This work investigates the impact of different aerosolization techniques on E.coli within an atmospheric simulation chamber. The topic is surely interesting since it is relevant to many experimental protocols. Also, the analysis is quite thorough and bacteria viability is analyzed in multiple ways to better assess the impact of the aerosolization techniques. The reviewer suggests publication of the paper with only some minor modification as expressed below:

P. 3, Line 85: I suggest reporting also the aerodynamic diameter of E. coli (which should be smaller than 2 um) to make clearer the following choice of 0.5 um as a cut-off for the fragmentation range.

Thanks for the comment. We replaced the typical size with the aerodynamic diameter of *E. Coli* and rephrased as: with an aerodynamic diameter of around 1 μm (Zhen et al., 2014b)

P.5-6: Were the aerosol generators cleaned between replicates to avoid a carry-over effect in bacterial cells?

Thanks for the comment. We added at row 168 the following sentence: To avoid cross-contamination between replicated experiments, at the end of each experiment, the two nebulizers were cleaned with a solution of Ethanol and MQ (70-30%) and rinsed with MQ.

P.5-6: While the droplet diameter of the FMAG is reported (0.8-12 um) the one from the SLAG is not, is there an approximate size of the droplets and is it compatible with E.coli size?

The SLAG is intrinsically designed to generate a polydisperse aerosol through a bubbling and film–jet breakup mechanism. For this reason, a single or nominal droplet diameter is not defined nor reported by the manufacturer, unlike the FMAG, which is specifically designed to produce monodisperse droplets. Consequently, the droplet size generated by the SLAG cannot be uniquely quantified.

P. 9: The WIBS peak is centered around 0.8 um vs. the OPS peak at 0.5 um. Couldn't this be simply an effect on how the different samples define the particles' binning?

We agree with the reviewer that the difference in the peak position is primarily related to the different lower size detection limits and binning schemes of the two instruments. In particular, the OPS measures particles starting from 0.3 μm, while the WIBS detection range begins at 0.55 μm. As a consequence, particles contributing to the smaller-size bins are only detected by the OPS, which shifts the apparent modal diameter toward smaller sizes compared to WIBS.

P. 9: Beside peak shifting WIBS seems to exhibit a higher (i.e.: non-overlapping error bars) maximum normalized concentration compared with OPS. Is this related to total particles counted by WIBS or to fluorescent-only particles? If so is that explainable by the dimensional shift or what alse could explain a higher number of fluorescent particles vs. total ones?

Thanks for the comment. Since only bacteria suspended in MQ are nebulized, all particles observed by WIBS are of biological origin; therefore, total and fluorescent particles counts measured by WIBS coincide. This difference can be attributed to the different size ranges of the two instruments, as explained in the previous comment. This difference in detectable size range and binning can therefore influence the shape and height of the normalized size distributions.

P. 10. Figure 3 is missing x-axis description (Midpoint bin (um)).

Thanks for the comment. We modified the figure following the suggestion, thanks

10P. 14, L. 297-301. While the presented study is surely valuable and a thorough characterization, it is far from being the first step in this field. For example Thomas et al. (2011) already provided data on survival and site of damage of E. coli nebulized with different techniques. Rather, I think that the major

strength of this work is the completeness of the analysis which goes way beyond simple viability (in a culturable sense). I suggest a rephrasing of this sentence.

Thanks for the comment. We added the reference to the manuscript and, considering also the comments of the other reviewers, we modified the conclusions as:

Line 337: This study demonstrated the effect of two different aerosol generators on the aerosolization of bacteria, not only on culturability but also on viability. The compact volume of the chamber represents a choice that prioritizes experimental control and reproducibility over direct representativeness of indoor environments. While larger systems, such as biological safety cabinets or full-scale chambers, allow longer aerosol residence times and closer analogy to ambient indoor conditions, they also introduce additional complexity and variability. The present setup was designed as an intermediate-scale system optimized for comparative evaluation of aerosol generators, rather than for exposure simulation. By isolating generator specific effects under controlled conditions, this work provides a basis for selecting aerosolization techniques in laboratory bioaerosol studies and for interpreting bacterial viability measurements in more complex atmospheric simulations. Future work will extend this approach to Gram-positive bacteria, in order to investigate how differences in cell wall structure may influence bacterial response to nebulization in terms of viability, culturability, and fragmentation.

Finally, this is not a comment on the quality of the paper itself, just an potential outlook for future work. Gram negative and gram positive bacteria differ in their structure, it would be interesting in the future to see if this also translates in differences when nebulized (in terms of viability, etc.).

We thank the reviewer for this valuable and forward-looking suggestion. We fully agree that structural differences between Gram-negative and Gram-positive bacteria, particularly in cell wall composition and thickness, may lead to different responses to nebulization-induced stress, potentially affecting viability, culturability, and fragmentation.

While the present study focuses on *Escherichia coli* as a well-established model organism, extending the analysis to Gram-positive bacteria represents a natural and highly relevant direction for future work, which we plan to address in subsequent studies.

We have added the following sentence to the Conclusion section:

Future work will extend this approach to Gram-positive bacteria, in order to investigate how differences in cell wall structure may influence bacterial response to nebulization in terms of viability, culturability, and fragmentation

Cited literature:

Thomas RJ Webber D, Hopkins R, Frost A, Laws T, Jayasekera PN, Atkins T.2011.The Cell Membrane as a Major Site of Damage during Aerosolization of Escherichia coli . Appl Environ Microbiol. 77:.https://doi.org/10.1128/AEM.01116-10