Title: Detection of Group A Streptococcal Biofilms 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 Streptococcal Biofilms 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.

Overall, the reviewers had a very favorable impression of the manuscript and made some thoughtful suggestions for improvement. We thank the reviewers for these suggestions, and they have been incorporated in the revised manuscript. 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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Reviewer 1:

1) The conclusions in the Abstract are not obviously correlated with the conclusions of the paper and this should be improved.

We agree and the conclusions in the Abstract have been reworded to closely match those of the manuscript.

2) The identification of the Group A Streptococcal biofilm has been carried out in the present paper using a quite complex, though rigorous, technique. Why didn’t the Authors use the Confocal Laser Microscopy?
Frankly, the microscopy took a significant amount of time. Use of the Confocal is on a fee for use basis and we have a Nikon Eclipse TE300 Light Microscope free for use in our lab. We may consider the use of confocal microscopy in the future. We note that the reviewer does not suggest confocal microscopy is required.

3) The final point is that the clinical implications of the findings are in my mind not very clear and should be better outlined and discussed. Should we treat all GAS carriers? and if yes how? Do we have less invasive diagnostic instruments to indentify biofilms?

Although the clinical implications from this finding are not yet clear, future work that builds on these observations may ultimately identify methods to diagnosis GAS colonization and differentiate it from GAS tonsilopharyngitis and possibly an effective treatment for GAS colonization. The following new text appears in the discussion:

“Specifically, our findings contribute to an understanding of GAS tonsillar colonization. Developing the capacity to distinguish patients with GAS tonsillopharyngitis from those with GAS colonization, or those with GAS colonization and viral tonsillopharyngitis is a clinically important goal that could greatly reduce unnecessary antibiotic use. This work may ultimately contribute to the development of clinically useful methods for identifying patients with longstanding GAS colonization.”

Reviewer 2:

1. Please address: has this antibody been previously used for immunofluorescence?

Yes. This has been noted in the Methods. Specifically, the antibody was used in this study: Roberts AL, Connolly KL, Doern CD, Holder RC, Reid SD: Loss of the group A Streptococcus regulator Srv decreases biofilm formation in vivo in an otitis media model of infection. Infect Immun 2010, 78(11):4800-8.

2. Please address if this antibody has any cross reactivity specifically for groups C, F and G streptococci.

The antibody is not cross-reactive with groups C, F, and G or other streptococcal species. This has been clarified in the Methods.

3. Authors should address that the cohort was an inherently biased sample- and findings may not be generalizable to pediatric GAS carriers that do not require adenoid/tonsillectomy.

We agree. The following text has been added to the Methods when the cohort is first described in detail: “It should be noted that we did not have access to samples from patients not requiring tonsillectomy. Thus, the cohort is biased and findings may not be applicable to pediatric GAS carriers that do not require such surgery.”