 |
| **BC** | 25 | 29 | 27 | 6 | 8 | 7 |

Key: FTS = Free Toe Shoe; BS = Breathable Shoe; NBS = Non-Breathable Shoe; BC = Barefoot Control.

As shown in Figure 1, the bacterial and fungal loads varied across different footwear types. Similarly, Figure 2 illustrates the relative proportions of bacterial and fungal isolates within each footwear types.

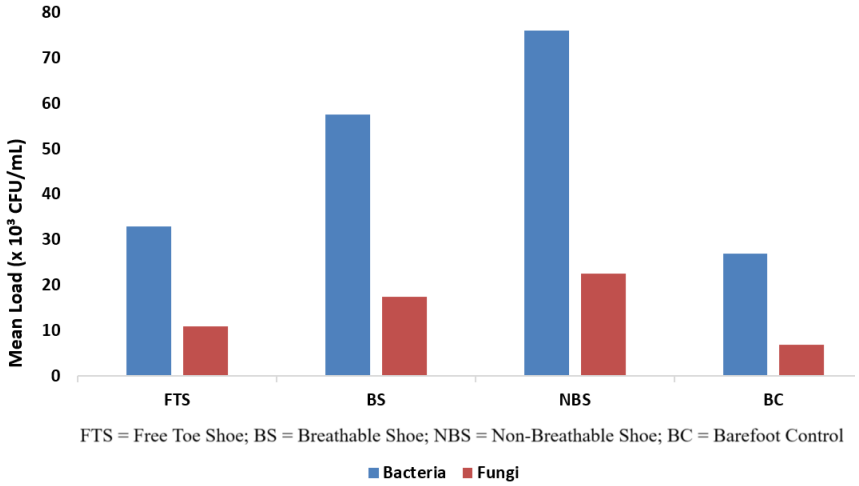


**Figure 1.** Comparison of bacterial and fungal loads in different footwear types.

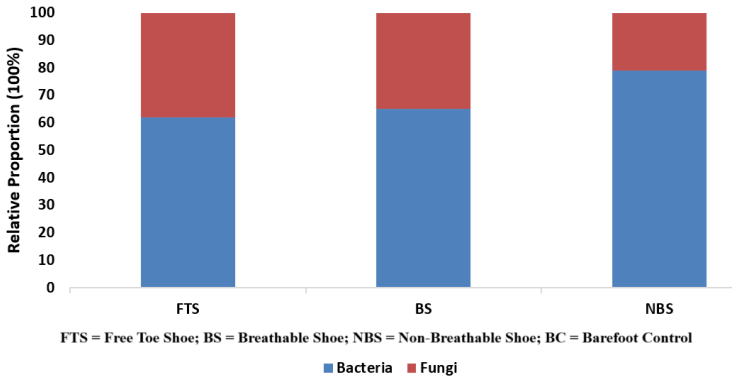


**Figure 2.** Relative proportions of bacterial and fungal isolates in different footwear types.

Tables 4 and 5 present the results of the biochemical tests performed on the isolates, including Gram staining, citrate utilization, catalase activity, motility, hemolysis, and sugar fermentation tests. Gram staining was used as a presumptive method for the identification of bacterial isolates. The probable organisms isolated from free-toe shoes were *Streptococcus* species, *Neisseria* species, and *Malassezia* species. From breathable shoes, the probable organisms were *Lactobacillus* species, *Aspergillus* species, and *Saccharomyces* species, whereas non-breathable shoes yielded *Streptococcus* species, *Malassezia* species, and *Candida* species.

**Table 4.** Biochemical characteristics of the bacterial isolates.

| ISO | MM | GS | CA | CU | M | H | GL | FR | GA | MA | LA | DE | MA | SO | PO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1st FTS** | Cocci in chains | + | - | - | Non motile | Beta | A | A | - | - | - | A | - | - | *Streptococcus spp* |
| **2nd FTS** | Cocci in cluster | - | + | + | Non motile | Gamma | A | A | A | - | - | - | - | - | *Neisseria spp* |
| **1st BS** | Rod-shaped bacilli | + | - | - | Non motile | Alpha | - | A | A | - | - | - | - | A | *Lactobacillus spp* |
| **1st NBS** | Cocci in chains | + | - | - | Non motile | Beta | A | A | - | - | - | A | - | - | *Streptococcus sp* |

MM = Microscopic Morphology, GSR = Gram Stain Reaction, CAT = Catalase Test Reaction, CUT = Citrate Utilization Test, MT = Motility Test, HT = Hemolysis Test, GLU = Glucose Test, FRU = Fructose Test, GAL = Galactose Test, MAN = Mannitol Test, LAC = Lactose Test, DEX = Dextrose Test, MAL = Maltose Test, SOR = Sorbitol Test, PO = Probable Organism, + = Positive, - = Negative, ISO = Isolates, FTS = Free Toe Shoe, BS = Breathable Shoe, NBS = Non Breathable Shoe

**Table 5.** Sugar fermentation of the yeast isolates.

| ISO | GLU | FRU | GAL | MAN | LAC | DEX | MAL | SOR | PO |
|---|---|---|---|---|---|---|---|---|---|
| 1st NBS | AG | AG | - | - | - | AG | - | - | *Malassezia spp* |
| 2nd NBS | - | - | - | - | - | - | - | - | *Candida spp* |
| 1st BS | AG | A | - | - | - | A | - | - | *Saccharomyces spp* |
| 1st FTS | AG | AG | - | - | - | AG | - | - | *Malassezia spp* |

Key: + = positive, - = negative, A = acid produced, G = gas produced, FTS = Free Toe Shoe, BS = Breathable Shoe, NBS = Non Breathable Shoe, GLU = Glucose, FRU = Fructose, GAL = Galactose, MAN = Mannitol, LAC = Lactose = DEX = Dextrose, MAL = Maltose, SOR = Sorbital Test, PO = Probable Organism, FTS = Free Toe Shoe, BS = Breathable Shoe, NBS = Non Breathable Shoe

This study emphasized microorganisms associated with different footwear types. *Lactobacillus* spp., a genus of Gram-positive bacteria, were isolated and identified from the toe web samples, consistent with the findings of [2]. The results regarding *Streptococcus* species agreed with the studies of [16, 17] on bacterial toe web infections and the impact of intrinsic and extrinsic factors on skin microbiota, respectively. The presence of *Neisseria* on the toe webs also corresponded with the findings of [2] on the foot microbiome. *Aspergillus* species, a genus comprising several mold species, were isolated from the toe webs, in agreement with the observations of [18].

Fungal species identified in this study included *Malassezia* spp., *Saccharomyces* spp., and *Candida* spp. These organisms are opportunistic pathogens that commonly colonize human mucosal surfaces as part of the normal microflora. However, under conditions of weakened host defenses or environmental disruption, they can proliferate and cause a range of infections, from mucosal candidiasis to systemic infections. They are also associated with health conditions such as allergies, rhinitis, asthma, and conjunctivitis. Furthermore, these microorganisms may contribute to the onset of sick building syndrome, as reported by [19]. A concise overview of the dominant microorganisms, their health implications, and recommended preventive measures is presented in Table 6.

**Table 6.** Summary of dominant microbes associated with footwear types and their possible health implications.

| Footwear Type | Dominant Microbial Species | Observed Microbial Trend | Likely Health Implications | Recommended Preventive Measures |
|---|---|---|---|---|
| **FTS** | *Streptococcus spp.*, *Malassezia spp.* | Moderate microbial load; high species diversity; balanced bacterial–fungal composition | Usually non-pathogenic flora; possible mild irritation if hygiene is poor | Maintain regular foot washing |
| **BS** | *Lactobacillus spp.*, *Aspergillus spp.*, *Saccharomyces spp.* | Intermediate microbial load; moderate diversity | *Aspergillus* may cause opportunistic infections in humid conditions | Keep footwear dry; use breathable socks; ensure proper ventilation |
| **NBS** | *Streptococcus spp.*, *Candida spp.*, *Malassezia spp.* | Highest bacterial and fungal loads; lowest diversity | Risk of *Tinea pedis*, erythrasma, interdigital dermatitis, and malodour due to microbial overgrowth | Alternate shoes to allow airing; disinfect closed shoes regularly |
| **BC** | Commensal flora | Low microbial load | Normal microbiome balance | Maintain basic hygiene |

Key: FTS = Free Toe Shoe BS = Breathable Shoe NBS = Non-Breathable Shoe BC = Barefoot Control.

The results further highlighted the importance of considering not only personal hygiene but also the choice of footwear in maintaining a balanced and healthy skin microbiome [20]. By understanding these microbial dynamics, public health guidelines and consumer choices can be better informed, potentially reducing the incidence of skin infections.

## 4. Conclusions

The composition and diversity of microorganisms in the toe web were influenced by the type of footwear worn. Understanding the implications of the unique toe web microbiome is important for both dermatological research and everyday personal care. The findings suggest that individuals prone to skin conditions should carefully consider their choice of footwear, as maintaining a balanced microbiome in the toe web may contribute to overall skin health and reduce the risk of dermatological issues such as dermatophytosis. From a practical perspective, the findings can guide dermatologists and podiatrists in advising patients on shoe hygiene and preventive care. Footwear manufacturers could help reduce odor and infection risk by designing shoes with improved ventilation, moisture-wicking materials, and antimicrobial linings. Future research could explore the mechanisms by which different types of footwear influence the skin microbiome. Investigating the long-term effects of specific footwear choices on skin health and microbial communities would provide valuable insights for preventive and therapeutic strategies in dermatology. Subsequent studies should involve larger and more diverse cohorts and incorporate molecular sequencing and statistical analyses to clarify microbial dynamics and functional interactions within the toe web environment. Based on the current study, alternating between different pairs of shoes to allow them to air out may reduce moisture accumulation and the growth of odor-causing bacteria, thereby supporting the maintenance of a healthy toe web microbiome.

## Author Contributions

Stephanie Ifunanya Nkamigbo: Writing—original draft, data collection, funding acquisition, data analysis, and experimentation; Ugochukwu Chukwuma Okafor: Contributed to drafting and critically revising the final manuscript; Onyekachukwu Izuchukwu Udemezue: Conceptualization, supervision, and methodology.

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## Competing Interests

The authors declare that they have no competing interests related to the publication of this research.

## Data Availability

The data that supports the findings of this study are not publicly available due to institutional privacy resrictions but are available from the corresponding author upon reasonable reasonable request.

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