NAAC Accredited

Savitribai Phule Pune University
(Formerly University of Pune)
DEPARTMENT OF ZOOLOGY
(UGC-CAS-Centre of Advanced Study in Zoology)

Ref. No. Zool /

Date :

To,

Prof. Katrine Whiteson,
Editorial Office
PeerJ life and Environmental

17th October, 2021

Subject: Submission of the revised manuscript PeerJ (#2021:03:59656:0:2:REVIEW)

Dear Editor,

On behalf of co-authors, I am submitting the revised manuscript entitled "Skin microbiota diversity among genetically unrelated individuals of Indian origin" to be considered for publication in 'PeerJ'.

We thank the reviewers for their generous comments on the manuscript. We have edited the manuscript and addressed their concerns in the rebuttal letter. We have also ensured that all the comments addressed in the response letter (corresponding page number and line number are mentioned) are also there in the revised manuscript.

We declare no conflicts of interests to disclose. The revised manuscript has been read and approved by all authors.

Please address all the correspondence concerning this manuscript to me at - richaashma@unipune.ac.in

Thank you for your consideration of this manuscript.

Sincerely,

Richa Ashma.

Richa Ashma
ASSOCIATE PROFESSOR
Department of Zoology
Savitribai Phule Pune University
Pune - 411 007

Response to the reviewers comments on PeerJ
(#2021:03:59656:1:1:REVIEW)

Skin microbiota diversity among genetically unrelated individuals of Indian origin

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Reviewer 1 (Anonymous)

Basic reporting

1) ***Please rename Propionibacterium to Cutibacterium throughout the manuscript. Why rename it in the table and not text?***

Response- We would like to thank reviewer for their effort in reviewing the manuscript**.** As suggested *Propionibacterium* to *Propionibacterium/Cutibacterium* have been renamed throughout the revised manuscript.

Changes in Manuscript-

1) Page no. 3, line no. 68
2) Page no. 4, line no. 90
3) Page no. 9, line no. 283
4) Page no. 9, line no. 297

2) ***The weather of Ahmednagar is comparatively hot and dry than Pune --> Please rephrase.***

Response- The sentences has been rephrased in the revised manuscript as, "Of the tree locations, Nashik has the hottest and driest weather; and in Ahmednagar the weather is hotter and drier compared to Pune."

Changes in manuscript- Page no. 10, line no. 336-337.

3) ***317 The average humidity and rainfall received in Nashik are more (67%, ~99.3mm) than in Pune (60.3%, ~67.9 mm) and in Ahmednagar (59%, ~46.3 mm). --> please rephrase. Please check English grammar!***

Response- The sentences has been rephrased in the revised manuscript as, "Similarly, the average humidity and rainfall received in Nashik (67%, ~99.3mm) are higher than those in Pune (60.3%, ~67.9mm) and Ahmednagar (59%, ~46.3mm)."

Changes in manuscript- Page no. 10, line no. 339-340.

4) ***Among all three cities, the population and urbanization are more in Pune (~ 31 lacks), followed by Nashik (~ 16 lacks) and Ahmednagar (~ 3 lacks). --> please rephrase to proper English. What is "lacks"? 100 000? Please report in millions or report the full number.***

Response- The population size for each city has now been mentioned as suggested by the reviewer as, "Pune has the highest population size (~.3.1 million), followed by Nashik (~1.6 million) and Ahmednagar (~0.3 million)."

Changes in manuscript- Page no. 10-11, line no. 344-345.

5) ***Age: the alpha diversity for ppl aged 41-59 is half as much as those aged 16-40y. Are you sure there is no impact seen for age? The only reason for me to see no sign difference is the low sample size.***

Response- The sentence has been rephrased in both result and discussion part of revised manuscript.

Rephrased text in results:

Statistically significant differences were noted between the adult and middle age (p=0.003) groups. We did not observe differences in alpha diversity between the elderly age group and adults (p=1.000) and or middle aged (p=0.360) volunteers i.e., we have reported a significant change in alpha diversity (i.e., differences in taxonomic diversity) between these two age groups. However, we did not observe significant difference in beta diversity (i.e., differences in taxonomic composition). We have now clarified the text.

Rephrased text in discussion:

"In order to investigate the effect of age on skin microbiota composition, we categorized the volunteers into three age groups. Significant association was observed between alpha diversity and age, in particular between the young and middle-aged individuals."

Changes in manuscript- Page no. 8, line no. 239-242 and page no.11, line no. 374-379.

6) ***254 Staphylococcus and Corynebacterium were abundant on the skin of individuals from the Pune district (Figure 6I-J). --> These results resemble the most with the results found in Europe (Callewert et al 2013) and USA (Council et al 2016; Grice et al 2010). Pune is also the most urbanized location of the three; a city with many urbanization elements. I think the authors should discuss this better in the discussion section.***

For instance on this location: 325 individuals residing in Pune are inhabitants of a metropolis, adapted to the western lifestyle, developed industrial area and infrastructure.

Response- Our result has been discussed in line with Callewaert et.al 2013 and Council et al., 2016 in revised version of the manuscript as,

"Our results indicating differences in skin microbiota composition among the individuals of three geographical locations could also be due to urbanization status and population size per city. Pune has the highest population size (~.3.1 million), followed by Nashik (~1.6 million) and Ahmednagar (~0.3 million). Likewise, Pune being a metropolitant city has developed industrial area and infrastructure and individuals residing in Pune are adapted to urbanization and the western lifestyle. They use skin ointment and cosmetics like moisturizers, deodorants, antiperspirants etc. which could lead to the alterations in axillary bacterial communities. The axillary studies of Grice et al. (2010), Callewaert et al. (2013), and Council et al. (2016) found dominance of *Corynebacterium* and *Staphylococcus* genera in individuals resides in cities which complies with our results of differential abundance analysis. Our analysis indicated the presence of *Corynebacterium* and *Staphylococcus* genera were higher in Pune than other two studies areas, although we ensured that volunteers did not apply deodorants and cosmetics to their skin/axilla for 24 hours before sample collection.".

Changes in manuscript- Page no. 10-11, line no. 342-357.

7) ***Did the authors ask the participants for deodorant use? Did the people in Pune use deodorants more frequently as compared to the other 2 locations? I believe deodorant use is more common in Pune, which correlates to more Staph and Coryne presence in that city.***

Response-

We asked participants not to use deodorant 24 hours before sampling while explaining the study and taking their consent of participation. This has also been ensured orally while sampling. This has been mentioned in the revised manuscript as,

"Likewise, Pune being a metropolitant city has developed industrial area and infrastructure and individuals residing in Pune are adapted to urbanization and the western lifestyle. They use skin ointment and cosmetics like moisturizers, deodorants, antiperspirants etc. which could lead to the alterations in axillary bacterial communities. The axillary studies of Grice et al. (2010), Callewaert et al. (2013), and Council et al. (2016) found dominance of *Corynebacterium* and *Staphylococcus* genera in individuals resides in cities which complies with our results of differential abundance analysis. Our analysis indicated the presence of *Corynebacterium* and *Staphylococcus* genera were higher in Pune than other two studies areas, although we ensured that volunteers did not apply deodorants and cosmetics to their skin/axilla for 24 hours before sample collection."

Changes in manuscript- Page no. 11, line no. 347-357.

8) ***Figure 2: I appreciate the effort, but it still remains very difficult to interpret these results. I would really urge the authors to make a bargraph, as done in f.i. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3337431/ Fig 3. It would be great to see the individual bargraphs per person, and clustered per location. That is the only good way to represent the bacterial composition.***

Response- Figure 2 has now been reconstructed and placed in the panel of Figure 1 as Figure 1C i-iii. A bar graph of bacterial compositions at phylum level per person and clustered per location done in the revised manuscript.

Changes in manuscript- Page no. 7, line no. 229-230.

New figure- Figure 1 C i-iii

9) ***Also: Staphylococcus abundance in Fig 2 is around 1-1.5%, while in Table 3 this is 23.2%. Where does the difference come from?***

Response- Thank you for the suggestion this has been corrected in revisited manuscript. Figure 2 has been reconstructed as figure 1C i-iii wherein, individual barplots sorted by their geography at phyla level is now evident. Now, there is no correspondence in Figure 2 and Table 3 as Table 3 represents relative abundances and prevalence at genus level.

Changes in manuscript- Page no. 7, line no. 229-230.

10) ***Figure 6: please add statistics in the figures. I would suggest to mention the y-axis as done in panel I. (no scientific number - just a comma number, for easy interpretation) Can the authors also put the most abundant one in panel A, followed by the second abundant one in panel B, etc?***

Response- Figure-6 has been changed to Supplementary figure-1 A-L and as suggested statistics has been added and explained in statistical analysis section of materials and method as, “The post hoc Dunn test was performed using Kruskal-Wallis test for pairwise multiple comparisons on subgroups to compare median similarities of genus within individuals. p values were adjusted and reported in using the Benjamini-Hochberg (BH) method.”

and in the result section as, “Pairwise comparisons using Dunn test indicated high abundance of *Staphylococcus* and *Corynebacterium* on the skin of individuals from the Pune district

(Fig. S1A and *Fig. S1C).* A high abundance of *Paenibacillus, Geobacillus, Virgibacillus, Jeotgalicoccus, Pullulanibacillus, Delsulfosporomusa, Citinovibrio,* and *Calditerricola,* was observed on the skin of individuals from the Nashik district (*Fig. S1B* and *Fig. S1F-L).* In Ahmednagar individuals, *Pseudomonas* and *Anaerococcus* were observed in abundance *(Fig. S1D* and *Fig. S1E."*

In statistics pairwise comparisons were done using Dunn test and boxplots has been arranged as per most abundance in panel A-L.

Changes in manuscript- Page no. 7, line no.210-212 and Page no. 8, line no.266-274

11) Figure 4: CST nr 1, 2, 3. Can the authors say what locations are 1, 2 and 3? Actually, I don't understand the CSTs, I'm afraid. I also did understand after looking it up in the manuscript. Can the authors explain more on this?

Response- Community state type (CST) is a standard cluster analysis for microbial community analysis (DiGiulio et al. PNAS 2015; PMID: 26283357). Each CST represents one community type, with a peculiar community composition that is shared by individuals who fall into that cluster. We have numbered the CSTs from one to three; each CST is abundant in a different set of taxonomic groups as shown in Figure 4. Naming the clusters by the abundant taxa would be problematic because each CST has a combination of multiple abundant taxa as shown in Figure 4. These (CST) clusters are defined purely by taxonomic community composition but we observed a significant association between the CST clusters and geography. We have now clarified CST in the revised manuscript as suggested.

Changes in manuscript- Page no.8, line no.251-256.

12) Figures: I would suggest to combine a couple of figures into multipanel figures.

Response- figure-2 has been reconstructed with bar plots and changed into multipanel figure-1C i-iii as suggested, and we have also taken this into account when we updated the other figures in the revised manuscript version.

13) 288 Bangalore (India) detected dominance of four phyla viz., Actinobacteria, Firmicute, --> FirmicuteS

Response- Correction has been done as "Similarly, a study on facial microbiota of healthy females (N=30) from Bangalore (India) detected dominance of four phyla viz.,

Actinobacteria, Firmicutes, Proteobacteria, and *Bacteroidetes* (Mukherjee et al., 2016)" in the revised manuscript.

Changes in manuscript- Page no. 9, line no. 305-307.

14) 335 Callewaert et al., in 2013 studying interpersonal axillary diversity, did not observe a significant correlation with geographical location --> This study was done in Belgium, which is a small country and entirely urbanized. So it is difficult to say something about geographical location here.

Response- We have removed the sentence in the revised manuscript.

Changes in manuscript- Deleted from page no.11, line no.366-368.

Can I ask the authors to provide the track change word document next time? It was hard to find all the differences in the manuscript as compared to previous version.

15) Experimental design- /

16) Validity of the findings- /

17) Additional comments- /

Response: As suggested track change word document has been uploaded to compare the revised manuscript from the previous one. Sorry for the earlier inconvenience.
