This manuscript "Measurement Report: Effects on viability, culturability, and cells fragmentation of two bioaerosol generators during aerosolization of E. coli bacteria" provides valuable insights into bioaerosol sources for simulation chamber–based research. However, several major concerns remain. The authors are encouraged to address the following recommendations to strengthen the clarity, rigor, and interpretability of the manuscript.

Major points:

1) From the title and abstract, I understood that this study aims to provide a comparative investigation of two bioaerosol generators: the Sparging Liquid Aerosol Generator (SLAG) and the Flow Focusing Monodisperse Aerosol Generator (FMAG). However, the manuscript primarily evaluates the performance of the overall system, which includes aerosol generation, aerosol residence within the chamber, and subsequent bioaerosol sampling. As a result, the manuscript lacks a step-by-step evaluation that would help clarify which specific process(es) may be responsible for the observed effects on bacterial viability.

For example, lines 61–68 describe various stresses associated with the use of nebulizers; however, the nebulization step itself should be more explicitly isolated and evaluated.

We thank the reviewer for this thoughtful comment. We agree that the experimental setup evaluates the bioaerosol generation process as a system, including aerosolization, resident time within the chamber, and subsequent sampling, rather than isolating each step independently. Our primary objective was to perform a comparative assessment under similar and realistic experimental conditions, representative of typical chamber-based bioaerosol studies, where aerosol generation, transport, and collection are coupled. For this reason, the performance of SLAG and FMAG was evaluated at the system level rather than through fully decoupled step-by-step tests. Nevertheless, several elements of the experimental design allow us to disentangle the dominant processes responsible for the observed effects on bacterial viability. In particular:

- The stability tests performed on *E. coli* resuspended in MQ water (Fig. 2) demonstrate that neither the suspension medium nor the experimental time scale significantly affects the viability or the culturability of bacteria.
- The aerosol resident time inside the chamber is short for both generators due to the small chamber used and the sampling flow is the same; therefore, the effect of hardware configuration can be considered the same for both nebulizers, and the results can be compared in relative terms.
- The differences observed in particle size distributions and fragmentation rates, especially the presence of a submicron fragmentation mode in SLAG but not in FMAG, provide evidence that mechanical stress during nebulization is an important contributor to bacterial damage.

Based on these observations, we conclude that the comparative approach adopted here allows us to identify aerosol generation as the primary process influencing bacterial viability, with distinct stress mechanisms associated with the two generators.

To better explain the goals of our work, we modified the end of the introduction, and we added a new paragraph to the Results and Discussion section as follows:

Line 68-74: Although bacterial aerosolization involves multiple sequential stages, including nebulization, aerosol transport, chamber residence, and sampling, these processes are coupled in

atmospheric simulation experiments. Therefore, this work adopts a comparative approach, evaluating the two generators under similar and controlled experimental conditions representative of typical chamber-based bioaerosol studies. This methodology enables the identification of generator-specific impacts on bacterial integrity and performance, while preserving experimental relevance for laboratory and simulation chamber applications.

Line 187: "Methodological framework for data interpretation

The experimental setup, while not permitting a fully decoupled, sequential quantification of each process in bacterial aerosolization, includes numerous design characteristics that facilitate the identification of the primary stressors impacting bacterial survival. The current setup links aerosol generation, chamber resident time, and sampling, mirroring operational settings of atmospheric modeling research. Bacterial culturability and viability were independently assessed in the nebulized solution. Short chamber resident times were ensured, and identical sampling flow was applied for both aerosol generators. In addition, particle size distributions and fragmentation patterns were systematically examined. Under these controlled conditions, the principal differences observed between SLAG and FMAG can be mainly ascribed to the aerosolization phase. This framework provides a basis for interpreting the following results and for identifying generator-specific effects on bacterial integrity. Potential stress associated with aerosol resident time in the chamber and with the Biosampler sampling is therefore considered a common background contribution, equally affecting all experiments and not biasing the comparative evaluation of the two nebulizers."

In addition, the remaining liquid within the nebulizer reservoirs after aerosolization should be examined more carefully to assess potential impacts of the nebulization process on bacterial viability.

In the SLAG operating principle, only a fraction of the liquid film disrupted by the air jets is converted into aerosol. Larger droplets generated during the bubbling process revert to the liquid reservoir and are not reintroduced into the aerosol flow and, therefore, don't contribute to the airborne bacterial population. As a result, the residual liquid in the SLAG reservoir is not representative of the aerosolized fraction and cannot be directly used to assess aerosol-phase stress or viability loss. To explain this sentence, we added to the manuscript the following sentence:

Line 148: The larger droplets, generated during the process, gravitationally return to the liquid reservoir and are not aerosolized, nor reintroduced into the circulation.

2) The physiological status of the bacteria at each experimental step should also be considered more carefully. For instance, lines 290–291 mention … the bacteria probably tend to cluster, leading to droplets devoid of individual bacteria…. Such clustering could lead to prolonged bacterial viability, as cells located within aggregates may be shielded from environmental stresses. This protective effect could also result in higher CFU/mL values. Further clarification and discussion of this possibility would strengthen the interpretation of the results.

We thank the reviewer for this comment. We agree that bacterial clustering may influence physiological status and survival during aerosolization. This aspect has now been explicitly discussed in the revised manuscript

Line 260: The cell aggregation, occurred during the nebulization, may experience partial shielding from environmental stresses such as dehydration and mechanical damage, potentially enhancing survival

compared with isolated single cells (Flemming and Wingender, 2010; Tang, 2009; Vejerano and Marr, 2018).

Flemming, H.-C. and Wingender, J.: The biofilm matrix, Nat Rev Microbiol, 8, 623–633, https://doi.org/10.1038/nrmicro2415, 2010.

Tang, J. W.: The effect of environmental parameters on the survival of airborne infectious agents, J R Soc Interface, 6, https://doi.org/10.1098/rsif.2009.0227.focus, 2009.

Vejerano, E. P. and Marr, L. C.: Physico-chemical characteristics of evaporating respiratory fluid droplets, J R Soc Interface, 15, 20170939, https://doi.org/10.1098/rsif.2017.0939, 2018.

3) The nebulization and sampling periods (20–30 minutes) may themselves contribute to bacterial damage. Prolonged aerosol residence in the chamber, as well as high-velocity airflow into the BioSampler liquid accompanied by vortex motion, may impose additional mechanical stress on bacterial cells. These potential effects should be considered when interpreting the results, as part of an evaluation of the system as a whole.

We thank the referee for this comment. We agree that both aerosol resident time within the chamber and the sampling process itself can contribute to additional mechanical stress on bacterial cells during experiments and should be considered to interpret the results.

To take into account this comment (and comment 2), we added the new paragraph “Methodological framework for data interpretation” as reported above.

Minor points:

Line 92–93: The manuscript states that 20 mL of bacterial suspension was centrifuged at 5000 rpm for 10 minutes. Please specify the applied relative centrifugal force (× g), as rpm alone is insufficient due to rotor-dependent variation. It would also be helpful to clarify whether the potential effects of this centrifugation step on bacterial viability or physiological status were considered.

Thank you for your suggestion. Our intention is to clarify a technical error regarding the rpm reported in the manuscript. The correct value, as specified in our protocol published in Vernocchi et al. (*Atmospheric Measurement Techniques*, 16, 5479–5493, 2023), is 3000 rpm, RCF 1560 x g. This setting was achieved using 12436 centrifuge rotor in the MPW-352 centrifuge model by Med Instruments. The suitability of these parameters is supported by our viability assays under control conditions, as documented in Figure 2 of the manuscript.

We rephrased the line 99 as: At this stage, 20 ml of the bacterial suspension was centrifuged at 3000 rpm, relative centrifugal force (RCF) 1560 x g, for 10 minutes, using a 12436-centrifuge rotor in an MPW-352 centrifuge (Med Instruments Warsaw, Poland). Finally, the pellet was resuspended in 20 ml of sterile distilled water (MQ).

3–114: The particle size range is given as “0.55 ÷ 10 µm.” Please confirm whether the symbol “÷” is a typographical error and clarify the intended size range (e.g., 0.55–10 µm).

Thanks for the comment. It was a typo. We intended the size range 0.55 – 10 mm.

Line 147–148: Please specify the medium used to prepare the bacterial suspension for the nebulizers. A brief description of the bacterial solution, including CFU mL^{-1} values before and after the

experiments, would improve transparency and allow better assessment of potential losses during the experimental procedures.

Thanks for the comment. We added the following sentences to the revised manuscript

Line160: For all experiments, the bacterial suspension used for nebulization was prepared by washing the overnight culture and resuspending the cells in MQ (as described in Bacteria strain section), which was used as the injection medium for both SLAG and FMAG. The CFU in the injection solution was approximately 10^9 CFU mL^{-1}, as determined by plate counting prior to nebulization. After aerosolization, CFU concentrations measured in the impinger collection liquid ranged between 10^6 and 10^7 CFU mL^{-1}, depending on the nebulizer and operating conditions (see Table 2).

Line 149: A nebulization duration of 20 minutes may be sufficiently long to affect bacterial viability. Please clarify whether bacterial viability within the nebulizer reservoir was assessed before and after nebulization. In particular, the SLAG system involves recirculation, which may impose additional stress on bacteria remaining in the reservoir. This potential effect should be considered when interpreting the results.

Thanks for the comment. We note that *E. coli* stability tests in MQ water show no significant loss of viability over time scales longer than the nebulization duration, indicating that liquid-phase residence does not contribute to bacterial damage. In the SLAG system, bacteria remaining in the reservoir are not aerosolized, as previously explained. As mentioned above, we modified line 148 as: "The larger droplets, generated during the process, gravitationally return to the liquid reservoir and are not aerosolized, nor reintroduced into the circulation."

Line 156–157: The manuscript states that all experiments were performed at (22 ± 1) °C and (49 ± 1) % relative humidity. Please clarify whether these values refer to conditions inside the chamber. In addition, indicate how and where temperature and relative humidity were measured, and reflect this information in Figure 1 if appropriate.

Thanks for the comment. Fig.1 was edited to reflect the measurement of T and RH inside the chamber. Finally, the sentence was modified as follows: "All experiments were performed at a temperature of (22 ± 1) °C and a relative humidity of (49 ± 1) %, as recorded by a sensor inside the chamber."

Line 160: The BioSampler uses Milli-Q (MQ) water as the collection liquid and operates with a relatively high airflow rate into the liquid phase, conditions that may impose additional stress on bacterial cells. This potential influence should be discussed when interpreting bacterial viability and culturability results.

Thanks for the comment. We have discussed this aspect in the new paragraph "Methodological framework for data interpretation" as described above.

Line 186-189: ... The results indicated that E. coli, resuspended in MQ, is not stressed during the short time required to run a chamber experiment (less than one hour). The potential loss of bacterial viability and culturability, as measured in the impinger liquid, can be attributed to the nebulization stress induced by nebulizers....

The physiological state of bacteria suspended in MQ water within the BioSampler is likely to differ substantially from the conditions implied by these statements and those represented in Figure 2. It remains unclear whether the bacterial concentrations measured in the BioSampler are directly

comparable to those shown in Figure 2. Clarification on this point is necessary for accurate interpretation of the results. Moreover, the above statements (line 188-189) the suggest that bacterial stress and loss of viability may occur in both the nebulizer and the BioSampler. This overlap in stress sources reduces the ability to clearly attribute observed differences in bacterial viability solely to the nebulization process, thereby weakening the basis for comparing the performance and efficiency of the two nebulizers.

We thank the reviewer for this detailed comment. We agree that the physiological state of *E. coli* suspended in MQ water within the BioSampler differs from that of bacteria maintained in static MQ conditions, as represented in Figure 2. Figure 2 establishes a baseline assessment of bacterial stability in MQ water over time**,** demonstrating that the suspension medium itself does not induce measurable stress on the time scale of the experiments. In addition, since the experimental conditions (i.e., short chamber resident time, temperature, humidity, and sampling flow) are similar, we can also compare the nebulization efficiency of the 2 nebulizers in relative terms.

This aspect is now discussed in the revised manuscript in the introduction section, in the new paragraph of Results and Discussion, and at line 221: “Viability and culturability measured in the BioSampler liquid therefore reflect the combined effects of aerosolization, chamber residence, and sampling, which are considered as a common background contribution in comparison of the two nebulizers.”