

Research Article

Isolation, Phenotypic Characterization, and Distribution of Normal Bacterial Flora from the Hand Skin of Healthy Female Students at Wolkite University, Ethiopia

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Abstract

The cutaneous microbiota serves as a critical first line of defense through bacterial interference, but can transition into opportunistic pathogens if introduced into deep tissues via mechanical trauma. This study aimed to isolate, phenotypically characterize, and assess the distribution profile of normal hand skin bacterial flora among healthy female students at Wolkite University, Ethiopia. A laboratory-based cross-sectional study was conducted from December 2024 to June 2025 at the Department of Biotechnology Laboratory. Hand skin swab samples were collected from 60 healthy female students using simple random sampling. Isolated colonies were purified and classified to the genus level based on macroscopic morphology, Gram reaction, cellular shapes, and standard biochemical verification arrays. A total of 27 distinct bacterial isolates were recovered from the 60 samples. Phenotypic and biochemical profiling identified seven distinct bacterial groups. The family *Enterobacteriaceae* was the most prevalent group (10 isolates, 37.07%), followed by *Staphylococci* (6 isolates, 22.22%) and *Lactobacilli* (4 isolates, 14.81%). The findings demonstrate that while female hand skin maintains protective resident commensals (*Staphylococci* and *Lactobacilli*), it frequently harbors transient enteric and environmental bacteria (*Enterobacteriaceae*) due to continuous exposure to shared institutional touch points. This underscores the critical importance of implementing consistent personal hygiene, systematic hand-sanitation protocols, and enhanced public health awareness within the university campus ecosystem to minimize hand-borne opportunistic infections.

Keywords

Enterobacteriaceae, Hand Skin, Normal Flora, Phenotypic Characterization, *Staphylococci*

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1. Introduction

The mixture of microorganisms regularly found at any anatomical site of humans is referred to as normal flora, and those which reside on the human skin are specifically known as skin flora or skin microbiota [1, 2]. Usually skin flora are non-pathogenic and they are either of commensal (harm full to their host) or mutualistic (without causing detectable harm) [3]. In a healthy individual, this normal microflora is relatively stable, with specific genera populating particular body regions during predictable periods of life [4]. This normal flora is established in an orderly succession after birth, eventually developing into the complex adult ecosystem observed across different skin boundaries.

The skin acts as an intricate microbial ecosystem driven by interactions between diverse microbial populations and host immune responses [5]. Culturable bacteria widely isolated from normal skin surfaces include *Staphylococci*, *Micrococci*, *Corynebacteria*, *Brevibacteria*, *Propionibacteria*, and *Acinetobacter* species [6, 7]. The primary factors determining both the absolute quantity and species quality of the local microbiota depend directly on the anatomical site and its regional microenvironment specifically fluctuating micro-gradients of pH, moisture, temperature, oxygen availability, and surface lipid concentrations [8]. Consequently, the composition of the cutaneous flora varies drastically between sebaceous (oily), moist, and dry skin areas [6]. Human hands function primarily as dry environments with intermittent moisture, representing a unique ecologic zone heavily exposed to external environments [9].

The skin barrier provides essential selective advantages for survival, concurrently preventing the escape of physiological moisture and halting the deep invasion of toxic chemical substances or infectious agents. Because of this, normal skin flora contributes significantly as a critical component of the first line of innate host defense. On outer epithelial cells and mucous membranes, the established resident normal flora blocks the colonization of incoming pathogenic invaders, successfully preventing clinical disease through a process known as bacterial interference. The functional mechanisms driving bacterial interference are multi-faceted; they involve direct competition for cellular receptors or binding sites on host tissue, competitive consumption of limited local nutrients, mutual metabolic inhibition via acidic or toxic by-products, and the targeted secretion of specialized antimicrobial proteins or bacteriocins [3, 6].

Despite their protective evolutionary roles, members of the normal flora can shift behaviors to become pathogenic under specific micro environmental or systemic circumstances. Cutaneous microbes are highly adapted to non-invasive lifestyles defined by the strict structural limitations of the outer skin layers. However, if these organisms are forcefully displaced from their natural boundaries and introduced into deep tissues or the bloodstream via mechanical trauma, compromised skin barriers, burns, or wounds, they rapidly

transition into highly aggressive opportunistic pathogens [6]. This risk is severely amplified in hospitalized populations or individuals experiencing profound immunosuppression. In clinical settings where opportunistic infections emerge directly from the patient's own commensal community, immediate and precise phenotypic isolation is required to differentiate normal flora from true contaminants and guide effective antibiotic selection.

Furthermore, external environmental and behavioral pressures can rapidly alter the homeostatic equilibrium of the skin, causing abrupt disruptions in community structures. In female populations, the frequent and varied use of topical cosmetics, moisturizing agents, and chemical cleansers alters local pH and lipid barriers, often reducing or shifting the baseline distribution of protective normal flora. While international programs have deeply characterized human internal mucosal populations, our fundamental understanding of localized hand microflora dynamics across specific institutional environments remains surprisingly limited [6, 10]. Cutaneous flora can be operationally categorized into transient (highly fluctuating, non-reproducing contaminants), temporary resident, and true resident (actively replicating and growing) flora [11]. On human hands, transient floras are constantly deposited through contact with shared public touch points. At present, the structural composition and changing dynamics of these bacterial communities have not been established under localized laboratory baselines at Wolkite University. To address this gap, this study focuses strictly on evaluating the hands of healthy female university students. This targeted phenotypic evaluation maps out exactly what commensal and transient bacterial genera are distributed across the hand skin under standard institutional conditions, utilizing classical Gram staining alongside detailed biochemical validation arrays.

2. Materials and Methods

2.1. Description of Study Area

The study was conducted at the Biotechnology Laboratory, Department of Biotechnology, Wolkite University, Ethiopia. Wolkite University is located in the Gurage Zone, Central Ethiopian Region. Wolkite, the capital town, is located 155 km southwest of Addis Ababa. The Gurage Zone comprises altitudes ranging from 1001 to 3500 meters above sea level. Based on the local agro-climatic classification, the zone is divided into three agro-climatic zones. Dega (high altitude) covers 28.3% of the area and ranges between 2500 and 3662 masl, Weinadega (mid-altitude) at 1500–2500 masl encompasses about 64.9% of the area, and Kola (lowland) at 1000–1500 masl covers 6.8% of the area. The mean annual temperature ranges from 14 to 24 °C, with an average of 20.5 °C. The rainfall pattern in the Gurage Zone is bimodal, with 80% occurring during the Belg period from February to May.

2.2. Sampling Design and Period

The design of this research was a laboratory-based cross-sectional study to conduct isolates and phenotypically characterize normal bacterial flora from the hand skin of healthy female regular students at Wolkite University with complete random design and each experiment had three replications and each experiments were repeated twice. The study was carried out from December 2024 to June 2025.

2.3. Target Population and Sample Size

The target population included all healthy female regular students residing on the main campus of Wolkite University during the study period. Students who were apparently healthy and volunteered to participate were included in the study. Those with visible skin infections, open wounds on their hands, or those who had used topical antibiotics within the previous two weeks were excluded from the study. A total of 60 healthy female students were selected using a simple random sampling technique. Swab samples were systematically collected from the hand skin surface of each selected participant.

2.4. Experimental Reagents, Material and Equipment

The major equipment used in this study included an autoclave, a hot plate with a magnetic stirrer, a compound light microscope, a bacteriological incubator, a digital weighing balance, a laminar airflow hood, and a refrigerator. The primary culture media and biochemical reagents utilized for isolation and characterization consisted of Nutrient Agar, MacConkey Agar, Mannitol Salt Agar, Triple Sugar Iron (TSI) Agar, Simmon's Citrate Agar, Urea Agar Base, and Hydrogen Peroxide (H₂O₂) for the catalase test, Crystal Violet, Gram's Iodine, 95% Acetone-Alcohol decolorizer, and Safranin counterstain. Additional laboratory consumables included sterile cotton swabs, normal saline (0.9% NaCl), microscopic glass slides, immersion oil, inoculation loops, and Petri dishes.

2.5. Measurement and Data Collection Procedure

Before sample collection, the purpose of the study was explained to each participant. Swab samples were obtained by rubbing a sterile cotton swab, pre-moistened with sterile normal saline, firmly over the palm and dorsal surfaces of the participant's hands. After collection, the swabs were immediately transported to the biotechnology laboratory for processing.

2.6. Isolation of Bacteria

The collected swab samples from the selected anatomical

sites of healthy female student's hand were directly inoculated onto Nutrient Agar plates by using the streak-plate method to obtain isolated colonies. To select for specific groups, samples were also streaked onto MacConkey Agar (selective for Gram-negative bacteria) and Mannitol Salt Agar (selective for *Staphylococci* species). The inoculated Petri dishes were then inverted and incubated at 37 °C for 24 to 48 hours under aerobic conditions. After the incubation period, distinct, isolated colonies were selected based on their macroscopic appearance for further purification and phenotypic identification.

2.7. Morphological, Cultural, and Biochemical Characterization

The isolated bacterial colonies were systematically classified based on their morphological, cultural, and biochemical characteristics. The macroscopic features of the isolated colonies, such as shape, size, color, margin, elevation, and opacity, were recorded. Microscopic examination was conducted using Gram staining techniques to determine the Gram reaction (Gram-positive or Gram-negative), cellular shape (cocci or bacilli), and structural arrangement of the bacterial cells under the oil immersion objective (100 times) of a compound light microscope.

2.7.1. Gram Staining Identification

Gram staining is a method that differentiates bacteria in two large group gram positive and gram negative. This method differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, in gram positive it is present as a thick layer. A Gram positive results in a purple/blue color while a Gram negative results in a pink/red color [12]. For each bacterial isolate a heat fixed smear from 24 hours- old cultures were prepared, stained with crystal violet solution for 1 minutes, rinsed rapidly with water and iodine solution was added and the smear was blot dried. The slides were washed with 95% ethanol 1 min, rinsed with tap water and stained with safranin for 30 seconds. The slide was air dried and examined under compound light microscope.

2.7.2. Catalase Test

The catalase test was performed to detect the presence of catalase enzyme by transferring a loopful of pure colony to clean microscope slide and addition of one drop of 3% of hydrogen peroxide solution. Positive test was indicated by formation of effervescence or appearance of bubbles, due to the breaking down of hydrogen peroxide to O₂ and H₂O [13].

2.7.3. Citrate Utilization Test

This test determines the ability of bacteria to convert citrate into oxaloacetate. Citrate is the only carbon source available to the bacteria in this media. If bacteria cannot use citrate, it was not grow. Positive result was seen if the bacterium grows

and the media turns into bright blue color as a result of an increase in the pH of the media [14].

2.7.4. Triple Sugar Iron Agar Test

Triple Sugar Iron agar slant tubes are used for this test, this multi-test medium contains glucose (at a concentration that is 1/10th that of the other sugars), sucrose and lactose. The inoculation was performed by using a straight wire; the test organism was inoculated deep into the butt of the medium and streak up the slant. These were incubated at 37 °C for 24 hr [13].

2.7.5. Simmon's Citrate Test

Pure isolated colonies were streaked onto the surface of sterile Simmons Citrate Agar slants using a sterile inoculating loop according to Varney *et al.*, [14]. Care was taken to inoculate only the surface of the slant without stabbing the butt of the medium. The inoculated tubes were incubated aerobically at 35–37 °C for 24–48 hours. After incubation, the tubes were examined for bacterial growth and color change of the medium. Utilization of citrate as the sole carbon source resulted in alkaline by-products that changed the bromothymol blue indicator from green to deep blue, indicating a positive result. Absence of growth and no color change (medium remaining green) were considered a negative result.

2.7.6. Urease Test

Pure bacterial isolates were inoculated into sterile Urea Agar slants using a sterile inoculating loop by streaking the surface of the medium. The inoculated media were incubated at 35–37 °C for 24–48 hours and observed for color development. Production of the urease enzyme hydrolyzed urea into ammonia and carbon dioxide, resulting in an alkaline reaction in the medium. A positive urease test was indicated by a change in color of the medium from yellowish-orange to bright pink due to the phenol red indicator, whereas no color change indicated a negative result [15].

2.8. Data Analysis and Presentation

The collected laboratory data regarding colony features, staining results, and biochemical reaction profiles were compiled systematically. The distribution, absolute frequencies, and percentage isolation rates of the identified bacterial genera among the participants were analyzed and

presented using descriptive statistical summaries, including frequency tables and graphical charts.

3. Results and Discussion

3.1. Socio-Demographic Characteristics of Participants

A total of 60 healthy female regular students from the main campus of Wolkite University were enrolled in this study. Hand skin swab samples were successfully collected from all 60 participants (100% response rate) and processed at the Department of Biotechnology Laboratory for the isolation and phenotypic characterization of normal bacterial flora. The baseline results demonstrate that human hand skin surfaces maintain a highly diverse and complex microenvironment capable of supporting both permanent resident commensals and transient opportunistic microbial communities [9].

3.2. Isolation and Distribution Rate of Bacterial Genera

From the 60 hand swab samples analyzed, a total of 27 distinct bacterial isolates were isolated (Figure 1; Table 1). Phenotypic characterization based on colony morphology, Gram reaction, and an array of standard biochemical tests identified seven distinct bacterial groups distributing on the hand skin of the study cohort. The distribution of the isolated bacteria showed that the family *Enterobacteriaceae* was the most frequently isolated group, accounting for 10 out of the 27 total isolates (37.04%). This was followed closely by the genus *Staphylococci*, which represented 6 isolates (22.22%) and the genus *Lactobacilli*, which accounted for 4 isolates (14.81%). The remaining bacterial isolates were distributed among the genera *Bacillus* (2 isolates, 7.4%), *Micrococci* (2 isolates, 7.4%), *Pseudomonas* (2 isolates, 7.4%), and *Enterococci* (1 isolate, 3.70%).

This broad distribution pattern is structurally consistent with established cutaneous ecological principles, which state that the dry, acidic, and nutrient-limiting nature of human skin naturally selects for resilient Gram-positive organisms [6, 16]. The cell wall composition of Gram-positive bacteria allows them to better withstand high osmotic pressures, dehydration, and desiccation compared to thin-walled Gram-negative bacilli [7].

Table 1. Frequency and percentage distribution of bacterial genera isolated from the hand skin of participants.

No.	Identified Bacterial Genus	Frequency (Number of Isolates)	Percentage Distribution (100%)
1	<i>Enterobacteriaceae</i>	10	37.04
2	<i>Staphylococci</i>	6	22.22

No.	Identified Bacterial Genus	Frequency (Number of Isolates)	Percentage Distribution (100%)
3	<i>Lactobacilli</i>	4	14.81
4	<i>Bacillus</i>	2	7.41
5	<i>Micrococci</i>	2	7.41
6	<i>Pseudomonas</i>	2	7.41
7	<i>Enterococci</i>	1	3.70
Total	All Genera Combined	27	100%

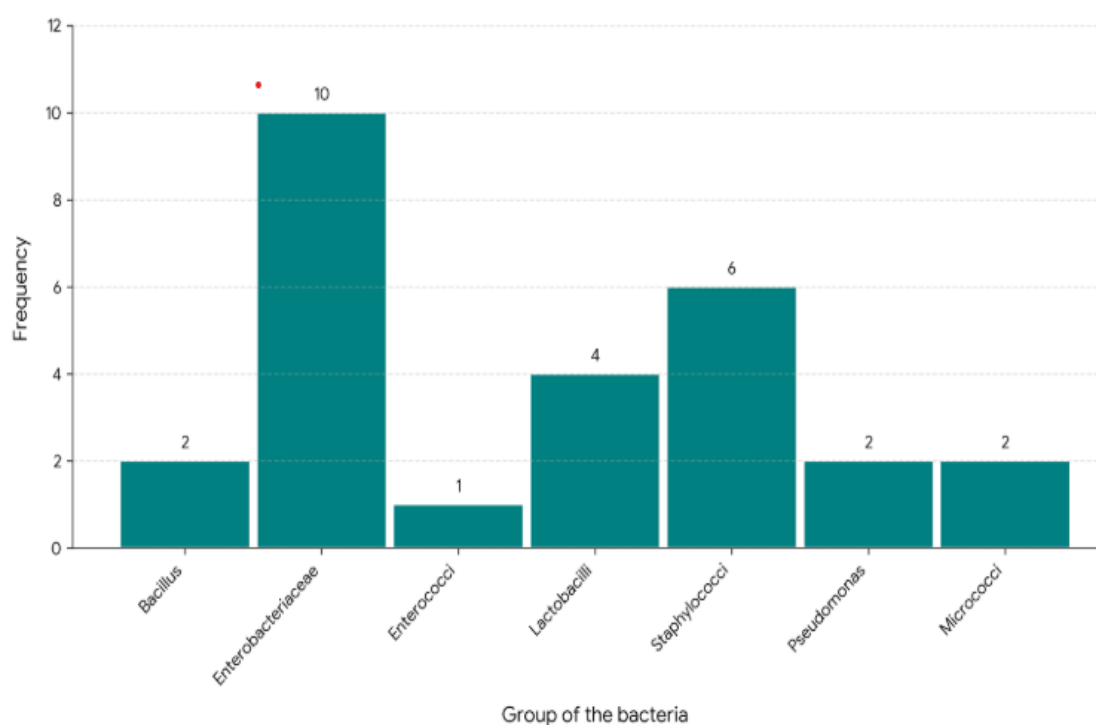


Figure 1. The frequency distribution profile of isolated bacterial groups.

3.3. Morphological and Biochemical Characteristics of Isolates

Out of the 60 hand swab samples processed in the laboratory, a total of 27 distinct bacterial isolates were successfully recovered and classified to the genus level based on their cultural, morphological, and biochemical profiles (Figure 2; Table 2). Gram-positive isolates predominated the sample ($n = 15$, 55.56%), distributed across five distinct genera/groups, while Gram-negative isolates accounted for the remaining 44.44% ($n = 12$). Among the Gram-negative isolates, *Enterobacteriaceae* represented the largest group ($n = 10$), characterized as motile, medium-sized rods capable of fermenting both lactose and glucose with associated gas production. These isolates tested positive for catalase, citrate utilization, and the Methyl Red (MR) test, but were negative for starch hydrolysis and the Voges-Proskauer (VP) reaction.

The remaining Gram-negative isolates were identified as *Pseudomonas* ($n = 2$), which exhibited a motile, rod-shaped morphology, tested positive for catalase, citrate, and glucose utilization, but failed to ferment lactose or produce gas. The Gram-positive cohorts were predominantly cocci, led by *Staphylococci* ($n = 6$) and followed by *Micrococci* ($n = 2$) and *Enterococci* ($n = 1$). Both *Staphylococci* and *Micrococci* exhibited positive reactions for catalase, glucose, citrate, and Methyl Red, while remaining negative for motility and starch hydrolysis. However, *Staphylococci* were differentiated by their ability to ferment lactose and a positive VP reaction. Conversely, the single *Enterococci* isolate was catalase-negative, VP-negative, and non-motile, but positive for lactose, glucose, and MR testing. Rod-shaped Gram-positive isolates comprised *Lactobacilli* ($n = 4$) and *Bacillus* ($n = 2$). The *Lactobacilli* isolates were non-motile, catalase-negative, and exhibited variable sizes, showing positive fermentation profiles for lactose, glucose, and MR. *Bacillus* isolates were

characterized as large, motile rods that unique among all tested groups, demonstrated robust starch hydrolysis alongside positive reactions for catalase, glucose, citrate, and Methyl red.

Among the recovered organisms, *Enterobacteriaceae* was the single most frequently isolated bacterial group on the hands of the participants, representing 10 out of the 27 total isolates (37.04%). The frequent isolation of *Enterobacteriaceae* which are traditionally classified as enteric or environmental rods presents an interesting ecological finding. Because human hands serve as primary tools for physical interaction, they are highly dynamic zones continuously exposed to external touch points [9].

In an institutional university setting, students frequently contact shared public surfaces such as cafeteria door handles, lecture hall desks, library books, and restroom fixtures. This dense interaction network facilitates the rapid mechanical acquisition of transient fecal-oral or environmental contaminants, explaining the elevated distribution of *Enterobacteriaceae* on the participants' hand surfaces [17].

The genus *Staphylococci* was the second most prevalent bacterial group identified, accounting for 6 out of the 27 isolates (22.22%). This high isolation rate directly aligns with foundational dermatological studies identifying *Staphylococci* (particularly coagulase-negative species like *Staphylococcus epidermidis*) as universal, core components of the human resident skin micro biota [2, 6]. These resident *Staphylococci*

play a critical evolutionary role in the first line of innate host defense. They engage in bacterial interference by physically blocking epithelial binding receptors, competing for limited surface nutrients, and actively secreting specialized antimicrobial peptides or bacteriocins that inhibit the colonization of highly virulent, incoming pathogenic invaders [3, 6]. The remaining microbial distribution on the hand skin included *Lactobacilli*, *Bacillus*, *Micrococci*, *Pseudomonas*, and *Enterococci*. The presence of *Bacillus* and *Micrococci* represents typical, dry-adapted environmental or soil-borne contaminants that temporarily adhere to the skin as transient flora [4, 8].

However, the presence of genera such as *Pseudomonas* and *Enterobacteriaceae* highlights a critical clinical duality. Under homeostatic baseline conditions, these organisms live completely harmlessly as commensal or transient surface residents on an intact stratum corneum. Yet, if these bacteria are mechanically displaced from their natural cutaneous boundaries and introduced into deep tissues or the systemic bloodstream via physical trauma, accidental punctures, severe burns, or open surgical wounds, they can rapidly transition into highly aggressive opportunistic pathogens [6, 16]. This structural finding underscores the profound public health importance of introducing consistent personal hygiene, systematic hand-sanitation protocols, and enhanced public awareness within dense campus student ecosystems [17].

Table 2. Morphological and biochemical characterization of bacteria species isolated from female student's hands.

Biochemical test	Bacterial species						
	<i>Enterobacteriaceae</i>	<i>Staphylococci</i>	<i>Lactobacilli</i>	<i>Bacillus</i>	<i>Micrococci</i>	<i>Pseudomonas</i>	<i>Enterococci</i>
Number of Isolates	10	6	4	2	2	2	1
Shapes	Rods	Cocci	Rods	Rods	Cocci	Rods	Cocci
Gram rxn.	-	+	+	+	+	-	+
Size	Medium	Small-Medium	Variable	Large	Small-Medium	Medium	Small
Catalase	+	+	-	+	+	+	-
Lactose	+	+	+	-	-	-	+
Glucose	+	+	+	+	+	+	+
Gas prod.	+	-	-	-)	-	-	-
Citrate	+	+	-	+	+	+	-
Starch	-	-	-	+	-	-	-
Methyl red	+	+	+	+	+	-	+
VP	-	+	-	-	-	-	-
Motility	+	-	-	+	-	+	-

+ = positive, and - = negative

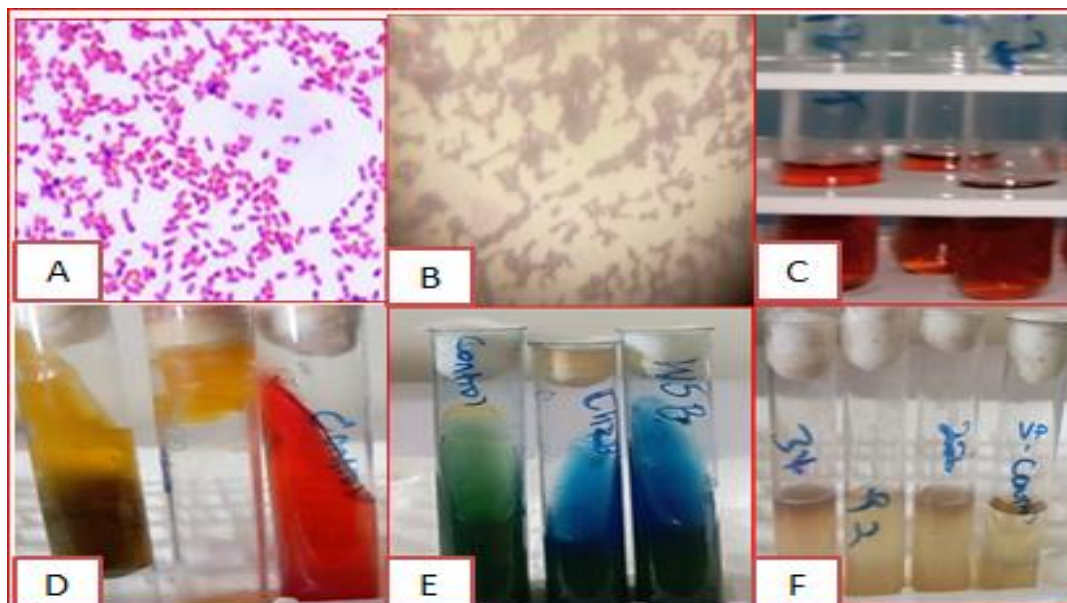


Figure 2. Morphological and biochemical characterization of bacterial isolates: (A): gram-positive isolate; (B): gram-negative; (C): positive methyl red test; (D): TSI positive test; (E): citrate positive; and (F): VP-negative test.

4. Conclusion

This study was successfully conducted to isolate and phenotypically characterize the normal bacterial flora from the hand skin of healthy female regular students at Wolkite University. Out of the 60 hand swab samples processed, a total of 27 bacterial isolates were recovered and identified to the genus level using morphological, staining, and standard biochemical verification arrays. The laboratory findings reveal that *Enterobacteriaceae* was the most frequently distributed bacterial group on the hands of the participants, accounting for 10 out of the 27 total isolates (37.04%). This was followed closely by the genus *Staphylococci*, which represented 6 isolates (22.22%). The remaining bacterial distribution consisted of *Lactobacilli*, *Bacillus*, *Micrococci*, *Pseudomonas*, and *Enterococci*. These results demonstrate that while the human hand skin maintains a rich community of protective, non-pathogenic commensal resident flora (such as *Staphylococci* and *Lactobacilli*), it also frequently harbors transient environmental and enteric bacteria like *Enterobacteriaceae*. While these organisms exist harmlessly on intact skin surfaces, they possess significant clinical potential to act as opportunistic pathogens if they are forcefully introduced into deep tissues through compromised skin barriers, mechanical trauma, or open wounds. Further research should be carried out with a larger sample size and extended study periods at Wolkite University. Future studies should incorporate advanced molecular identification techniques, such as 16S rRNA gene sequencing, and include antimicrobial susceptibility testing (AST) to map out the resistance profiles of these commensal and transient skin isolates.

Abbreviations

AST	Antimicrobial Susceptibility Testing
MR	Methyl Red Test
VP	Voges-Proskauer Reaction
TSI	Triple Sugar Iron Agar

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Author Contributions

Debebe Landina Lata: Conceptualization, Methodology, Software, Writing – original draft, Writing – review & editing

Abera Kenea: Conceptualization, Data curation, Visualization

Tesfaye Giza: Funding acquisition, Investigation, Supervision

Eyasu Milkias: Formal Analysis, Project administration, Resources

Kaleb Kamayla: Data curation, Resources, Validation

Dawit Regasa: Conceptualization, Data curation

Conflicts of Interest

There are no conflicts of interest to disclose regarding this current paper.

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Research Field

Debebe Landina Lata: biotechnology, plant biotechnology, plant microbe interactions, plant molecular biology, microbiology and microbial biotechnology, nanotechnology, and bioinformatics

Abera Kenea: Biotechnology, bioinformatics, molecular biology, nanotechnology, and biochemistry

Tesfaye Giza: plant science, coffee science, agronomy, plant breeding, and plant pathology

Eyasu Milkias: chemistry, biochemistry, medical chemistry, analytical and organic chemistry

Kaleb Kamayla: Biology, medical microbiology, microbiology, entomology, and biotechnology

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