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# Molecular Characterization and Antibiogram Studies of Bacterial Flora of Human Facial Skin

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**Abstract**

The skin is one of the largest organs in human body having multiple functions which is composed of three layers, epidermis, dermis and subcutaneous tissue or hypodermis. A diverse microflora is associated with the skin and mucous membranes of every human being which is normally established immediately following the birth of the newborn. That includes *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Micrococcus*, *Pseudomonas*, *Malasezzia* and *Demodex* mites. Here, in this study, bacterial flora of human facial skin was isolated and identified on the basis of conventional biochemical profiles along with molecular characterization of bacterial isolates using universal target sequence analysis of 16S rDNA. The amplified products were sequenced using commercially available sequencing facilities. *In silico* studies was carried out to find out nucleotide sequence identity and homology of the obtained bacterial sequences using online application BLASTn. The sequences were submitted to GenBank for accession numbers. Finally, the antibiogram studies were performed to find out antimicrobial susceptibility of bacterial isolates. In the present study a total of 50 samples were collected from facial skin of the students at Microbiology Department, GCUF. All samples were positive for bacterial growth. Out of all positive samples different bacterial pathogens were isolated which included 35 (70%) gram-positive cocci.

10 (20 %) were gram-positive rods and 5 (10%) were gram-negative rods. The biochemical characterization of isolated yielded *S. aureus*, *S. epidermidis* and *Streptococcus pyogenes. Staphylococcus epidermidis* was the most common pathogen among all isolates followed by *S. aureus* and *Streptococcus pyogenes*. *S. epidermidis* was resistant to tetracycline and Cephalexin. *S. aureus* was resistant to Amoxicillin, Tetracycline and Ampicillin where *Streptococcus pyogenes* was found resistant against Tetracycline.

## Introduction

At every step of life, the humans are in steady interaction with the microbial world. Life on earth could not proceed without micro-organisms as these are responsible for reusing the components that are imperative for human life. Human skin is colonized by huge number of microscopic organisms. There is a lot of intricacy in communications amongst the bacterial cells. These communications incorporate pathogenic, focused and harmonious connections (Becker et al., 2014).

The largest organ in human body is the skin in terms of surface area and having multiple functions which includes an obstructer between the extrinsic environment and the intrinsic organs. The skin also protects against different environmental factors including chemicals, different microorganisms and radiations etc. Skin also has many sensory functions affiliated to sense of touch, insistence, temperature, pain sensation and alerts for potential tissue damage. Skin also releases waste products from the body like urea, water, ammonia and uric acid. Skin also provides mechanical support. In addition, it also has a potential function in exchange of salts, fluids and heat (Garcia et al., 2017).

The epidermis is composed of keratinized stratified squamous epithelium. Depending on its location in the body, it is made up of nearly five layers of epithelial cells. It isn't able to receive blood. "Thin skin" is described as skin that has four layers of cells. The stratum, stratum granulosum, stratum spinosum, and stratum Basale are the layers in order from deep to shallow. Thin skin might be delegated to the bulk of the body. Only the palms of the hands and the soles of the feet are covered in "tough skin." Between the stratum granulosum and the stratum corneum is the fifth layer, the stratum lucidum. Keratinocytes are cells that can be found in most levels except the stratum Basale (Rousso et al., 2015). A keratinocyte is a type of cell that produces and stores the keratin protein. Other non keratinocytic cells of the epidermis incorporate melanocytes, Langerhans cells, and merkel cells (Kolar sick et al., 2011).

The stratum lucidum is sandwiched between stratum corneum and stratum granulosum. The layer is composed of dead and flattened cells and it is only present in hard skin of the soles, digits and palms. Stratum corneum is the outermost layer of the epidermis due to increased keratinization or cornification of the cells in this layer. Almost 15 to 30 layers are present in the stratum corneum. After 4 weeks, whole layer is replaced (Schommer et al., 2013).

A diverse microbiota is associated with the skin and mucous membranes of every human being which is normally established immediately following the birth of the newborn. These microorganisms are termed as normal microflora and are either beneficial or in some cases causes localized infections or remain as commensal. The normal microflora includes several bacteria, fungi and mites. Most of the normal microflora is considered as nonpathogenic in healthy individuals; however, these are frequently associated with infections in immune compromised hosts. This is also a well-established fact that viruses and parasites are not normal microflora as these are not commensal and do not help the host (Moestrup et al., 2017).

## Materials and methods

### Sample collection

Using sterilized cotton swabs, 50 skin samples were taken from people of all ages. Proceed to the GC University Faisalabad Microbiology Laboratory.

### Isolation of Skin Bacteria

Use of commercially available culture media was used to isolate bacteria from the skin. The swab samples were first infected in broth culture, then on agar plates. Finally, the streak plate approach was used to achieve pure cultures, resulting in purified colony growth (Wayne, 2010).

### Skin Swab Culture in Liquid Media

Tryptic soy broth (Oxoid, UK™) was prepared and poured into sterile test tubes. The tubes were labeled properly. Keeping all the aseptic measures the swabs were inoculated into tubes containing broth. Following inoculation, the culture tubes were incubated at 37°C in incubator for eighteen to twenty-four hours (Fig. 3.1). Later on, the tubes were observed for bacterial growth; the tubes which turned turbid were considered positive and were put in refrigerator until further processing.

### Isolation of Bacteria on Solid Media

Isolation of bacteria was performed using different culture media like Tryptic Soy agar, Nutrient agar, Mannitol Salt agar and blood agar (Oxoid, UK™) respectively. Loopful of broth culture were inoculated by streak plate method on above mentioned agar plates.

All culture plates were incubated at 37°C for 24 hours. Later culture plates were observed for bacterial growth. The culture plates which were having bacterial colonies were considered as positive. Standard laboratory methods for morphological characteristics and biochemical suggestive tests, such as catalase, Bactrian test, oxidase, and analytical profile test, were used to identify positive growth of samples.

### Antimicrobial susceptibility

To determine the susceptibility of isolates, antibiotics such as amoxicillin, ampicillin, cloxacillin, kanamycin, tetracycline, and ciprofloxacin were utilized in accordance with the Clinical and Laboratory Standards Institute's standard method of disc diffusion.

Molecular Characterization and DNA Extraction

All the identified culture isolates were subjected to molecular characterization through Polymerase Chain

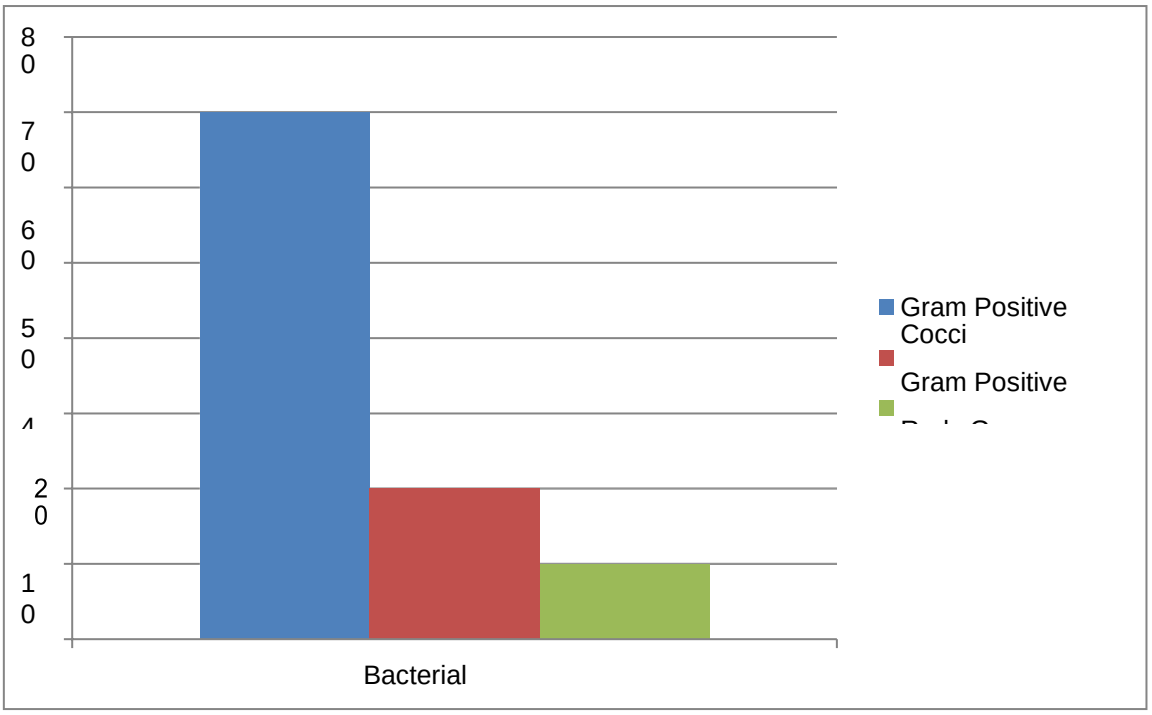
Reaction (PCR) using 16S rRNA universal primers.

Heating method was used for extraction of DNA. Firstly, Eppendorf tube was filled with 200ul distilled water and put single colony of desire sample. This work was done in bio-safety cabinet and Eppendorf tube vortexes for 30seconds. Furthermore, the tube was punctured and kept in hot water (96C°) for 10mins and frozen at 4C° for 5mint, followed by centrifugation at 13000rpm for 5 minutes. The 150ml supernatant was collected in separate tube. The extracted DNA was used for PCR.

## Results

### Assessment of samples

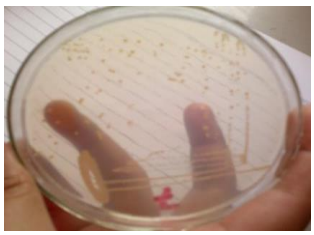
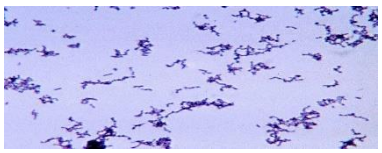
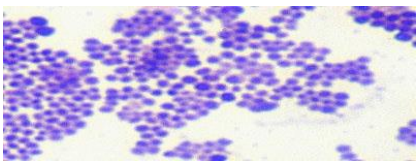
In the present study a total of 50 samples were collected from facial skin of the students at Microbiology Department, GCUF. All samples were positive for bacterial growth. Out of all positive samples different bacterial pathogens were isolated which included 35 (70%) Gram-positive cocci, 10 (20 %) were Gram-positive rods and 5 (10%) were Gram-negative rods. Distribution of bacteria is shown in Figure 4.1.




### Distribution of Gram-positive and Gram-negative bacteria in skin samples

### Cultural characteristics

All samples were inoculated on nutrient agar, blood agar, tryptic soy agar and mannitol salt agar and incubated at 37$^{O}$C for 24 hours. The colonies of *S. epidermidis* were white in color with 1-2 mm in diameter. The colonies of *S. aureus* were golden yellow in color with 2-3mm in diameter. Further, the colonies were opaque, smooth, and convex with entire margins.

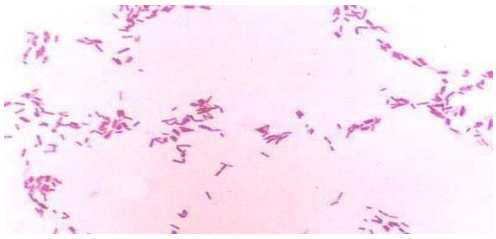
i ii iii

**i Figure Golden colonies of S. aureus on nutrient agar ii- hemolytic colonies of S. aureus on blood agar, iii*White colonies of S. epidermidis on blood agar***

**Bacterial Morphology**

All the positive isolated pathogens were characterized on basis of their morphology and arrangement. Most of the isolates were Gram positive cocci whereas some were Gram positive rods. Very few bacteria were Gram negative and rods.


**I Gram staining of *S. aureus* *ii* Gram staining of *Propionibacterium acne***



**Iii Gram staining of *Pseudomonas aeruginosa***

Biochemical Tests

The biochemical characterization of *S. aureus*, *S. epidermidis* and *Streptococcus pyogenes* is shown in the Table

Biochemical characteristics of Gram-Positive Cocci

| **Biochemical test** | ***S. aureus*** | ***S. epidermidis*** | ***Streptococcus pyogenes*** |
|---|---|---|---|
| Catalase | Positive | Positive | Negative |
| Coagulase | Positive | Negative | Negative |
| Bacitracin | Negative | Negative | Positive |
| Oxidase | Negative | Negative | Negative |

**Antibiotic Susceptibility Testing (AST)**

## Antibiotic susceptibility of Normal Microflora of Skin

| Antimicrobial Agent | *S. epidermidis* | *S. aureus* | *S. pyogenes* |
|---|---|---|---|
| Amoxicillin | Sensitive | Resistant | Sensitive |
| Tetracycline | Resistant | Resistant | Resistant |
| Ampicillin | Sensitive | Resistant | Sensitive |
| Cloxacillin | Sensitive | Resistant | Sensitive |
| Ciprofloxacin | Sensitive | Sensitive | Intermediate |
| Kanamycin | Sensitive | Sensitive | Sensitive |
| Cephalexin | Resistant | Sensitive | Sensitive |

**Sequencing Data of 16S rRNA gene**

The sequencing data of two isolates is described below.

Isolate 1: ***Staphylococcus aureus***

ATGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTA
CCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCAGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGA
CGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAG
CCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTT
CCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGG
AAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCG
CGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGA
AAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGT
AGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCG
AAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTC
CGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGA
CGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTG
ACAACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCG
GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAA
TACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACT
ACATGAAGCTGGAATCGCTAGTAATCGTAGAT CAGCATGCTACGGTGAA

Isolate 2: ***Staphylococcus aureus***

CATGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCT
ACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAG
ACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATA
GCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCT
TCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGG
GAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGC
AAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGT
GGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGA
TATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAG
GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTA
ACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCC
TTCCCCTTCGGGGGACAAAGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTT

AGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA
TGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCA
TAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGTAGA
TCAGCATGCTACGGTGA

## Discussion

The knowledge of normal skin facial flora is important to better understand the role of these bacteria in human skin disorders and in order to chart down the strategies to for treatment and cure of such problems. Therefore, it is invincible that we should assess the antibiotic sensitivity of these bacteria as well (Todar, 2013; Wilson, 2009).

All samples were positive for bacterial growth. Out of all positive samples different bacterial pathogens were isolated which included 35 (70%) gram-positive cocci, 10 (20 %) were Gram-positive rods and 5 (10%) were Gram-negative rods, which are consistent with the earlier findings (Lockhart et al., 2007; Elsner, 2006). The Gram- negative rods did not make significant part of human skin microbiome as compared to their significance in GIT where these in outstanding numbers (Lockhart et al., 2007). Probably this is due to dry skin conditions which limit their proliferation and survival (Elsner, 2006). A few Gram-negative organisms like *Pseudomonas* and *Pasteurella spp. etc.* do exist but these are not part of regular microbiota of human skin (Larson et al., 1986).

The biochemical characterization of isolated yielded *S. aureus*, *S. epidermidis* and *Streptococcus pyogenes. Staphylococcus epidermidis* was the most common pathogen among all isolates followed by *S. aureus* and *Streptococcus pyogenes* which is similar to the findings of Grice and Segre (2011) that CoNS (coagulase-negative staphylococci) and

*S. epidermidis* and other Gram positive are considered as primary skin inhabitants while some other bacteria which are also commonly present include members of genera *Propionibacterium, Corynebacterium, Micrococcus* and *Brevibacterium*.

The skin isolates were confirmed using 16S rDNA sequencing. Identification of skin microbes using genomic techniques is more efficient in comparison to classical culture techniques. 16SrDNA sequencing and metagenomic studies revealed that skin microbiota belongs to different phyla: *Bacteroidetes, Actinobacteria, Firmicutes* and *Proteobacteria* (Gao et al., 2007; Grice et al., 2008; Grice et al., 2009; Costello et al., 2009).

*Staphylococcus epidermidis* was the most common pathogen among all isolates followed by *S. aureus* and *Streptococcus pyogenes*. *S. epidermidis* was resistant to tetracycline and Cephalexin. *S. aureus* was resistant to Amoxicillin, Tetracycline and Ampicillin where *Streptococcus pyogenes* was found resistant against Tetracycline. Altogether, the findings of the current study showed that *S. aureus* is predominant followed by *S. epidermidis* and *Streptococcus pyogenes*.

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