Title: Streptococcus intermedius causing infective endocarditis and abscesses: a report of three cases and review of the literature.

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Author's response to reviews: see over
Reviewers' Comments:

REVIEW 1

1. The title of the manuscript is somewhat audacious. It suggests extensive molecular analysis a spectrum of clinical cases. However, no molecular data is presented and the authors only mention the results on page 7. With only 3 cases the spectrum of disease also is quite limited. Please change the title into a more fitting one.

RESPONSE: We appreciate the reviewer’s comments regarding the title and have changed the title to better fit the scope of the manuscript.

2. The authors do not describe how the 16S-gene sequence was determined. What kind of primer set was used for PCR and what part of the 16S rRNA gene was sequenced? No extensive description is required, but the size and location of the analyzed product is important to assess the validity of the molecular identification.

RESPONSE: The primer set used was 8F and 1492R and a 647 bp fragment of the 16S rRNA gene was amplified. We have made this change in the manuscript. We are not sure what the reviewer means regarding the location of the sequence. We did find that our partial sequence was 100% identical to the S. intermedius strain in GenBank and can deposit our sequence in GenBank if it is desired.

3. The authors have used RAPD profiles to assess a possible epidemiological link. The patterns obtained are not shown, but apparently they were similar yet distinct. Based on this result the authors conclude that the cases were unlinked. RAPD is not a reliable genotyping technique, the method is not robust. How can the authors exclude differences in the profiles due to experimental difficulties? How was the method validated?

RESPONSE: We respect the reviewer’s comments. We could have used a more reliable genotyping technique to look for an epidemiological link. We, therefore, decided to delete this part of the manuscript and focus more on the different clinical manifestations of S. intermedius.

REVIEW 2

1. Does any other bacteria were isolated and in particular anaerobes? this must be mentioned, since anaerobes are frequently associated with milleri streptococci in abscess?

RESPONSE: No other bacteria were isolated from the cultures including anaerobes in all three cases. This was added to the descriptions of all three cases.
2. In cases 1 and 2, the strains are resistant to erythromycin and clindamycin, the presence of an erm-related gene (erm A or ermB) is probable; this could be easily confirmed by PCR using specific primers. Moreover, what is the susceptibility to tetracycline for these two strains, as it is well known that among streptococci, tetracycline and erythromycin resistant determinants are genetically linked on the same genetic mobile element such as a Tn916 related conjugative transposon. This must be added in the revised manuscript.

RESPONSE: Tetracycline was not tested in any of the three isolates and this was added in the manuscript. It apparently is not part of the routine antibiotics tested for viridans group streptococci in our hospital’s laboratory. It would be interesting to see if there is an erm-related gene in the isolates in light of the antibiogram of the strains; however, it is out of the scope of our manuscript as we focused more on the clinical manifestations of the infection.

3. It is mentioned that partial sequencing of 16rRNA encoding gene was performed, this is relatively imprecise and the coordinates of the primes used to amplify and sequence should be indicated.

RESPONSE: As stated above in the first reviewer’s comments, we used the 8F and 1492R primer set and produced a 647 bp 16S rRNA encoding gene fragment which was identical to the reference strain in GenBank. This has been added. If desired, we can deposit our sequences to GenBank.

4. The photo of the RAPD performed on all strains should be provided instead of the Gram staining showing chains of streptococci.

RESPONSE: In changing the scope of our manuscript, we deleted the section that discussed the possible epidemiological link of our cases and the RAPD PCR performed. We feel the picture of the gram stain showing streptococci reiterates the different clinical manifestations of S. intermedius infection and reflects its tropism for brain tissue.

5. In general, this paper is too long and could be transformed as a note or a letter, and all figures could be deleted since they do not provide major information.

RESPONSE: We understand that the manuscript describes the three cases in detail and has a review of the literature. However, we feel that our manuscript depicts the different infections S. intermedius can cause in otherwise healthy individuals. It is also the only case of infective endocarditis confirmed by gene sequencing. Shortening the manuscript or deleting the figures would not stress the severity and range of infection S. intermedius can cause.