The introduction sets the context of the study effectively, in particular the lack of standardised approaches for bioaerosol research and the impact of aerosolisation and bioaerosol sampling methods on the viability of airborne microorganisms. As with other areas of environmental microbiology, the issues of mechanical sampling stresses, also the question of viable, but non-culturable microorganisms and the biases these effects can bring to data collection, remain a challenge for aerobiologists. Any information concerning bioaerosol generation and its influence on experimental findings is therefore helpful.

Lines 84-87 – General comment - As a test organism, the use of an *E. coli* strain was understandable, if challenging, given the relatively lower robustness of Gram-negative, nonspore-forming bacteria compared to the Gram-positive species often used in aerosol test studies.

Thanks for the comment. Our *E. coli* bacterial strain (ATCC 25922; American Type Culture Collection, Manassas, VA, USA) was selected as the model organism for this study. E. coli is a premier bacterial model due to its rapid growth rate and cost-effective cultivation requirements. Furthermore, it can be handled under Biosafety Level 1 (BSL-1), conditions using standard laboratory practices. Our future goal is to adapt this protocol for other significant bioaerosol studies involving different microbial species.

Moreover, the endotoxins, components of the outer membrane of gram-negative bacteria are significant bioaerosol constituents known to trigger respiratory issues in exposed individuals. So could be interesting to investigate this cellular component of the outer membrane of the cell wall consisting of lipids and lipopolysaccharides (LPS). Endotoxins are found in high concentrations in the air at sites that handle organic material such as composting facilities, intensive farms, and wastewater operations.

- Wouters, I.M.M. et al. "Overview of Personal Occupational Exposure Levels to Inhalable Dust, Endotoxin, β (1→3)-Glucan and Fungal Extracellular Polysaccharides in the Waste Management Chain". Ann. Occup. Hyg. 2005, 50, 39–53.
- Lawniczek-Walczyk, A. et al. "Occupational exposure to airborne microorganisms, endotoxins and beta-glucans in poultry houses at different stages of the production cycle". Ann. Agric. Environ. Med. 2013, 20, 259–268.
- Thorn, J et al. "Measurement Strategies for the Determination of Airborne Bacterial Endotoxin in Sewage Treatment Plants". Ann. Occup. Hyg. 2002, 46, 549–554.

Line 93 – as mentioned by at least one other reviewer, it would be helpful to have the centrifugation conditions expressed as xg to present a standardized value and to allow reproducibility, should the method be applied by others across different machines.

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).

Lines 107-108 – the test chamber was small at 20l. Even a class II biological safety cabinet would have been more specious and perhaps more representative of an ambient indoor atmosphere where particles can remain airborne at least for minutes prior to collection. BSCs can also be cleaned very effectively, have HEPA filtered inlet air and have a reasonable internal volume, typically, of 0.8 to 1.0m^3. If not described elsewhere it would be useful if any perceived limitations of this very compact chamber choice were presented, compared with other obvious options such as a BSC, or just a larger steel vessel of the type used. Maybe this could be added to the Conclusions section - see comment below?

We thank the reviewer for this comment. We agree that the relatively small volume of the test chamber (≈20 L) represents a methodological choice that differs from larger systems such as Class II biological safety cabinets or large-scale atmospheric simulation chambers.

The compact chamber was selected as a compromise between benchtop setups and large-volume facilities, with the primary objective of enabling a highly controlled and reproducible comparison of aerosol generators under well-defined conditions. The reduced volume minimizes wall-cleaning and decontamination times and facilitates systematic replication while maintaining full control over resident time, humidity, and sampling conditions.

To respond to this comment, we added the following sentence to the conclusion:

"The compact volume of the chamber represents a methodological 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.

Line 113 – I presume that the aerosol range should read, "…in the range 0.55 -10µm", rather than "….in the range 0.55 ÷ 10µm."

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

Line 115 and elsewhere – It would be useful to have the rationale for the choice of sterile water (for E. coli suspension and bioaerosol collection). I can see that this might eliminate any risk of crystal formation, which might interfere with some of the applied assays, but the use of freshly prepared, sterile isotonic buffer (such as phosphate buffered saline) would perhaps have conferred increased protection for the aerosolised and sampled particles, and is perhaps the cell suspension medium that many others would have preferred. Having read on, I do note that there is further comment on this between lined 169 and 189 and the journal editor may feel that this explanation is sufficient. Figure 2 does go some way to indicating that viability was retained within the same order of magnitude (recoverable CFU/ml), throughout the short course of the experimentation.

Thanks for the comment. We leave it to the Editor to judge if our explanation is enough.

Line 116 – The sampling pump flow rate is given as 12.5 l/min. Given the limited test chamber volume did this have any implications for the testing? For sample, very limited exposure of the bioaerosols to the airborne state prior to sampler entrapment? From the test setup diagram I assume that any negative pressure effects in the chamber were avoided by use of the balancing effect of the HEPA filtered air inlet, but please comment on this further on the set-up if you can.

We thank the reviewer for this comment. We acknowledge that, given the limited chamber volume, the BioSampler flow rate implies relatively short aerosol resident times before collection. This experimental condition was selected to enable rapid attainment of steady-state concentrations and to ensure sufficient bacterial recovery above detection limits. And yes, the chamber was operated under pressure-balanced conditions. This configuration ensured stable aerosol conditions and avoided additional stress related to pressure gradients.

In the present study, aerosol resident time and sampling conditions are treated as systematic background contributions, common to all measurements, and we have clarified these aspects in the revised manuscript to better describe the implications of chamber volume, sampling flow rate on the experimental conditions (the new paragraph in Results and Discussion called "Methodological framework for data interpretation").

Finally, for a better understanding, we added the following sentence at Line 133: The BioSampler flow rate was balanced by a HEPA-filtered air inlet to maintain ambient pressure inside the chamber, resulting in short and well-defined aerosol resident time across all experiments.

Lines 144-145 – Is this assumption based on the manufacturer's information, or a fact established by other independent evaluation? It would be useful to have that qualified in the text, or to modify the sentence in a way such as, "This modest shear stress typically **allegedly** allows biological cells to maintain viability, even following dispersion into uniform particles."

We agree with the referee. We modified the text as suggested

Figure 3 required additional labelling on the x-axis - at least one other reviewer has commented on this.

Thanks for the comment. We modified figure 3 by adding the labelling on the x-axis

Lines 297-301 - Conclusions section – are there any lessons learnt in terms of any experimental weaknesses identified? The MQ water issue is well outlined, but there are few comments on experimental weaknesses or biases otherwise. Also, the starting volume for each nebuliser is quite different – do the authors have any comments on the implications of this for wider aerosolisation testing and the practical choices to be made? The paper of Gatta et al., 2025 is referred to several times throughout this manuscript; but are there any comparisons or reflections to be drawn between this earlier paper and the new manuscript. As this earlier published work is clearly important to the current paper, it would be useful to get that perspective

Thanks for the comment. We have revised the conclusion, and here is the new version:

The experimental results indicated that the MQ did not induce stress in the *E. coli* for the duration required to complete the nebulization experiments. The viability and CFU of *E. coli* resuspended in MQ were relatively stable and did not decline within 24 hours following the MQ resuspension of the bacteria. The two FMAG settings yielded identical bacterial size distributions, suggesting that FMAG frequency did not affect size distribution but solely impacted experimental repeatability. The size distribution of FMAG was associated with the bacterial concentration in the injection solution. Utilizing the identical FMAG setup, the nebulization of MQ with ON *E. coli*, diluted by a factor of 100, resulted in a shift of the size distribution peak from roughly 2.8 µm to 0.8 µm. This indicates that with an ON MQ injection liquid (total bacterial concentration > 10^9 #ml^{-1}), the bacteria probably tend to cluster, leading to droplets devoid of individual bacteria. The SLAG nebulizer generated a significant concentration of fragments: using a setting previously employed in our earlier tests (Agarwal et al., 2024; Gatta et al., 2025;

Vernocchi et al., 2023), the fragmentation rate is roughly 40% of the total nebulized particles. Both nebulizers induced stress throughout the nebulization process, resulting in a twofold reduction in the viability of the bacteria collected in the impinger. Finally, the FMAG had a nebulization efficiency approximately 20 times greater than that of the SLAG.

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.