The authors wish to express their thanks to the referees for the helpful comments pertaining to the review of the manuscript entitled “Genotypic diversity and phenotypic traits of Streptococcus mutans isolates and their relation to severity of early childhood caries”. Below are our responses, with precise details, to each comment made by the referees. We are secure that the modifications made in the manuscript based on those comments significantly improved its quality and adequacy to the Journal’s format. All modifications on the manuscript are marked in yellow for the reviewer 1.

Editor Comments:
In this study, the authors investigated the relationship between severe early childhood caries and characteristics of S. mutans clinical isolates. A focus on this relationship will be of interest to the
readers of the journal. As the comment of reviewer 1, the reasons for selection of S. mutans clinical strains and additional detailed information about of in vitro assays using these strains should be added.

Response: Thanks for the opportunity to answer the reviewer’s questions. Follow below the responses for the reviewer 1.

Reviewer reports:
Georg Conrads (Reviewer 1): 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 represents 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?

Response: Thanks for your questions. We collected up to six colonies with MS morphology for each child. However, some of these strains did not grow well on BHI broth and others were discarded because Gram-staining showed that these cultures were not pure. Therefore, for some patients, less than 6 colonies were isolated and frozen for posterior genetic analysis. We worked with 6 colonies for each patient based in the results of previous studies (Li & Caufield, 1995; Emanuelsson & Thornqvist, 2000; Klein et al., 2004; Moser et al., 2010; Cheon et al., 2011; Cheon et al., 2013), which found a limited diversity of S. mutans in dental plaque or biofilm (mean genotypes less than 2 per child). Additionally, in this present study, children had complete deciduous dentition and several studies have demonstrated that S. mutans infections are established in the early stages of dentition development and remain stable for several years
(Alaluusua et al., 1994; Emanuelsson & Thornqvist, 2000; Klein et al., 2004). Cheon et al. (2011) showed that 7 S. mutans isolates would provide 83% power of identifying up to 3 genotypes in younger individuals (5-6 years), close to the 6 isolates proposed in this present study evaluating very young children (3-5 years). We inserted the paragraph described below in the Discussion (page 11) to comment genetic diversity of S. mutans observed in the present study.

A limited number of S. mutans genotypes (53.6% of children with 2 genotypes and 35.7% with one genotype) was observed in this present study. This result is consistent with other reports (Emanuelsson & Thornqvist, 2000; Klein et al., 2004; Cheon et al., 2011; Cheon et al., 2013), which found less than 2 genotypes in dental plaque per child. Additionally, in this present study, children had complete deciduous dentition and several studies have demonstrated that S. mutans infections are established in the early stages of dentition development and remain stable for several years (Alaluusua et al., 1994; Emanuelsson & Thornqvist, 2000; Klein et al., 2004).


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.").
Response: Thanks for your suggestion. I changed the sentences on page 2 (Abstract).
S mutans strains with genotypes more characteristic for ECC and S-ECC children formed more biofilms than those identified in CF children. The strains isolated from S-ECC children were highly acid tolerant.

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.
Response: Thanks for your suggestion. I included the recent study published by Momeni et al. (2016) on page 3 (Introduction).


Materials & Methods:

Line 106: you mean "patients received dental…"?
Response: Thanks for your question. We explained better what kind of treatment children with
dental caries were submitted. See on page 4. All children with dental caries were submitted to
restorative/surgical treatment performed by a pediatric dentist, after samples collection.

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?
Response: Thanks for your observation. When we commented that no biofilm was collected
from caries cavities, it means that there was no contamination of our samples from intact enamel
surface around dental caries with biofilm presented inside caries cavities. Samples from caries
cavities were also collected separately and frozen, but S. mutans strains were not isolated at that
moment for financial reasons. The studies that focused on diversity of S. mutans in dental plaque
have been used mesial, distal, buccal, lingual and occlusal surfaces of primary teeth to collect
pooled plaque samples, excluding caries cavities (Emanuelsson & Thornqvist, 2000; Klein et al.,
2004; Cheon et al., 2011; Cheon et al., 2013). Cheon et al. (2011) collected both types of biofilm
samples: dental plaque by passing a sterile cotton swab over the teeth and bacterial plaque
samples from caries lesions of children and analyzed separately.

See the sentence on pages 4-5. “No biofilm was collected from caries cavities in order to avoid
contamination with S. mutans strains harboring this environment”.

Line 119: "mutans (written in italics) streptococci" change to "mutans streptococci": italics for
species name only; here, the group is meant.
Response: Thanks for your observation. We corrected the term “mutans streptococci” on page 5.

Line 139: "The DNA was ressuspended" please correct
Response: Thanks for your observation. We replaced for “resuspended”. See on page 5.

Results and Discussion:
"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 (duplicates, did not grow, looked similar)?

Response: Thanks for your questions. We defrost all 98 S. mutans strains, but some of them did not grow. Then, we decided to randomize S. mutans strains, which had grown in order to obtain a representative number of strains per group – CF, ECC and S-ECC.

"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.

Response: Thanks for your observation. We revised the statistical analysis of data from aciduricity assays. We considered the percentage of bacterial growth on time 60 in relation to time zero (100%) in pH 7.0, pH 5.0 and pH 2.8. The same analysis was made by Damé-Teixeira et al. (2014). We replaced the Figure 3 and modified the text of Material and Methods (page 8) and Results (page 10).


"...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).

Response: Thanks for your observation. There was not a direct comparison between MS-isolates (total of 108 isolates) and SM-strains (total of 59 strains) because we randomly selected 21 CF, 20 ECC and 18 S-ECC SM-strains from 98 SM-isolates. The comparison was made only among the groups: CF, ECC and S-ECC considering each situation separately: MS-isolates and SM-strains.