Author’s response to reviews

Title: Differences in Gram-positive bacterial colonization and antimicrobial resistance among children in a high income inequality setting

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Author’s response to reviews:

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Dear Editor in Chief,

We have revised the manuscript and accommodated the reviewers’ suggestions. The main changes were:

(i) inclusion of more information about the significance of MRSA, SCCmec types and PVL genes in Introduction, Results and Discussion;

(ii) inclusion of the antimicrobial susceptibility profiles of the MRSA strains;
Inclusion of a major limitation statement explaining why children with symptoms were included in the analysis.

Several additional changes/revisions were incorporated in the revised manuscript (highlighted in yellow). The point-by-point responses to each reviewer are submitted below.

We would like to thank the reviewers for their valuable comments and suggestions, which were helpful to improve the manuscript and make it clearer.

We look forward to receiving feedback from the editor and the reviewers on this manuscript.

RESPONSE TO REVIEWERS
Deniz Gur (Reviewer #1)
Reviewer's report:

This manuscript is interesting and suitable for publication in your journal however I think it needs some minor revisions.

Authors’ comments:
Many thanks for your valuable comments.

1. The authors have investigated the SCCmec types and the presence of PVL genes among methicillin-resistant S. aureus (MRSA), and the antimicrobial resistance profile and serogroups of BHS in different pediatric populations. However, they have not given any information about the significance of SCCmec types, MRSA or PVL genes in the introduction section. They should add more information to the introduction and discuss the results accordingly.

Response: We agree and added more information about the significance of MRSA, SCCmec types and PVL genes in Introduction (lines 70-74), Results (lines 200-204) and Discussion (lines 261-267).

2. There are some microbiological procedures that should have been given in more detail, such as: Isolation of bacteria; how nasal specimens were obtained, e.g. which media? Antibiotic susceptibility tests; e.g. which medium?
Response: We included that “Swabs were immediately placed into a tube containing Stuart medium until transported to the laboratory on the same day” (lines 117-118).

We stated that we used mannitol salt agar to isolate S. aureus (line 122), and 5% sheep blood agar plates to isolate beta-hemolytic streptococci (line 128).

Regarding the culture medium for antimicrobial susceptibility testing, we included the following statement: “Antimicrobial susceptibility testing was performed by the disk-diffusion method on Mueller-Hinton agar (MHA) plate for MRSA strains and on MHA with 5% sheep blood agar plate for BHS” (lines 130-132).

3. Antibiotic susceptibility testing was performed for BHS but not for S. aureus. Antibiotic resistance patterns in MRSA and SCCmec types is significant and should have been given in these isolates.

Response: We included the antimicrobial susceptibility profiles of the MRSA strains for nine drugs in appropriate sections (Abstract: lines 35, 40-42; Methods: lines 130-135; Results: lines 184-190 and Discussion: lines 261-267).

4. Line 127 data analyses (analysis)

Response: Done (line 138).

Cezar Vinícius Würdig Riche, M.D., MSc (Reviewer #2)

The article presents epidemiological data of children colonized with S. aureus and BHS in a city with "high income inequality", in Southeastern Brazil, and discusses the influence that different socioeconomic status could have on it. The design is appropriate, the proper references were included and the data is original. However, it has two major concerns that must be reviewed. First, it must be made clear whether the written consent of the child's legal guardian was obtained. Second, the paper focuses on colonized patients, and there are patients with symptoms and/or illness in the analysis. These patients have to be excluded and the necessary corrections should be made.

Response: We appreciate your comments and they will help improve the manuscript. The point-by-point responses to all questions you raised are below.
Abstract: no comments.

Background: no comments.

Methods:

In line 93: "part of the Greater Rio de Janeiro Metropolitan Area, in the state of Rio de Janeiro" correct for "in Rio de Janeiro metropolitan area, Rio de Janeiro state, Brazil".
Response: Done (line 100).

In line 100: "The clinic is part of a public health post" did you mean Primary care facility? In must be corrected.
Response: Yes. We corrected it (line 107).

In line 106: "except from June 12th to June 24th due to logistical constraints during the 2014 World Cup, which took place in Rio de Janeiro." Should be suppressed.
Response: Done.

In line 111: Was the child's legal guardian written consent obtained? If yes, it must be stated.
Response: Yes. Legal guardians of all participants provided written consent. It is already stated in the appropriate section ("Ethics approval and consent to participate") at the end of the manuscript between “Authors’ contributions” and “Consent for publication” (lines 341-344). However, we stated it in line 116 (Following legal guardians’ written consent…”) to make it clearer.

Lines 112-113: "A mobile questionnaire… by a trained interviewer." Should be rephrased for "A questionnaire for socioeconomic data was completed by child's legal guardian."
Response: Done (lines 118-120).

In line 118: "mecA gene via PCR" correct for "mecA gene via polymerase chain reaction (PCR)".
Response: Done (line 126).

In line 120: What did you mean with "In turn"? Please, make it clear.
Response: We deleted it.

Lines 133-137: "For this analysis, we classified pediatric patients into tree SES groups…” this criteria implies in a high chance of misclassification, since the "residence location / attendance clinic" does not assure the socioeconomic status. This must be discussed as a possible limitation.
Response: We agree and included this point as a possible limitation: “Finally, the classification of pediatric patients into three SES groups may be a possible limitation, since self-reported residence location and type of clinic attended do not assure the socioeconomic status of the patients; however, the criteria we used to divide the patients into three subpopulations are very well described and support our analyses” (lines 291-294).

Results:
In line 159: "public clinics" correct it for singular.
Response: Done (line 170).

Lines 175-188: "Differences in BHS serogroups and S. aureus SCCmec types" The text repeats the same information presented in table 2, I suggest present the relevant information and suppress the second paragraph.
Response: We presented only relevant information to avoid repetition with data in the Table 2 (lines 192-204).

In table 2: Correct serotype for "Lancefield Group" to make the information presented unambiguous. A single isolate is either Lancefield group C or G, it cannot present both antigens. Please clarify this in the table.
Response: We changed “serotype” to “Lancefield group”. We also changed “Group C, G or C/G” to “Group C or G”.
Lines 209-212: This is the major concern of this article. Why were considered as colonized, children with "symptoms/illness"? This should have been considered an exclusion criterion. It becomes even more relevant once BHS are among the main causes of throat and upper respiratory tract infections in children. These patients must be excluded from the analysis - note that this implies the review and possible removal of tables 2 and 4, and corrections in relevant paragraphs should be made.

Response: We agree that BHS are among the main causes of upper respiratory tract infections in children, especially sore throat, and this is an important limitation of the study. However, colonization is usually the first step of the infectious process. Also, viruses cause most of these diseases and if we exclude all the symptomatic children, we will exclude patients with viral infections. In addition, in the regression analysis, characteristics (factors) that were not significantly different were not included. Therefore, if “symptoms/illness” was not included in the analysis, it means it did not differ between subpopulations. Removing it from the analysis would not change anything as far as our main conclusions. Also in Table 4, symptoms were included in the adjusted model and were not confounders, so not included in the final model.

We included this point as a major limitation of the study: “A major limitation of this study is that some children with symptoms may have the disease caused by the bacteria investigated, especially BHS and sore throat. However, colonization is usually the first step of the infectious process and viruses cause most of the upper respiratory tract diseases. If we excluded symptomatic children from the analysis, we would exclude patients with viral infections. Also, the prevalence of children with symptoms did not differ between subpopulations and, therefore, does not affect the main conclusions of this report” (lines 278-283).

We also changed the title of Table 4 to “Characteristics associated with the presence of beta-hemolytic streptococcal among pediatric patients examined at private and public clinics – Niterói, RJ, Brazil, 2014” to avoid misunderstanding between colonization vs. infection.

Discussion:

In line 220: Correct for "Bacterial isolates…"

Response: Done (line 236).

In line 224: "Geographically, they often form the barrier between these two groups." It must rewrite or suppressed.

Response: We suppressed it.
Lines 224-228: This part of the discussion must be reconsidered. How could it be that staying within a region without infrastructure (slum) a factor for less exposure to microorganisms?

Response: We suppressed it.

In line 240: "MRSA colonization among all SES groups (6%)…” Please, confirm the frequency. (8.1%?)

Response: We corrected it. It is 8.1% (line 254).

Lines 256-266: "Differences in antibiotic use… treatment regimen by SES groups." This information shouldn't be discussed once the data wasn't presented/evaluated neither referenced from a previous paper. Consider suppressing this paragraph.

Response: We suppressed it.

Lines 267-271: As previously stated, this population must be excluded from the analysis.

Response: We have addressed this point above and included it as a major limitation. “A major limitation of this study is that some children with symptoms may have the disease caused by the bacteria investigated, especially BHS and sore throat. However, colonization is usually the first step of the infectious process and viruses cause most of the upper respiratory tract diseases. If we excluded symptomatic children from the analysis, we would exclude patients with viral infections. Also, the prevalence of children with symptoms did not differ between subpopulations and, therefore, does not affect the main conclusions of this report” (lines 278-283).

Conclusion: no comments.