Author's response to reviews

Title: Prevalence of Staphylococcus sp and S. aureus in wounds of hospitalized patients in inland regions of the Northeast of Brazil and associated factors: a cross-sectional study

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Author's response to reviews: see over
Dear Editor,

We are submitting the article with the corrections suggested.

As suggested, the introduction and discussion were reduced. On the other hand, suggested additions or changes have been exposed in the text in red to facilitate the review process.

Below, responses will be exposed to each item of reviewers:

Reviewer: Meghan Davis

Major Compulsory Revisions

1. Interpretation of the results is hampered by gaps in reporting the methodology. Minor points below address specific gaps. Particular attention should be given to the statistical analysis section, since, as written, the reader cannot necessarily replicate the analysis. Of equal concern is the use of growth on mannitol salt agar to identify S. aureus, which ideally should be done using PCR amplification of aureus-specific nuclease gene if possible. Other staphylococci may appear with the same phenotype as S. aureus on MSA. Other staphylococci, such as the staphylococcal intermedius group (SIG) should be differentiated from S. aureus if possible because their epidemiology and outcomes may differ. If this is not possible, changing “S. aureus” to “coagulase-positive staphylococci (CPS)” or “presumptive S. aureus” would be appropriate to help the reader understand that the assumption that these staphylococci are S. aureus is exactly that, an assumption, particularly given the geographic novelty of this study. In particular, bite wounds from animals may be seeded with non-aureus staphylococci – were there any such wounds in this evaluation?

   - Were added information on methodology in an attempt to enlighten the reader on aspects questioned.
   - The PCR can be carried out only with MRSA. The identification of S. aureus was really through growth in mannitol salt agar, characteristic of the colony in the middle and by the Gram method, and positivity in tests for catalase and coagulase. Due to this limitation was accepted the suggestion of the reviewer to put "presumptive S. aureus" rather than S. aureus. The term "presumptive" was added in the methodology.
   - There was only 1 case of animal bite (snake bite) and therefore was not reported in the results.

2. The discussion is quite lengthy and reads as a literature review more than a true discussion. Consider moving the second paragraph to the top of the discussion section and leading with your most important findings. Consider trimming the verbage in the discussion section overall. Important limitations should be discussed, such as the choice to perform just nasal sampling when the implications of the work may be for community MRSA and MSSA strains. CA-MRSA in other countries has been shown to be associated with skin carriage.

   - As requested, the second paragraph became the first to guide the discussion. The first paragraph was deleted, as well as parts of the discussion regarding the location of the wound and other insignificant data in statistical tests and with less clinical significance were excluded.
   - The main limitations of the study, including the one suggested above, were inserted at the end of the discussion (last paragraph).
Minor Essential Revisions

1. Methods gaps:

a. General – what was the age range? Were children included? If participants under 18 were included in the study, these should be examined separately to determine if they have higher risk than the 18-65 group (as has been demonstrated in other studies), and as a sensitivity analysis, they should be dropped and the models re-run to determine if they are influential on the tested associations. If they are not, this could be indicated through inclusion of a single line to the results indicating that this sensitivity analysis was performed and no changes in inference were observed.

Only patients older than 18 years were included in the study. So, as requested, this information has been added. In addition, a single line was included in the results indicating that sensitivity analysis was performed.

b. Sampling days – how were these chosen (convenience vs regular schedule), and how many days were allocated to sampling? This is important to understand if the choice of sampling days introduced bias.

Data collection occurred at the beginning of each week. This information was added in the methodology. This point was also addressed in the discussion as a limitation of the study.

c. Culture and microbial identification – seen major points above. Also, please indicate how much (e.g. 10ul) was transferred to MSA.

As requested, the information has been added in the study. 0.1mL was transferred to MSA.

d. MRSA and PCR screening – were only cefoxitin resistant strains tested for mecA? If all were screened regardless of resistance phenotype, please note this. If only cefoxitin-resistant CPS were screened, please indicate, because some non-aureus staphylococci, e.g. S. pseudintermedius, are less likely to exhibit phenotypic cefoxitin resistance (indeed, CLSI recommends oxacillin screening instead) but may harbor the mecA gene.

Yes. Only cefoxitin resistant strains were tested for mecA. This information was added in the study.

e. Statistical analysis: Overall, please reduce description of the descriptive analysis and focus on adding detail about the variables and models. How were prevalence ratios calculated (what model? Are these PORs or PRs?) Please explain “to be included in tests of association, the quantitative variables were categorized by their median values” more directly – were these converted to categorical variables? Include (College Station, TX) after the reference to Stata 10.0. For Poisson regression, I suspect you mean (in stata code) poisson y x1 x2...xn, vce(robust)” for which there are references to consider including:


All suggestions were accepted. Changes were made in the statistical analysis as requested topic.

2. Results section: recommend displaying the demographic data presented in text form as a table instead – a classic Table 1 would be appropriate. This will result in renumbering the remainder of the tables. Also, why “widows” and not “females” to be parallel to the men, or does this mean married versus unmarried?

- As suggested, the sociodemographic data were presented in table.
- There are 3 categories: Married; Single and widow or widower. For the association tests, the variable was transformed into 2 categories: Married and Unmarried (Single + Widower / Widow).

3. Discussion section, paragraph starting “The most common location…” – in the presentation of bacilli versus staphylococci, please comment on the mechanism by which findings of bacilli would exclude staphylococci, with referencing. Do you mean through competitive exclusion?

This explanation was just an inference (hypothesis) of the researcher. It was based on the literature. This part of the text was deleted.

4. I’m uncertain if reference 4 is the most appropriate to use for line three in the introduction, since this references a potentially highly-exposed worker population and not the general public.

This part of the text was deleted.

5. Copy edits:

a. Abstract: end of results section, change “from individuals that died” to “from individuals who died” – this change needs to be made elsewhere in the paper as well, e.g. page 8, second-to-last line.

The suggested change was performed.

b. Introduction, sentence 3, change “commonly found in skin” to “commonly found on skin” – also note that the progression of logic from sentence 2 to sentence 3 is unclear.

The suggested change was performed.

c. Introduction, paragraph 2, capitalize Pseudomonas.

The suggested change was performed.

d. Methods, “Data Collection” section, recommend changing “such as” to “including” for lists of variables. Please explain what consumer goods are. Consider changing “contained questions” to “evaluated factors” in the second sentence. Consider changing “serum” to – “saline” (?) since serum suggests blood serum and you likely mean saline. If you do mean blood serum, please elaborate.

The suggested change was performed.

e. Methods, “Culture” section, recommend deleting “allowing the growth of colonies” in the second sentence as this is redundant. For the acronym for coagulase-positive staph, SCN is used – is this supposed to be CNS for the English abbreviation?
It was a mistake. We put the abbreviation in Portuguese. The suggested change was performed.

f. Please standardize your use of significant digits throughout the manuscript. For example, in results, “a mean of 3.84 years of education (SD = 3.7)” could be reframed with both using two significant digits as “a mean of 3.8 years of education (SD 3.7)” In particular, consider presenting all PRs to three significant digits instead of four, e.g. 1.48 not 1.476. P-values greater than 0.10 can be provided with three significant digits, e.g. 0.13 not 0.126.

The suggested change was performed.

Reviewer: Jennifer S McDanel

Reviewer's report:

Major Compulsory Revisions

1. The length of the Introduction and Discussion sections should be shortened, and the sections should highlight the main points/concepts of the manuscript. Some of the information included within these sections does not pertain to the main points of the manuscript, and this information draws the reader away from the focus of the paper.

As requested, the introduction and discussion were reduced.

Despite being added information suggested by the reviewers, the discussion was reduced. After the suggested adjustments, the discussion was with 1964 words and now has 1498 words.

2. This study is unique because it examined wound infections caused by Staphylococcus sp. in a population that resides in a remote, rural, and undeveloped region of Brazil that has extreme impoverishment. I think this aspect could be emphasized more throughout the manuscript and abstract.

As suggested, this aspect was evidenced in the abstract, in the introduction and discussion.

3. A main result of this project that is mentioned in the Discussion section but needs more emphasis is that wound infections caused by MRSA are not common in this region and many of the patients who got an MRSA wound infection have had previous exposure to the healthcare setting by either/having a prior hospitalization or antibiotic exposure. Therefore, interventions could be targeted at preventing the spread of MRSA in the healthcare setting. Additionally, mortality rates were high for patients who acquired a MRSA wound infection (50%). Are there any other interventions that can be implemented to potentially reduce the mortality rate? 4. In the Methods section under paragraph three, there are a few terms that need more explanation. What are considered consumer goods? What information was included for living conditions? When did the previous hospitalizations occur, 6 months or a year since the hospitalization due to the wound infection? When did previous antibiotic use occur, 6 months or a year since the hospitalization due to the wound infection?

• A paragraph was added in the discussion addressing the issues relating to MRSA.
• In the methodology, were added with information intended to clarify what was asked:

“number of consumer goods (cars or motorbikes, internet access, as well as electrical or electronic goods including television, cell phone, computer, DVD player and stereo)
and type of property (rural or urban area). The questionnaire also evaluated factors relating to the hospitalization and the wound, such as previous hospitalization (in one year), previous antibiotic use (in six months) and duration of wound.”

5. Please give a detailed description on how often samples and data were collected throughout 2012. How many patients had wounds that were potentially missed? Were some of the patients with wounds seen at outpatient clinics instead of being admitted to the hospital?

The description on how often sample and data were collected was added in the methodology. The potential losses were described in the discussion as a limitation of the study. There was no inclusion of outpatients in the study and this information was added in methodology and discussion.

6. How many patients had multiple wounds? If a patient had multiple wound infections, were all the wounds swabbed or was only one wound selected? Were patients included only once in the study?

These issues were clarified in the methodology:

“Most patients (71.8%) had only a wound, while 28.2% had two wounds or more. Thus, some patients were included more than once in the study (all wounds were swabbed), whereas the sampling unit of the survey was wound.”

7. Please include more information on the patients with wound infections caused by MRSA. Were the wounds of patients with MRSA infections started at home or in the hospital? Did the patients with MRSA infections have necrosis or septicemia? How many patients with an MRSA wound infection also had nasal colonization?

As requested, this information on MRSA were added.

Minor Essential Revisions

8. In Table 1, define urban and rural areas.

Below the table (currently Table 2), the definitions of urban and rural areas were added.


The definition was added.

10. In the Methods section under Statistical analysis, consider changing “multiple analysis” to “multivariable analysis”.

The change was performed.

Discretionary Revisions

11. How many beds does the Hospital Regional do Serido have or approximately how many patients per year does this hospital admit?

The information suggested were added

12. Which antibiotics were prescribed to the patients for their Staphylococcus wound infections?
Data from main groups of antibiotics used were added to the results

13. Could some of the wound infections be caused by multiple organisms (polymicrobial infections)? If patients are not infected with Staphylococcus sp. what other organism may be causing the wound infection?

Yes. This information has been added in the discussion.

14. Another potential direction of the manuscript is to only include wound infections caused by Staphylococcus aureus and MRSA since there is currently more clinical interest on these organisms.

Were removed from the discussion paragraphs related to Staphylococcus in general, trying to focus on S. aureus and MRSA.

15. How many of the Staphylococcus sp. wound infections may have been acquired in the hospital? How would you define hospital-acquired wound infections?

Some information were added to the manuscript in an attempt to elucidate these issues.

16. Can molecular typing (such as pulsed-field gel electrophoresis or spa typing) be performed on the S. aureus isolates specifically the MRSA isolates? This information would identify which strains are circulating within the region and if the strains are related.

No molecular typing was performed by problems in the laboratory. The PFGE was started but there were problems in the lab (lack or inactivation of enzymes breaking device) which prevented the completion of this part of the research. The absence of molecular typing was cited as a limitation of the study.