Author's response to reviews

Title: Detection of Group A Streptococcus in Tonsils from Pediatric Patients Reveals High Rate of Asymptomatic Streptococcal Carriage.

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Author's response to reviews: see over
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*BMC Pediatrics*

RE: MS 1210872181587310

To whom it may concern:

On behalf of myself and the other authors, I include the resubmission of the manuscript entitled: “Detection of Group A *Streptococcus* in Tonsils from Pediatric Patients Reveals High Rate of Asymptomatic Streptococcal Carriage,” for publication in *BMC Pediatrics*. In submitting this work, I assert that the manuscript has not been submitted elsewhere for publication and that all authors have contributed to and approve of the work.

Kindly, the Editor had some remaining comments that we needed to address. We thank the Editor for the comments and believe that we now have a better manuscript because of them. A point-by-point response to the reviewer comments follows this letter.

Please do not hesitate to contact me if I can provide further information or if a list of potential reviewers is needed.

Sincerely,
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Editor's comments:

A) The authors have revised the conclusions to include that: 1) GAS carriage may persist in patients long after symptoms have passed despite the use of antibiotics to treat recurrent GAS pharyngitis, 2) high rates of asymptomatic GAS carriage contribute to the failure to identify viral pharyngitis due to positive detection of GAS in virally infected children and 3) the results support a hypothesis that GAS carriage may contribute to tonsillar hypertrophy and the development of clinical syndromes previously unassociated with GAS." The conclusion also states "Now that we have established the ability to rapidly detect the presence of GAS within the tonsillar crypt..."
Nothing is presented in the manuscript to support any of these assertions - the manuscript should be revised to reflect the actual experiments that were performed.

Response: Thank you for this valuable input. We have now included any conjecture in the Discussion. The Conclusions now read, “Our study revealed the presence of GAS within the tonsillar reticulated crypts of approximately one-third of children who underwent tonsillectomy for either adenotonsillar hypertrophy or recurrent GAS tonsillopharyngitis at the Wake Forest School of Medicine.”

While we believe we have presented strong evidence for the presence of GAS biofilms, we have removed this from the conclusions and discussed this possibility in the Discussion. This ties into the Editor’s second comment.

B) The comment "Whether or not the bacteria observed in biofilms are S. pyogenes is a critical point. Confirmation of the identity of these bacteria by culture or amplification of a specific gene (e.g. emm) would greatly strengthen the paper¹s conclusions" has not yet been addressed.

Response: Originally, we were confused by this comment because we thought the Editor, and the Reviewers, were suggesting the immunofluorescent detection of GAS was somehow deficient. The anti-GAS antibody only recognizes GAS and not other streptococcal groups. We now take the comment to mean that we have not directly shown that the cocci in the SEM and Gram-stain images are GAS. While these tonsils were shown to be positive for GAS by immunofluorescence, it is true that we cannot rule out that we are seeing another Gram-positive community in these images. We have not undertaken amplification of the emm gene or culture because while this would confirm the presence of GAS, we cannot directly test the Gram-stained or SEM samples because they have already been processed. Thus, we would confirm that the tonsil is positive for GAS, but not the image. Immunogold staining would allow us to prove the SEM samples were positive, but we are not positioned to undertake these experiments at this time. Thus, rather than conclude GAS were present in biofilms, we instead present the data as strong evidence that GAS biofilms were present. This does not diminish the impact of the paper in our opinion.

If the Editor would like to further discuss this point, I would be happy to do so.