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UNCOVERING THE MICROBIAL LANDSCAPE OF HUMAN SKIN: A MULTAN-BASED STUDY

Original Research

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Acknowledgement: The authors sincerely thank Dr. Malika Uzma and all participants for their cooperation and support during sample collection.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: Human skin, the body's largest organ, serves as both a protective barrier and a microbial ecosystem hosting bacteria, fungi, and viruses. These microorganisms, collectively known as the skin microbiota, play a crucial role in maintaining skin homeostasis and immune regulation. Factors such as hygiene practices, environmental exposure, occupation, and antibiotic use influence microbial diversity and load. Understanding microbial distribution helps in assessing hygiene levels and identifying potential reservoirs of opportunistic pathogens.

Objective: This study aimed to evaluate the microbial diversity and distribution across different skin sites of students at Times University, Multan, and to determine gender-based differences in bacterial and fungal colonization.

Methods: A total of 150 samples were collected from 50 healthy participants (25 males and 25 females), aged 21–29 years. Skin swabs were obtained from three anatomical sites: the palm, neck, and antecubital fossa. Samples were inoculated onto nutrient, blood, and MacConkey agar for bacterial isolation, and potato dextrose agar for fungal identification. Isolates were characterized through morphological assessment, Gram staining, and biochemical testing including catalase, coagulase, oxidase, methyl red, Voges-Proskauer, citrate, and urease tests.

Results: The study identified seven bacterial species—*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus* spp., *Corynebacterium* spp., *Pseudomonas* spp., and *Micrococcus* spp.—and three fungal species—*Tinea*, *Candida*, and *Alternaria*. In total, 59 bacterial and 51 fungal isolates were recovered. The highest bacterial load was observed in the palm (mean 6.36 ± 0.017 CFU/ml), followed by the neck (5.65 ± 0.023 CFU/ml), and antecubital fossa (3.52 ± 0.030 CFU/ml). *Staphylococcus epidermidis* was the most prevalent bacterium (97 isolates), while *Tinea* showed the highest fungal prevalence (+++).

Conclusion: The findings demonstrate significant microbial heterogeneity across different skin sites, with palms exhibiting the highest load due to frequent environmental contact. The detection of *E. coli* and *S. aureus* highlights the importance of hand hygiene in reducing infection risk and preventing microbial transmission in communal settings.

Keywords: Bacteria, Fungi, Hand hygiene, Microbial load, Skin microbiota, Swabbing technique, University students.

INTRODUCTION

The human skin, the largest organ of the body, functions as both a protective barrier and a dynamic ecosystem hosting a diverse microbiota that includes bacteria, fungi, viruses, and archaea. These microorganisms coexist in a delicate symbiotic relationship with the host, contributing to cutaneous and systemic homeostasis (1). Recent advances in next-generation sequencing (NGS) and metagenomic technologies have redefined the understanding of the skin from being merely a physical barrier to an active immunological interface that plays a critical role in health, disease prevention, and immune regulation (2). This paradigm shift has underscored the skin microbiome's influence on wound healing, inflammatory responses, and the pathogenesis of dermatological disorders such as acne, psoriasis, and atopic dermatitis (3). The skin supports trillions of microorganisms, with the dominant bacterial phyla including Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes (4). Among these, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Cutibacterium acnes*, and *Escherichia coli* are frequently reported species that maintain commensal or opportunistic relationships with the host (5). The initial colonization of skin microbiota begins at birth, differing significantly between neonates delivered vaginally and those born via cesarean section, indicating early environmental and maternal influences (6). The composition and diversity of skin microbial communities are subsequently shaped by multiple factors including age, genetics, immunity, sex, hygiene practices, antibiotic use, and environmental conditions such as climate and pollution (7). For instance, *Malassezia* species predominate in humid environments, whereas *Actinobacteria* are more abundant in arid regions (8).

Sampling methodologies—such as tape stripping, swabbing, adhesive patches, and skin scraping—allow for varying degrees of microbial recovery, yet the lack of standardized procedures remains a challenge in comparative analyses (9). While scraping tends to yield higher microbial diversity, flocked swabs are often preferred in clinical settings for their non-invasive and practical application (10). Standardization of collection and analysis techniques is essential for accurate microbiome profiling and inter-study comparability. Beyond its dermatological implications, the skin microbiome has significant public health relevance. Healthcare workers, particularly dermatologists and nursing staff, can act as asymptomatic carriers of multidrug-resistant organisms (MDROs) such as methicillin-resistant *Staphylococcus aureus* (MRSA), facilitating healthcare-associated infections (HAIs) and contributing to antimicrobial resistance (AMR) transmission across community and hospital environments (11). Furthermore, the misuse of antibiotics and inadequate hygiene practices exacerbate microbial dysbiosis and the horizontal transfer of antibiotic resistance genes between commensals and pathogens including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *S. aureus* (12). Given these concerns, expanding AMR surveillance beyond hospital environments to include schools, workplaces, and community institutions is imperative. Such a comprehensive approach would enhance early detection of resistant strains and strengthen preventive strategies against microbial exchange and infection transmission (13). Therefore, the present study aims to explore the integral role of the skin microbiome in maintaining cutaneous and systemic health, its contribution to immune modulation, and its emerging association with antimicrobial resistance. The objective is to elucidate the interconnections between microbial diversity, host factors, and external influences to inform targeted strategies for infection control and microbiome preservation in both clinical and community settings.

METHODS

This cross-sectional study was carried out at Times University, Multan, with the objective of assessing microbial colonization patterns on human skin and evaluating the hygiene practices and prevalence of microflora among university students. Ethical approval for the study was obtained from the Institutional Ethical Review Committee of Times University, Multan. Written informed consent was obtained from all participants prior to sampling, and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki (1). The study population consisted of 50 healthy volunteers, including 25 males and 25 females, aged between 21 and 29 years. Inclusion criteria required participants to have healthy skin without any visible dermatological lesions, wounds, or autoimmune disorders. Individuals with a history of recent antibiotic use (within the preceding three months), chronic illness, or active infections were excluded to avoid factors that could alter microbial diversity. A total of 150 skin samples were collected from these 50 participants, with three anatomical sites sampled per individual: the palm, neck, and antecubital fossa. Each site contributed 50 samples, making a total of 150 (Palm = 50; Antecubital fossa = 50; Neck = 50). Sampling was performed using sterile cotton swabs moistened with normal

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saline, ensuring aseptic technique. The swabs were immediately transferred to sterile transport tubes and inoculated into nutrient broth for enrichment using the serial dilution method. The inoculated broths were incubated at 37°C for 24 hours (2).

Following incubation, samples were subcultured onto selective and differential media, including nutrient agar, blood agar, and MacConkey agar for bacterial isolation, and potato dextrose agar for fungal growth. The inoculated plates were incubated at 37°C for 24–48 hours and examined for colony formation. Colony morphology—including shape, pigmentation, texture, elevation, and hemolytic characteristics—was observed for preliminary identification. Fungal isolates were characterized based on macroscopic and microscopic morphological features (3). Gram staining was conducted on all bacterial isolates to determine Gram reaction, cellular morphology, and arrangement. Subsequent biochemical tests were performed to confirm bacterial identification. The biochemical assays included Catalase, Coagulase, Oxidase, Methyl Red (MR), Voges-Proskauer (VP), Citrate utilization, and Urease tests, all carried out following standard microbiological protocols (4). Identification was finalized based on test results interpreted against established diagnostic criteria for Gram-positive and Gram-negative bacteria. All laboratory procedures were performed in a biosafety level-2 (BSL-2) facility under sterile conditions. Data were systematically recorded and analyzed based on microbial species and their frequency across different anatomical sites and gender groups.

RESULTS

A total of 150 skin swab samples were collected from 50 participants (25 males and 25 females) aged 21–29 years. Each participant provided samples from the palm, neck, and antecubital fossa, contributing 50 samples per site. Bacterial growth was observed in all sample types, and seven distinct bacterial species were identified: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus* spp., *Micrococcus* spp., *Cutibacterium* spp., *Corynebacterium* spp., and *Pseudomonas* spp. The overall bacterial load, expressed as colony-forming units per milliliter (CFU/ml) at a 10^{-4} dilution, varied by anatomical site and gender. Among male participants, the highest mean plate count was recorded from neck samples (8.0 ± 0.15 CFU/ml), followed by palms (7.9 ± 0.54 CFU/ml) and antecubital fossa (6.8 ± 0.49 CFU/ml). The lowest bacterial counts were observed in palm (1.2 ± 0.34 CFU/ml), neck (3.8 ± 0.24 CFU/ml), and antecubital fossa (2.5 ± 0.10 CFU/ml) samples. Among females, neck swabs exhibited the highest mean count (12.40 ± 0.35 CFU/ml), followed by antecubital fossa (9.51 ± 0.40 CFU/ml) and palms (7.65 ± 0.60 CFU/ml). The lowest values were observed in palms (2.51 ± 0.19 CFU/ml), neck (6.62 ± 0.20 CFU/ml), and antecubital fossa (3.43 ± 0.42 CFU/ml). When mean plate counts were analyzed across all 50 participants, male samples demonstrated 6.36 ± 0.02 CFU/ml from palms, 5.65 ± 0.02 CFU/ml from necks, and 3.52 ± 0.03 CFU/ml from antecubital fossa. Correspondingly, female samples revealed 5.9 ± 0.43 CFU/ml, 6.12 ± 0.32 CFU/ml, and 4.3 ± 0.21 CFU/ml for palm, neck, and antecubital fossa respectively. Among the bacterial isolates, *Staphylococcus epidermidis* was the most frequently detected species (n = 97), distributed as 39 isolates from palm, 28 from antecubital fossa, and 25 from neck. *Escherichia coli* accounted for 83 isolates (28 palm, 25 antecubital fossa, 16 neck), *Pseudomonas* for 88 isolates (35 palm, 25 antecubital fossa, 22 neck), and *Staphylococcus aureus* for 43 isolates (16 palm, 15 antecubital fossa, 12 neck). Other detected bacteria included *Proteus* (n = 52), *Corynebacterium* (n = 42), and *Micrococcus* (n = 46).

Prevalence analysis indicated that *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas* exhibited the highest occurrence (+++), while *Escherichia coli*, *Cutibacterium*, and *Micrococcus* showed intermediate prevalence (++). *Proteus* and *Corynebacterium* displayed the least prevalence (+). Morphologically, *Staphylococcus aureus* appeared as golden-yellow convex colonies of cocci with buttery odor, *E. coli* formed pink convex colonies with fecal odor, and *Pseudomonas* produced flat pink-red colonies with a grape-like smell. Gram staining and biochemical assays confirmed Gram-positive cocci for *Staphylococcus* and *Micrococcus*, and Gram-negative rods for *E. coli*, *Proteus*, and *Pseudomonas*. Biochemical profiling further differentiated the isolates: *Staphylococcus* was catalase-, coagulase-, urease-, and VP-positive, whereas *E. coli* was catalase- and methyl red-positive but negative for oxidase, citrate, and urease. *Pseudomonas* was oxidase-, catalase-, and citrate-positive. Variable urease activity was observed in *Cutibacterium*, *Corynebacterium*, *Proteus*, and *Micrococcus*, indicating metabolic diversity among isolates. In total, 59 bacterial and 51 fungal isolates were recovered from all skin sites. The distribution of bacterial and fungal isolates varied significantly among anatomical areas. Palms exhibited 30 bacterial and 6 fungal isolates, antecubital fossa showed 6 bacterial and no fungal isolates, whereas neck samples demonstrated 23 bacterial and 45 fungal isolates, confirming higher fungal colonization in moist areas such as the neck. Three fungal species—*Tinea*, *Candida*, and *Alternaria*—were identified. *Tinea* and *Candida* were detected on both the palm and neck, whereas *Alternaria* was restricted to the palm. *Candida* was the only species present at all three sites, including the antecubital fossa. *Tinea* showed the highest prevalence (+++), followed by *Candida* (++), and *Alternaria* (+). Colony morphology revealed creamy-white granular colonies for *Tinea*, smooth and elevated creamy colonies for *Candida*, and white-grey lobed colonies for *Alternaria*.

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Table 1: Gender-Based Expression of Minimum, Maximum, and Mean Plate Count (CFU/ml) in 10^{-4} Dilution Across Different Swab Areas Among Fifty Participants

Swab Area	N	Male (CFU/ml)			Female (CFU/ml)		
		Max	Min	Mean ± SD	Max	Min	Mean ± SD
Palm	50	7.9 ± 0.544	1.2 ± 0.336	6.36 ± 0.017[a]	7.64 ± 0.604	2.51 ± 0.185	5.9 ± 0.43[b]
Antecubital fossa	50	6.8 ± 0.491	2.5 ± 0.100	3.52 ± 0.03[c]	9.51 ± 0.404	3.43 ± 0.423	4.3 ± 0.21[c]
Neck	50	8 ± 0.148	3.8 ± 0.240	5.65 ± 0.023[b]	12.40 ± 0.348	6.62 ± 0.201	6.12 ± 0.32[a]

Note: Values represent mean ± standard deviation of colony forming units per milliliter (CFU/ml) in 10^{-4} dilution. Superscripts (a, b, c) indicate comparative significance within each gender group as reported.

Table 2: Table expressing the prevalence of isolated bacteria.

S.No.	Isolated bacteria	Prevalence
1	Staphylococcus	+++
2	Staphylococcus aureus	+++
3	Escherichia coli	++
4	Cutibacterium	++
5	Corynebacterium	+
6	Proteus	+
7	Pseudomonas	+++
8	Micrococcus	++

Table 3: Table expressing the morphological identification of isolated bacteria.

Strains	Colony morphology				Cell morphology			
Bacteria	Shape, size(mm)	color	margin	elevation	odor	Shape	Gram staining	Spore staining
Staphylococcus	Spherical(cocci)	Golden yellow	entire	convex	Buttery or a little musty	Dome	+	-
Escherichia coli	Straight rods	Pink colonies	entire	convex	fecal	Bacilli	-	-
Cutibacterium	Pleomorphic rods	White-creamy	entire	convex	A bit musty	Short club like	+	-
Corynebacterium	Club shaped rods	Grayish white-cream	Entire	Little convex	mild	Club shaped	+	-
Proteus	Rods	Pink-red	irregular	Low convex-flat	Fishy or burnt chocolate	Bacilli	-	-
Pseudomonas	Rods	Pink-red	entire	flat	Grape like	Bacilli	-	-
Micrococcus	cocci	Bright yellow	Entire	convex	odorless	Cocci	+	-

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Table 4: Table expressing the Screening of biochemical tests of selected bacterial.

Strains	Catalase	Coagulase	Oxidase	Methyl red	Voges-Proskauer	Citrate	urease
Staphylococcus	+	+	-	-	+	Variable	+
Escherichia coli	+	-	-	+	-	-	-
Cutibacterium	+	-	-	+	-	-	Variable
Corynebacterium	+	-	-	-	-	-	Variable
Proteus	+	-	-	+	-	-	+
Pseudomonas	+	-	+	-	-	+	Variable
Micrococcus	+	-	+	-	-	+	Variable

+ showing positive for results, - showing negative for results

Table 5: Table expressing the Total Number of bacteria and fungi expressed in inoculations of swabs taken from different areas of skin (Palm, Antecubital fossa and Neck).

Zones	No. of participants	Bacteria	Fungi
Palm	50	30	6
Antecubital fossa	50	6	-
neck	50	23	45
total	150	59	51

Table 6: Distribution and Prevalence of Isolated Fungal Species Across Different Skin Sites

Fungal Isolates	Palm	Antecubital Fossa	Neck	Prevalence
Tinea	+	-	+	+++
Candida	+	+	+	++
Alternaria	+	-	-	+

Note: "+" indicates positive detection; "–" indicates negative detection. Prevalence scale: (+) minimum, (++) intermediate, (+++) maximum.

Table 7: Table expressing the Colony morphology of isolated fungi.

Fungi	Color	Colony shape	Margins
Tinea	creamy white -powdery white	Granular	Regular-Irregular
Candida	Creamy	Round, smooth, elevated	Entire
Alternaria	White-grey	circular	lobed

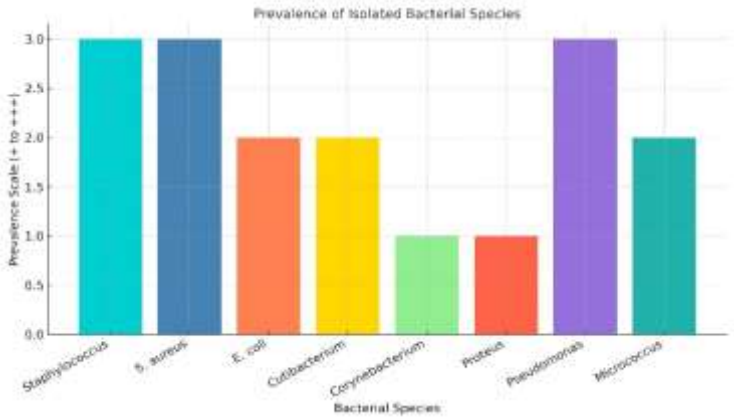


Figure 1 Prevalence of Isolated Bacterial Species

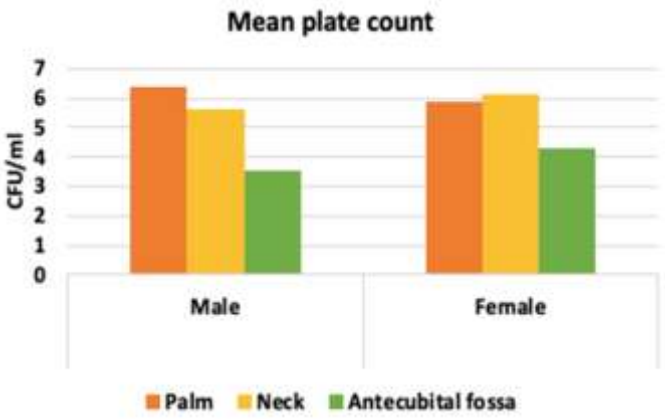
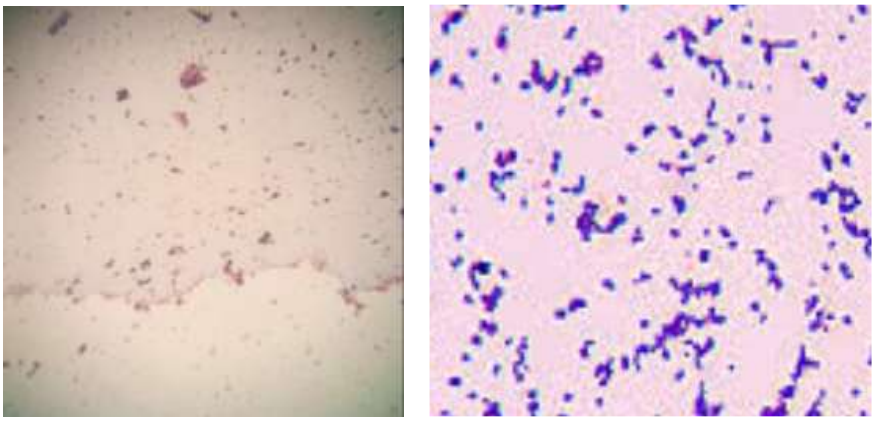
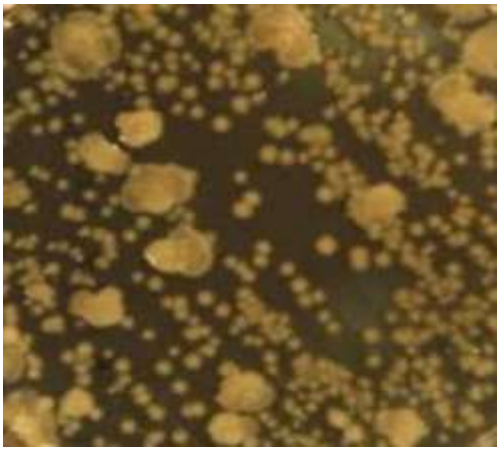


Figure 2 Mean Plate Count



Gram negative and Gram -positive bacteria in staining

Figure 3 Gram Negative and Gram-Positive Bacteria in Staining



Bacterial Colonies on Spread Plates

Figure 4 Bacterial Colonies on Spread Plates

DISCUSSION

The current investigation explored the diversity and distribution of microorganisms on the skin among university students, providing valuable insight into the ecological variability of the cutaneous microbiota. The findings confirmed that the human skin harbors a rich and complex community of microorganisms, whose composition varies across anatomical sites according to local physiological and environmental conditions. The observed variation in microbial load and colony-forming unit (CFU) counts across the palm, neck, and antecubital fossa supported the concept of site-specific microbial ecology, a well-established phenomenon in skin microbiome research (14). The palm exhibited the highest mean CFU count, followed by the neck, whereas the antecubital fossa demonstrated the lowest microbial density. This pattern reflected the influence of environmental exposure and sebaceous activity on microbial colonization. The hands, being in frequent contact with external surfaces, accumulate transient as well as resident microorganisms, explaining their higher bacterial load. Sebaceous regions such as the neck showed relatively greater microbial abundance due to lipid secretions that provide an enriched substrate for bacterial proliferation, particularly for *Cutibacterium acnes* and *Staphylococcus aureus*. Conversely, the antecubital fossa, being a dry and less sebaceous region, harbored fewer microorganisms. These findings are consistent with earlier dermatological microbiome models that describe the skin as comprising distinct ecological niches—dry, moist, and oily—each supporting characteristic microbial communities (15,16). The dominant bacterial isolates identified in this study included *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus aureus*, and *Cutibacterium acnes*. The predominance of *S. epidermidis* reaffirmed its role as a major commensal organism contributing to skin defense by competing with pathogens and producing

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antimicrobial peptides. Its presence in both moist and sebaceous sites aligned with its ecological versatility and protective function within the skin ecosystem. The isolation of *S. aureus*, an opportunistic pathogen often associated with folliculitis, abscesses, and impetigo, was notable even among healthy participants, indicating asymptomatic colonization that poses potential risks for horizontal transmission in communal environments. The detection of *E. coli*, typically an enteric organism, suggested transient contamination possibly arising from inadequate hand hygiene or contact with contaminated surfaces. Such findings highlight the public health relevance of monitoring hygiene practices in institutional settings, as hands can serve as vectors for pathogen dissemination (17-19).

The identification of *Cutibacterium acnes* predominantly from sebaceous regions corroborated its known preference for lipid-rich environments. Although this bacterium contributes to maintaining skin homeostasis by producing bacteriocins and modulating pH, its overgrowth has been implicated in acne pathogenesis. The distribution pattern of bacterial species across anatomical sites thus reinforced the concept of niche specialization and adaptive colonization, which are critical for understanding host–microbe interactions. The detection of fungal isolates—*Tinea*, *Candida*, and *Alternaria*—further emphasized the diversity of the skin microbiome. *Tinea* species were most prevalent, particularly on the neck and palm, reflecting the affinity of dermatophytes for moist areas. The presence of *Candida* across all sampled sites suggested its capacity for opportunistic colonization under favorable conditions. These observations supported the view that the skin microbiome is not limited to bacteria but also includes a significant fungal component that can influence skin health and disease dynamics (20,21). In relation to gender differences, females demonstrated slightly higher mean bacterial counts than males, possibly attributable to variations in hormonal influence, skin pH, and cosmetic use. However, the absence of statistical analysis to determine the significance of these differences limited the interpretability of this observation. The study's results were broadly comparable to those of other investigations reporting similar gender- and site-specific microbial variations.

One of the key strengths of this research was its site-specific sampling approach, which allowed for direct comparison of microbial profiles across distinct ecological zones of the skin. The use of standard culture media and biochemical testing ensured reliable identification of cultivable bacteria. The inclusion of both bacterial and fungal isolates provided a more holistic view of the cutaneous microbial community, which is often overlooked in conventional bacterial-centric studies.

Nevertheless, several limitations must be acknowledged. The reliance on culture-based methods restricted detection to organisms capable of growth under laboratory conditions, thereby excluding a large fraction of the skin microbiome that remains unculturable. The absence of molecular characterization methods such as 16S rRNA gene sequencing or metagenomic profiling limited the taxonomic resolution and accuracy of microbial identification. Additionally, the study population was confined to a single university cohort, which constrained the generalizability of findings to broader demographic and geographical contexts. Future studies should employ an integrative approach combining culture-dependent and molecular-based analyses to achieve a more comprehensive understanding of skin microbial diversity. Expanding sampling to include participants of varying age groups, occupations, and environments would also enhance external validity. Furthermore, assessing the antimicrobial resistance profiles of isolated species could provide valuable insights into the skin's potential role as a reservoir for antibiotic-resistant organisms and its implications for community and hospital-acquired infections (22,23). In summary, the study confirmed that the human skin supports a diverse microbial ecosystem whose composition varies according to anatomical site and host-related factors. The findings highlight the significance of the skin as both a protective barrier and a dynamic microbial habitat. Understanding these microbial patterns is essential for improving hygiene strategies and developing targeted interventions to maintain skin health and prevent pathogen transmission in public environments.

CONCLUSION

This study concludes that human skin hosts a diverse and dynamic microbial ecosystem whose composition varies across anatomical locations in response to physiological and environmental factors. The palm exhibited the highest microbial burden, reflecting its frequent exposure to external surfaces and potential role in microbial transmission, while sebaceous areas such as the neck and forehead supported commensals favored by lipid-rich conditions. Conversely, the dry nature of the antecubital fossa contributed to lower microbial abundance. The predominance of *Staphylococcus epidermidis* emphasized its role as a beneficial commensal maintaining skin homeostasis, whereas the presence of *Staphylococcus aureus* and *Escherichia coli* underscored the possibility of opportunistic colonization and the public health importance of maintaining hand hygiene. Overall, the findings affirm that the skin functions not only as a protective barrier but also as a complex microbial habitat essential to both health and disease, highlighting the need for improved hygiene practices and continued research on skin microbiota diversity and its implications for infection control.

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AUTHOR CONTRIBUTION

Author	Contribution
Malika Uzma*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Atif Nisar	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Iqra Awais	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Abdul Salam	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Ayesha Malik	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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