Author's response to reviews

Title: Persistence survey of Toxic Shock Syndrome toxin-1 producing Staphylococcus aureus and serum antibodies to this superantigen in five groups of menstruating women

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Version: 2 Date: 16 March 2010

Author's response to reviews: see over
Dear Drs. Alam and Cassidy-Cain

We appreciate the helpful comments from the reviewers regarding the manuscript “Persistence Survey of Toxic Shock Syndrome Toxin-1 producing Staphylococcus aureus and Serum Antibodies to this Superantigen in Five Groups of Menstruating Women” (1579702396300429). We have addressed their comments as follows with their concern presented first followed by our response. We have also addressed the questions from the editor and will start with those. We hope that these revisions have strengthened the manuscript and will allow its acceptance for publication in BMC Infectious Diseases.

Editor
Please modify the background section of your abstract to include some context for your study. The aim should appear as the last sentence in this section.

The Background of the abstract section was modified per the editor’s suggestion and now appears below.

Background
Menstrual Toxic Shock Syndrome (mTSS) is thought to be associated with the vaginal colonization with specific strains of Staphylococcus aureus TSST-1 in women who lack sufficient antibody titers to this toxin. There are no published studies that examine the seroconversion in women with various colonization patterns of this organism. Thus, the aim of this study was to evaluate the persistence of Staphylococcus aureus colonization at three body sites (vagina, nares, and anus) and serum antibody to toxic shock syndrome toxin-producing S. aureus among a small group of healthy, menstruating women evaluated previously in a larger

Please more explicitly state the competing interest for JEH.
The authors did not include anyone with the initials JEH, thus, we believe that the editors mean JEW. The following clarifying point was added
JEW: Hilltop Research received financial support for clinical work. JEW was employed as the clinical investigator and executed the clinical portion of the study

It was also noted that the second author M. Hansmann did not list her full name and initials as did the other authors.
This draft changes it to Melanie A. Hansmann.
Reviewer 1:
Recommendations for the Methods Section
It is not clear the criteria for sampling the groups…Maybe this is a limitation for the conclusion that there is no difference regarding the status of persistent or transient when the colonization sites are compared for this type of strain (toxigenic) and can introduce bias for data interpretation. As shown in the Table 2 the people in the nasal carriage subgroup (n=561) are more than the double of the people in both vaginal and anal group/subgroup (n=13 and n=12, respectively).

Clarification was added to the methods section as to the number of subjects in Group 1 of the follow up study. The following sentences were added to the manuscript: The total number of subjects who met the Group 1 criteria (S. aureus TSST-1 vaginal carriers) from the previous study was very low (33 subjects/3012 total subjects). Every attempt was made to recruit all Group I subjects. Some of the subjects could not be located and others did not wish to participate in this follow up study.

A table was added to the manuscript that provides details on the inclusion/exclusion criteria for the previous study and follow up study.

Table 2: Inclusion/Exclusion Criteria for the previous study and this follow up study

<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Had a regular menstrual cycles (minimum of 21 days and maximum of 35 days);</td>
<td>Had participated in another clinical study</td>
</tr>
<tr>
<td>Had used tampons at least occasionally</td>
<td>Were pregnant, actively trying to get pregnant or suspected they were pregnant</td>
</tr>
<tr>
<td>Refrained taking a bathe or shower within the 2 hours prior to their scheduled visits</td>
<td>Had a gynecological abnormality as judged by the study medical personnel</td>
</tr>
<tr>
<td>Refrained from using douching substances, vaginal medications, suppositories, feminine sprays, genital wipes, or contraceptive spermicides for 48 hours prior to their scheduled visits</td>
<td>Had an infection of the genitals within the past 6 weeks</td>
</tr>
<tr>
<td></td>
<td>Had been medically diagnosed as having diabetes, kidney failure, hepatitis, AIDS (HIV positive) or toxic shock syndrome</td>
</tr>
<tr>
<td></td>
<td>Were currently taking (within the last 30 days) immunosuppressive drugs, chemotherapy, systemic antimicrobial</td>
</tr>
</tbody>
</table>
Recommendations for the Results display

I believe that Table 2 should display the number of culture-positive among the group 4. I suggested also that not only percentages were displayed but also the number of subjects in which category of colonization (transient, intermittent, persistent).

Please note that the body of the text outlines the definition of the groups as transient, intermediate and persistent. A footnote was added to Table 2 regarding the definition of the carrier index “CI”. The number of subjects in each category was added to the table.

Recommendations for the Discussion section

I believe that would be interesting to emphasize that the find of at least 29.3% (17/58) of previous stated as non carriers showed to be positive during the follow up reinforces the concept that the state of carrier does not have steady behavior at the human body and can have huge variation along its life.

Excellent suggestion regarding carriage and will be added to the conclusion (Page 15– second paragraph). It is interesting that at least . . . . . was added to the manuscript.

I would like to comment that there are some cases in which the state of persistent carriage are in fact transient, because the patient have successive colonization by different strains. It was demonstrate by a genomic study using pulsed-field gel electrophoresis of Staphylococcus aureus strains from HIV patients. [Padoveze MC, de Jesus Pedro R, Blum-Menezes D, Bratfich O, Moretti ML. Staphylococcus aureus nasal colonization in HIV outpatients: persistent or transient? Am J Infect Control 2008; 36(3):187-91].

The reviewer makes an important note regarding colonization. The authors chose to characterize strains of S. aureus by the phenotypic production of TSST-1 since the focus was to investigate the colonization of S. aureus TSST-1 strains and their relationship(s) to serum antibody titers to this antigen over time. We did not include molecular characterization of the strain via techniques such as PFGE.

This concept of changing the pattern of colonization is also observed in the present study, pointed on page 12 when stated that there was 21 subjects became colonized with TSST-1 producing S. aureus by “converting” from non-toxigenic strains. The potential clinical usefulness of the results found in the study should be more explored in the Discussion section.

This is an excellent suggestion. The following sentence was added to the Discussion Section of the paper.
It is interesting that at least 29.3% (17/58) of previous stated as non carriers showed to be positive during the follow up reinforces the concept that the state of carrier does not have steady behavior at the human body and can have huge variation along its life.

**Recommendations for Conclusion section**

I recommend that data relating the category of colonization (transient, intermittent or persistent) with titers levels should be displayed in the Results section. Otherwise, the following statement on the page 15 seems to be not adequately supported: “From these findings, it appears that antibody titers in women found to be colonized with toxigenic *S. aureus* remained skewed toward higher titers whether or not the colonies were found to be persistent or transient in nature.

This suggests that colonization at some point in time is sufficient to elevate antibody titer levels and those levels appear to be persistent.”

We agree with Reviewer’s comments and information was added to the Discussion and the following figures were added to the manuscript.

Figure 1

**Antibody Titer Distribution for Groups 1 & 2**
**Figure 2**

*Antibody Titer Distribution for Groups 3 & 4*

**Recommendation regarding limitations statement**

I suggest the inclusion of comments on the criteria for sampling people to be recalled from the previous study, as the size of sample can be a limitation to the conclusions.

Subjects participated in the initial study and meet all of the inclusion/exclusion criteria. A table which summarizes the Inclusion/Exclusion Criteria was added to the manuscript. The subjects were recruited from more than 3000 subjects who participated in the previous study (JCM, 2005 citation). It should be noted that this is the largest study of its kind that has been published and that every effort was made to recruit a balanced study population among the five groups. Several years had passed since the initial study visit so it was difficult to find subjects since a large time span had passed (e.g. move, changed phone numbers, etc.).

**Reviewer 2**

Comment 1. The authors compare their study with the study and a review by VandenBergh et al. (1999). However, the present study is clearly different, because subjects were selected based on the presence or absence of a positive S. aureus strain; therefore, carriage rates cannot be compared with cross-sectional studies.
We thank the reviewer for noting this and the difference between our study and the VandenBergh study is stated in the first paragraph of the discussion section.

**Comment 2.**
*From the literature, it is already clear that* S. aureus *is carried in the nares. Therefore, the study of anal or vaginal carriage does not seem to add much to the existing literature.*

The authors disagree with the reviewer’s comment that the study of anal and/or vaginal carriage does not add much to the existing literature. The presence of the organism (*S. aureus* – TSST-1) vaginally is the initial step in the pathogenesis for menstrual Toxic Shock Syndrome therefore this is an important clinical aspect. The authors hypothesized that the gastrointestinal tract colonization was an important reservoir for subsequent vaginal colonization and chose to investigate that site as well.

See references below:


**Comment 3.** *As was recently demonstrated, classification can be simplified into either persistent carrier or others (intermittent carriers and non-carriers) (see Van Balkom et al. 2009).*

The authors agree that the classification is arbitrary classification but decided that the differences were important to express in this manner.
The following was added to the conclusion section of the paper: This reinforces the concept that the carrier state for the human is not always absolute and this has been noted by other investigators. Serum IgG levels against TSST-1 has also been found to be higher in persistent carriers than in non-carriers of *S. aureus*. (reference to van Belkum)

**Comment 4.** To identify whether TSST-1 positive strains interact with a person, one should determine T-cell expansions and not antibodies (see for instance Popa E. et al. Clinical Exp. Immunol. 2003).

This is a very interesting comment. Antibody is an accepted marker for exposure from an immunological perspective. This was the focus of the paper – the use of antibody titers to determine exposure to the TSST-1 toxin. Newer literature suggests the specific V beta repertoire expansion signature for different toxins. This would necessitate access to Tcells. These were not collected in the study nor identified to the participants in the informed consent document that this type of analyses would be completed.

**Comment 5: The presence of risk factors for *S. aureus* carriage such as psoriasis, diabetes mellitus, Wegener’s granulomatosis, intravenous drug abuse, is not reported in the subjects that were induced in the current study.**

The authors agree with reviewer. We added a table explaining/clarifying the inclusion/exclusion criteria for the previous study and this follow up study.

**Comment 6: The use of antibiotics before or during the study is not reported.**

We added a table explaining/clarifying the inclusion/exclusion criteria for the previous study and this follow up study. See Table 2 that was added to this version of the document.

**Comment 7. The purpose of the study of anti-TSST-1 antibodies is not clear. What do the authors want to demonstrate?**

The objective was stated is the background section of the manuscript. Seroconversion is an important aspect of the primed immune system against infectious agents and/or toxins. Seroconversion correlated with actual exposure to the toxin has not been previously noted in the literature and this paper serves as providing information regarding this important mechanism associated with protection from developing menstrual TSS. This was added to the background section of the abstract.

**Comment 8. Unfortunately, data on antibody titres are not provided. Could a figure clarify findings?**

The authors agree with reviewer. Please see the addition of Figures 1 and 2 per Reviewer 1 comment.
Comment 9. During the observation period only one subject converted from antibody-negative to antibody-positive. In this subject TSST-1 positive S. aureus strain was cultured. Whereas the authors claim that there were no TSS-like symptoms, it is also of interest whether this subject (and the other 4 who seroconverted before the start of the study) did have any other disease (bacterial and/or autoimmune).

The authors agree with reviewer. Subjects meet Inclusion/Exclusion criteria. We added a table explaining/clarifying the inclusion/exclusion criteria for the previous study and this follow up study.

Comments 10. In the discussion the effect of the vaginal environment for TSST-1 positive S. aureus carriage is only briefly discussed without citations. This part should be improved.

The authors agree with reviewer and citations 19, 20, 21 & 22 were added to paragraph 3 of discussion.

Comment 11. What was the intra- and inter-observer variation of obtaining vaginal and anal swabs for the presence of S. aureus?

A video was developed prior to the start of the study by a physician to provide training to the clinicians for consistent sample collection and for quality sample collection. One laboratory conducted all analyses for S. aureus isolation and identification. A second laboratory conducted all analysis for antibody titers. The same technicians completed the assays.

Comments 12. What was the inter- and intra-observer variation for the antibody test?

All antibody titers were conducted at one laboratory with one laboratory technician who had the expertise for conducting the analysis.

Comments 13. Numbers in table 1 and table 2 are hard to compare. For instance, it is stated that in group 2 78 subjects were enrolled and 64 subjects completed the study, whereas in table 2 data of 61 subjects are reported.

Table 2 included only those subjects who completed a minimum of 3 visits as stated in the header.