

AIRBORNE BACTERIA AND PARTICULATE MATTER IN POULTRY ENVIRONMENTS: A DOUBLE THREAT TO WORKERS' HEALTH

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Abstract

The poultry industry poses a global health risk due to microbial contamination. Also, particulate matter (PM) may act as a vehicle for microbial dissemination. Understanding PM dynamics in poultry production environments is crucial for evaluating occupational exposure to airborne pathogens. This study aims to quantify PM contamination and assess occupational exposure to airborne bacteria in poultry pavilions (PP) throughout different stages of the production cycle. Air samples from indoor (n=47) and outdoor (n=20) PP were collected across various stages of the growth cycle (empty pavilion- sanitary control, 1st, 2nd, and 3rd weeks). For bacteria characterization the impactor MAS-100 was set to 100 L/min for 5 minutes and culture-based methods were performed. PM size fractions (PM_{0.5}, PM₁, PM_{2.5}, PM₅, and PM₁₀) were measured using a Lighthouse Handheld Particle Counter at a flow rate of 2.83 L/min during 5 min. The highest bacterial loads and PM contamination occurred during the 3rd week, indicating a link between PM and the dissemination of airborne bacteria. PM₁₀ and PM₅ were dominant throughout the production cycle, highlighting their potential role in carrying pathogens to workers' respiratory systems. These findings emphasize the need to monitor PM and microbiological contamination in poultry farms to reduce worker health hazards.

Keywords: Particulate matter; Bacteria; Occupational health; Poultry farms; One Health.

Introduction

The poultry industry poses a global health safety risk due to putative microbial contamination. The management of emerging pathogenic microorganisms, resistant to antibiotics and food-related stressors, is a major concern. Regarding bacteria, *Escherichia coli*, *Staphylococcus* sp. (Bist *et al.*, 2022), and other species belonging to the WHO Bacteria Priority pathogens list (BPPL) (Gorny, 2022), were already isolated from poultry farms (Li *et al.*, 2021). Besides, several inflammatory agents are also present, including gram-negative bacteria metabolites - endotoxin, which has been linked to subjective symptoms and a reduction in respiratory airflow (Viegas *et al.*, 2013). Other concerns are related to Particulate matter (PM), which is regarded as one of the most harmful air pollutants in poultry production environments (Bist *et al.*, 2022; Derakhshani *et al.*, 2021). Wherever microbial sources exist, PM can serve as a vehicle for biological particles, functioning as a carrier for the respiratory system and boosting exposure to infections.

Particles that are significant to human health are inhalable particles with a diameter of less than 10µm referred to as thoracic particles, that are small enough to penetrate the respiratory tract (Gorny, 2022). Understanding PM dynamics is helpful when estimating occupational exposure to microorganisms, giving key data for the physical features controlling the transmission of airborne bacteria and exposure mitigation (Li *et al.*, 2021). Programs to reduce pathogens on farms helps to control issues related to the one health concept, since they improve workers and animals' health, reduce environmental contamination, and minimize water and vegetable pollution (Gomes *et al.*, 2022; 2023). Knowing the chemical and biological composition of the bioaerosols will help define the possible health effects related (Viegas *et al.*, 2014). Together, this information will support the development of mitigation techniques to reduce airborne transmission of PM and microorganisms from poultry production environments. Based on these assumptions this study aims to quantify particulate matter (PM) contamination and assess occupational exposure to airborne bacteria in PP.

Material and methods

This study is part of a broader exploratory study that aims to assess microbiological contamination and occupational exposure in Portuguese poultry farms (n=5). The sampling campaign was conducted during Winter. Air samples from indoor pavilions (n=47) and outdoor (n=20) were collected on several stages of poultries' growth cycle (sanitary control- empty pavilions; 1st; 2nd and 3rd weeks;) by the impactor MAS-100, set at 100 L/min for 5 min, and using Polycarbonate (PC) filters (0.2 µm pore; 47 mm; Merck Millipore Ltd., Cork, Ireland) as performed by (Lanier *et al.*, 2010). To avoid particle bounce, 150 µL of sterile glycerol 10% [v/v] was added to the filters (Ferguson *et al.*, 2021). Filters were kept in petri plates at -20°C during transportation. The airborne dust collected by polycarbonate (PC) filters was extracted following Frankel and team members' protocol (2012). Briefly, 5 ml of 0.1% Tween 80 saline (0.9% NaCl) solution was added to the filters and extraction was accomplished via orbital shaking (500 rpm, 15 min). After extraction, 100 uL aliquot was spread onto petri dishes containing Tryptic soy agar (TSA) supplemented with nystatin (0.2%) and MacConkey agar (MAC) to cultivate total bacteria and gram-negative bacteria, respectively. TSA plates were incubated at 30°C, during 7 days and MAC plates were incubated at 37°C, during 7 days. Bacterial densities (CFU.m⁻³) were obtained. The Lighthouse Handheld Particle Counter HH3016-IAQ was used to measure particulate matter at five different sizes (PM_{0.5}, PM₁, PM_{2.5}, PM₅, and PM₁₀), at a flow rate of 2.83 L/min during 5 min. Size fractions were used to estimate dust penetration within the respiratory system and their potential health effects (Viegas, *et al.*, 2012).

Results and discussion

Regarding airborne bacteria levels, bacterial loads indoor surpassed those outdoors in 79% of the air samples collected (43 out of 47), supporting the assumption that PP are most likely a point source of microbial contamination (Chen *et al.*, 2021). Regarding total bacteria load, the median values increased throughout the weeks (sanitary control: 2.27x10²; 1st week 1.10x10³; 2nd week 1.26x10³; 3rd week 2.79 x10³ CFU.m⁻³). The same frequency was observed on gram negative bacteria load, were the highest median value was observed on the 3rd week (sanitary control: 2.01x10²; 1st week: 5.46x10¹; 2nd week 7.23x10¹; 3rd week 2.47x10² CFU.m⁻³). Considering PM concentration, the highest median values were also obtained the 3rd week for PM₅ (sanitary control: 2.92x10¹ µgr.m⁻³; 1st week 5.53x10²; 2nd week 5.42x10²; 3rd week 6.94x10² µgr.m⁻³) and for PM₁₀ (sanitary control: 7.49x10¹; 1st week 1.43x10³; 2nd week 1.52x10³; 3rd week 1.98x10³ µgr.m⁻³) (Figure 1).

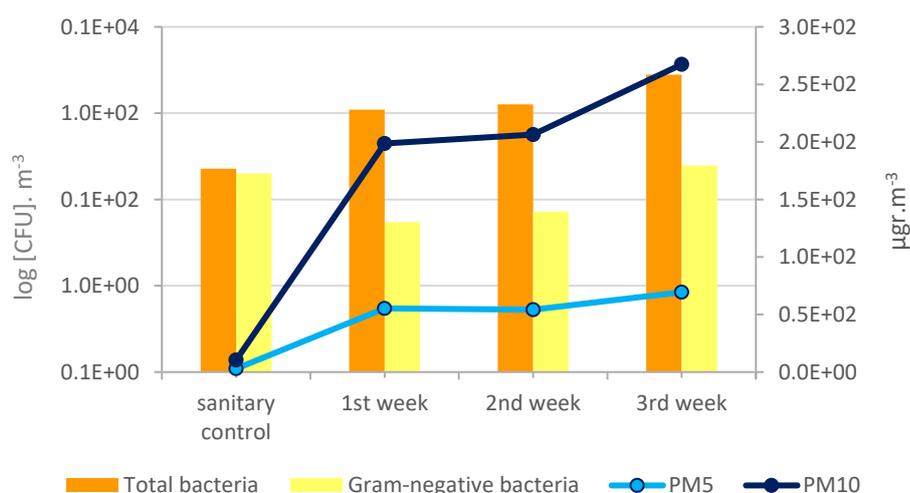


Figure 1. Airborne bacteria median load (log [CFU.m⁻³]) and particulate matter concentration (µgr.m⁻³) of the most prevalent size fractions (PM₅; PM₁₀) inside poultry pavilions following different stages of poultries growth cycle.

Several factors can influence bacteria load. High-density and poorly ventilated conditions may have contributed to a higher concentration of airborne bacteria during the 3rd week (Chen *et al.*, 2021; Peng & Biswas, 2020).

Assuming that PM contributes significantly to the spread of microorganisms (Chen *et al.*, 2021; Dias *et al.*, 2024), the highest level of PM contamination during the 3rd week might also justify the highest bacteria load during that stage. Regarding PM distribution indoors, from the different sizes evaluated (PM_{0.5}, PM₁, PM_{2.5}, PM₅, and PM₁₀), PM₁₀ were prevalent during the production cycle (51.25% sanitary control; 63.36% on 1st week; 65.56% on the 2nd week; 65.09% on the 3rd week;). PM₅ were also frequent (19.97% sanitary control; 24.46% on 1st week; 23.35% on the 2nd week; 22.82% on the 3rd week;). The data indicate that coarse particles (2.5-10 µm) are the most significant PM in this environment. These PMs have the potential to exacerbate respiratory conditions such chronic obstructive pulmonary disease (COPD) and asthma. Also, smaller PM (< 7 µm) can reach the lung's gas exchange zone, leading to systemic effects (Shen *et al.*, 2023; Viegas *et al.* 2013). These data should be considered since workers avoid using respiratory protection during their working routines and so health risks are emphasized. Microbial assessments of poultry farms have previously revealed the presence of several microorganisms, including zoonotic pathogens that can operate as carriers of airborne illnesses. Regarding microbiological components, endotoxin, a significant constituent of gram-negative bacteria's outer membrane, is a substantial health hazard (Gomes *et al.*, 2023). Inhalation exposure to non-viable microbe components, such as endotoxins may also occur (Huneau-Salaün *et al.*, 2011), hence, examining non-viable components may be also relevant in determining pulmonary disease risk. (Li *et al.*, 2023; Peng *et al.*, 2020). From a One Health perspective, quantitative and qualitative research is required to fully understand this environment and determine the measures that should be taken to minimize exposure indoors and outdoors and control risk.

Limitations

Air sampling is commonly used to evaluate occupational exposure. However, active sampling techniques have limitations in determining long-term exposure due to the brief sample duration of the device. The use of passive sampling methods on the other hand, allows accessing pollution levels over a long period. The potential of electrostatic dust cloths (EDC) and surface samples has already been well supported (Dias *et al.*, 2024; Viegas *et al.*, 2022) and future studies should follow this strategy.

Conclusions

The majority of studies covering exposure assessments on poultry farms are based on measurements taken at a single stage of the production cycle. For an accurate occupational exposure assessment to airborne bacteria and PM contamination, this study comprises a wide sampling campaign covering several stages of poultry growth cycle. The highest level of airborne bacteria was found during the 3rd week. Similarly highest contamination of PM was found in the third week which may reflect the idea that particulate matter acts as a vector for disseminating microbiological agents. Besides, particle fractions PM₅ and PM₁₀ were dominant throughout the production cycle, suggesting that PM can carry potential pathogenic bacteria and their metabolites and reach workers respiratory system. We strongly recommend enforcing strict hygiene protocols during sanitary control, identifying contamination sources within pavilions (eg: cracks on walls, contaminated materials), and improving ventilation systems. Regarding workers protection, we emphasize the importance of using PPE, particularly RPE to reduce inhalation of PM carrying potentially pathogenic airborne bacteria. Further research should focus on species identification to complement the data obtained. Also, metabolites assessment (endotoxins) and bacteria antibiotic resistance profile should be carried out. Overall, this study underlines the need of microbial exposure assessments in poultry farms.

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