Reviewer's report

Title: Genotypic diversity and phenotypic traits of Streptococcus mutans isolates and their relation to severity of early childhood caries.

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Reviewer: Georg Conrads

Reviewer's report:
OHEA-17-00036: In their manuscript entitled „Genotypic diversity and phenotypic traits of Streptococcus mutans isolates and their relation to severity of early childhood caries.” the authors isolated and stratified S. mutans genotypically and phenotypically (acidogenicity, acidotolerants, biofilm-production) originated from 10 (controls), 9 (EC) and 8 (S-ECC) children.

General comment:

This is an interesting and well written study.

You should discuss why your collection of strains really represent the SM genotype-richness in the oral cavity, especially as you did not take samples from caries directly. Something like a rarefaction analysis should be done to answer the question: how many colonies do you have to pick before finding almost no new genotype of SM or MS? Now you picked "up to six", why not more and why not the same number from every sample?

Some details:

Abstract:
"Genotypes…from CA…formed more biofilm" or "Genotypes…from S-ECC…were more acid tolerant": It is clear what you mean, but from the biological perspective these sentences sound
strange as you are directly attributing phenotypical reactions (biofilm/acid) to a genotype. It also sounds very categorical; I guess there were some exceptions and was some deviation. Please rephrase more accurate ("The strains with genotypes more characteristic for CA children formed…etc.).

Introduction:
Line 82: "Only few studies evaluated genetic diversity of S. mutans from ECC children [8, 11]." Please include the recent study by Momeni et al. Journal of Microb. Methods 2016 including one cohort of 90 children aged 9-18 months.

Materials & Methods:
Line 106: you mean "patients received dental.."?
Line 113: "No biofilm was collected from caries cavities." What is the ratio behind the exclusion of the caries-attached biofilm? Are you not afraid of missing most important strains/genotypes?
Line 119: "mutans (written in italics)streptococci" change to "mutans streptococci": italics for species name only; here, the group is meant.

Line 139: "The DNA was resuspended" please correct

Results and Discussion:
Line 240: "Fifty-nine out of 98 S. mutans (21 CF, 20 ECC and 18 S-ECC) were re-isolated and genotyped by AP-PCR." Why this selection? Why exclusion of 39 strains (dupicates, did not grow, looked similar)?

Line 254ff: "However, genotypes of S-ECC children presented higher counts of viable cells than CF and ECC genotypes at pH 2.8 at time zero and time 60": The difference at time 60 is due to acid-exposure but how do you explain the difference at baseline (T = 0)? Please discuss.

Line 341ff: "…we showed that the detection of S. mutans in biofilms increased depending on severity of dental caries, since all MS isolates from S-ECC children were positively identified as S. mutans." I do not see this in your data comparing MS-isolates (38 for CF, 35 for ECC and 36
for S-ECC) versus selected SM-strains (21 CF, 20 ECC and 18 S-ECC). There is a difference of 18 strains but you made further selection (exclusion of 39 strains).

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.
Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.
Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
No

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