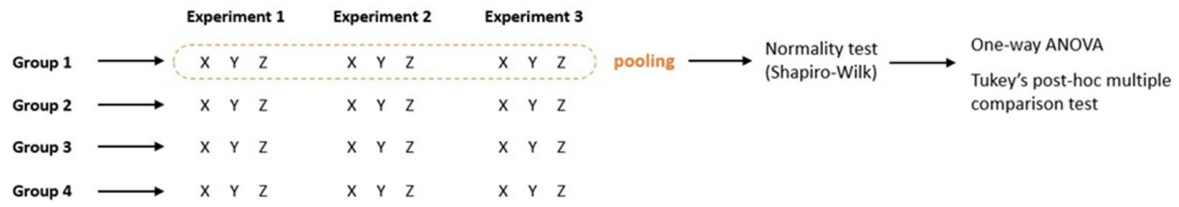
Responses to Reviewer 2's comments

We thank the Reviewer for their valuable comments and critiques. Please find below a point-by-point response to comments and critiques. We have highlighted the modifications in the revised manuscript with yellow color.

Reviewer 2

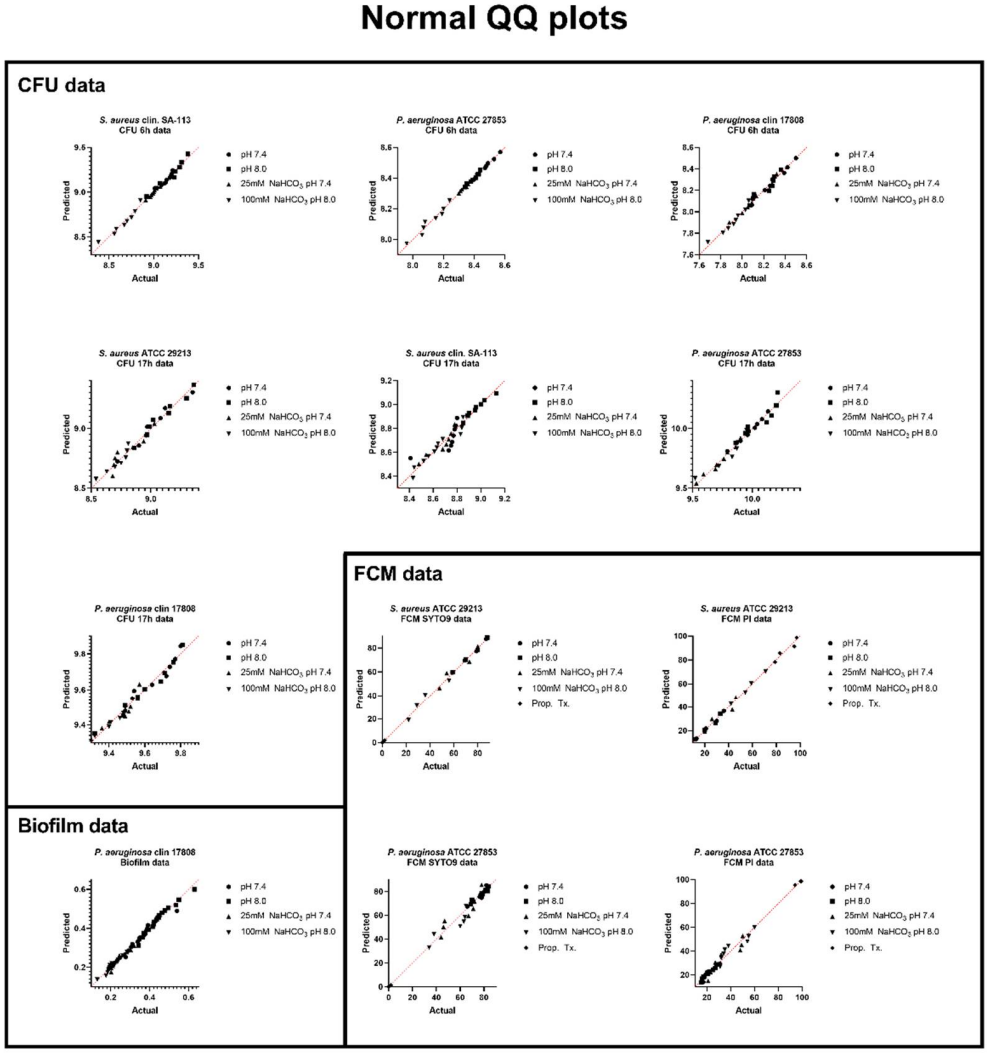
Most of the experiments have been done with only 3 replicates. This small number of replicates makes it impossible to determine whether the data are normally distributed, and consequently, their presentation as averages and standard deviations is invalid. On the other hand, the experimental groups are compared with a poor defined "one-way ANOVA and multiple comparison test". I should point out that the test of variances (ANOVA) is designed for normally distributed samples. In addition, there are a dozen different post-hoc multiple comparison tests; it would be important to specify which one has been used

We have made a clarification on the statistical analysis in the manuscript. In all experiments, we performed at least three independent experiments. In each treatment group for the CFU and flow cytometry experiments, the samples were always tested in triplicates, whereas for the biofilm crystal violet assay five parallels were used, with the exception of the CFU assay for *S. aureus* ATCC 29213 at 6 h having only one sample in each experiment.



All data (except for CFU assay *S. aureus* ATCC at 6 h) in each treatment group were pooled, so the total numbers of the samples were at least 9 replicates per treatment group. The pooled data were tested with Shapiro-Wilk test for the data distribution (see below). The means of each group were compared using one-way ANOVA, followed by Tukey's post-hoc multiple comparison test.

Data from the CFU assay *S. aureus* ATCC at 6 h (3 replicates) were analyzed by using Kruskal-Wallis test, followed by Dunn's post-hoc multiple comparison test.



In lines 51-62 it is emphasized that the presence of bicarbonate is necessary for the secretion of mucus. In reality, Garcia et al. Affirm that bicarbonate is essential for post-secretory mucus modification, and that bicarbonate efflux is necessary to maintain optimal conditions of secretion -a fluid mucus in the ASL-, and not that it conditions secretion itself.

We remove the sentence ("Similar findings have been reported in mouse small intestine *ex vivo* [Garcia *et al*., 2009; Gustafson *et al*., 2012]."") from the manuscript in order to reduce vague connections. We also specify that Garcia et al. reported that PGE_2 or 5-HT stimulated mucus release was reduced by approximately one half in the absence of HCO_3^-.

The experiments have been done with 0, 25 and 100 mM of bicarbonate. The concentration of bicarbonate in ASL is between 10 and 25 mM, while in CF patients it is between 5 and 8 mM. Values of 0- and 100-mM bicarbonate do not make much sense in the description of

CF. Although experimentally they make sense, it would be necessary to justify the choice of these concentrations in terms of the pathophysiology of CF.

We choose 0 mM HCO_3^- as control condition to assess the effects of 25 and 100 mM bicarbonate. 100 mM HCO_3^- was chosen because this concentration is well-tolerated by airway epithelial cells (Gróf *et al. Int J Mol Sci*. 2020). Furthermore, we speculate that inhalation of hypertonic (4.2 or 8.4%) $NaHCO_3$ aerosols might increase HCO_3^- concentrations temporarily up to these levels in the airways (Gomez *et al. Clin Drug Invest*. 2019).