A pilot study on showcasing the forensic relevance of skin microflora

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Abstract

Background

Utility of skin microflora is coming up now with the advancement of molecular techniques and the use of results in predicting infection transmission, genetic relatedness of the source of the origin, forensic use etc. Hence, preparing a catalogue of such endogenous and exogenous microbes would serve as a reference for future studies.

Results

Isolates identified showed typical pattern of having core microbial communities as found in all the samples and the typical ones which were found only in one individual and not in others. This study showed a potential to identify and classify microbes associated with skin of healthy individuals.

Conclusion

This can serve as a reference model for future studies and to employ the knowledge into forensic as well as other studies related to health. However, it would be worthwhile to consider similar study with large number of individuals with variable food habits and ethnicity for making an exclusive catalogue for skin microflora.

Background:

Skin microbiota is an important aspect of our health and routine work. Its role in multiple ailments and also in forensic applications is gaining wider grounds now. Our skin harbours both the exogenous or transient microbes due to the environment to which a person is exposed and also the endogenous or the resident microbes residing permanently over the body. Hence, preparing a catalogue of such endogenous and exogenous microbes would serve as a reference for future studies.

Skin microbiota is a diverse and complex community, composition of which is highly dependent on temporal changes, the chemical, biological, and physical surroundings and type of skin[1]. Some genera of microbes are commensal and are normally found associated with healthy skin. Different species of *Staphylococcus* are normally found on skin of healthy individuals. At the same time people handling soil etc. also accumulate a few species of *Bacillus* and *Pseudomonas* from there and these species remain in balanced state with our skin, until they get an opportunity to penetrate into the skin through wounds or cuts. The isolates obtained from each sample were classified using biochemical tests and confirmed further using molecular analysis of 16S rDNA sequencing (Table S2). The obtained results suggested that the individuals working in forensic DNA laboratory harboured more bacterial isolates followed by individuals associated with computers. This finding may also be treated as an alarm towards the health

and safety measure for forensic DNA experts and computer professionals. Earlier work about microflora associated with computers and mobiles and its forensic use has also endorsed the fact that high and variable number of microbial species remains associated with the keyboards of mobile and laptops, sometimes even depicting the signature microflora of its user [2]. The identified Isolates showed typical pattern of having core microbial communities as found in most of the samples and the typical ones which were found only in one individual and not in others. Interestingly, a few typical profiles were shown by just one or two bacterial species indicative of the profession of the individual from which they were obtained and were not present in others. For instance, individual 5, who worked in agricultural setup showed the presence of *Staphylococcus sciuri* that is considered as animal bacterial species and is commonly present on skin and mucosal surfaces of a wide range of pets and farm and wild animal[3], whereas *Exiguobacterium acetylicum* as a typical origin of rhizospheric soil which was found in almost all individuals as they were handling soil either being in their profession or being the homemakers. The isolates obtained before and after spirit wash could distinguish between normal residents and environmental species which temporarily inhabit the skin.

Results:

We classified the obtained bacterial population into commonly found bacteria on human skin and the ones which were found to be unique in this study as listed in the supplementary TableS3. Multiple species of *Staphylococcus* were found to be there with an unique one viz. *S. vitulinus*, while other unrelated bacteria as identified by 16S rRNA sequencing were *Citrobacterfreundii*, *Escherichia vulneris*, *Pseudonocardia*, *Kocuriarhizophila*, *Geobacillusstearothermophilus*, *Bacillus thuringiensis*, *Aerococcusurinae*, *Staphylococcus sciuri*, *Bacillus velezensis*, *Planococcus*, *Paenibacillusmucilaginosus*, *Bacillus pumilus*, *Cronobactersakazakii*, *Bacillus tequilensis* and *Staphylococcus saprophyticus*. The presence of these uncommon bacterial genus was correlated with the profession of the individuals and discussed further. The identified bacterial isolates were also analysed by using neighbour joining (NJ) and Principal Component analysis (PCA plot-PAST software v 3.2) methods to see their genetic relatedness as presented in Figure S1 and Figure S2 and also through a Table S4.

Discussion:

Species of *Staphylococcus* were omnipresent and are well documented to be present on the skin, however *S. vitulinus* has not been reported as skin pathogen and is normally found associated with food and animals, *Citrobacter freundii* is a pathogen normally found in urinary tract infections and is associated with wound, respiratory, meningitis, and sepsis, *Escherichia vulneris* is an opportunistic human pathogen, *Pseudonocardia* is a very typical one that is found associated with the cuticle of leafcutter ants, *Kocuriarhizophila* has also not been reported from human skin, *Geobacillus stearothermophilus* is a food contaminant normally found in soil, hot springs, ocean sediment etc., *Bacillus thuringiensis* found in agricultural produces, *Aerococcusurinae* is found in dust of occupied rooms such as grain storage etc., *Bacillus velezensis* belongs to the Rhizosphere community as like

Planococcus, *Paenibacillus mucilaginosus*, *Bacillus pumilus*, *Cronobactersakazaki* is an environmental pathogen, whereas *Bacillus tequilensis* is a plant pathogen. The obtained results also show the individual variation in the microbial profile of individual which is suggestive of use of bacterial profile of individual for forensic purpose (Fig. 1). The tree and PCA plot both have revealed clusters having genetic relatedness and segregated clusters differentiating between bacterial isolates. This classification is a miniaturized version of what all information could be obtained by having an extended study with meticulous observation of all details related to demography and profession of the participating individuals.

Conclusion:

This study showed a potential to identify and classify microbes associated with skin of healthy individuals. This can serve as a reference for future studies and to employ the knowledge into forensic as well as other studies related to health. However, a comprehensive study with higher number of individuals from all walks of life and with variable food habits and ethnicity may be a better approach for making an exclusive catalogue for skin microflora.

Methods:

We developed a model study where we collected swabs before and after alcohol wash from hands of 11 healthy individuals having different kind of work profiles and age groups (*Table S1*). Samples were collected from fingertips of all the individuals using sterile swabs soaked in Phosphate buffered saline(PBS) buffer (NaCl 8 gm, KCl 200 mg, Na₂HPO₄ 1.44 gm, KH₂PO₄ 240 mg volume made to 1 litre using distilled water). The samples were obtained before and after a spirit wash to separate endogenous microbes from exogenous ones. The collected swabs were washed in same PBS buffer and were plated on bacteriological media Brain Heart Infusion (BHI) to obtain pure cultures using standard microbiological practices. The cultures were subjected to the gram staining and the battery of biochemical tests for their primary identification. For confirmation of the strains the cultures were subjected to DNA isolation using standard protocol[4] and 16S rDNA PCR using universal 16S rDNA primers viz. 27F-5'-AGAGTTTGATCMTGGCTCAG and 1492R-5'-ACG GCTACCTTGTTACGA on ABI 9700 thermal cycler machine (ThermoFisher Scientific) [5]. The isolates were finally identified by sequencing the 16SrRNA amplicons on ABI 3730xl DNAAnalyzer (Thermo Fisher Scientific, CA, USA) and subsequently matching them with the similar sequences in the database using BLASTN[6].

Abbreviations

PBS	Phosphate buffered saline
BHI	Brain Heart Infusion
PCA	Principal Component analysis
NJ	Neighbour joining

Declarations

Ethics approval and consent to participate:

The study was approved by the ethical committee of Jiwaji University, Gwalior with letter number JU/IHEC/2017-A/06 dated 9.3.2017. Written informed consent was obtained from a parent or guardian for participants under 16 years old along with all other participants of the study.

Consent for publication:

Not applicable

Availability of data and material:

Available on reasonable request.

Competing interests:

Author (S) declares they have no conflict of interest.

Funding:

There was no funding body for this study.

Authors' contributions:

PS and MKG planned the study, TJ collected samples, conducted the experiment and did the analysis, RKK helped in the analysis, VN and PS drafted the manuscript, PKS, GC and NK reviewed the manuscript. All the authors read and approved the manuscript.

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Not applicable

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Figures

Figure 1

Bar diagram showing the percentage of individual bacterial species from the total bacterial profile of individuals taken in the study

Supplementary Files

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