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PM_{10}-BACTERIAL INFECTION INTERACTION IN A-549 CELLS: A ONE HEALTH PERSPECTIVE

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Despite efforts to promote decarbonization, there remains a heavy reliance on coal for domestic heating, especially in colder regions such as León (Castilla y León, Spain) (Vicente et al., 2021). Of the compounds derived from its combustion, there is growing concern about atmospheric particulate matter (PM_{10}). *In vivo* and *in vitro* studies show that this exposure can lead to serious pulmonary and cardiovascular conditions (Inesta-Vaquera et al., 2023). However, the potential consequences with antibiotic-resistant bacteria are yet to be studied. In this context, the synergistic presence of PM_{10} and *Staphylococcus aureus* (USA300), a facultative intracellular superbug that is the leading cause of death in more than 135 countries (Ikuta et al., 2022), represents an additional challenge.

Therefore, in this study, we start from the One Health approach, which recognises the interconnection between pollution and impacts on human and environmental health. The aim is to understand how tumour cells of basal alveolar epithelium (A-549) respond to two of the main environmental conditions to which humans are usually exposed: atmospheric pollution and bacterial infection.

PM_{10} samples were collected on quartz filters using a high-volume sampler (CAV-A) located in a public building in the centre of León (Spain). This is an area with a high number of homes that use coal in their heating systems. The samples were extracted using a flow of dichloromethane followed by filtration with methanol, to be subsequently evaporated under a flow of nitrogen. The final extract was diluted in dimethyl sulfoxide (DMSO) at a concentration below 1% (v/v).

As for the cells, they were cultured in 96-well plates, each with $4x10^4$ cells, and were maintained at 37 ºC in a 5% CO_2 atmosphere in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and 5% penicillin-streptomycin, until reaching 70-80% confluence. Subsequently, the effect on cell viability resulting from exposure to increasing concentrations of PM_{10} samples from 0.05 to 200 µg/mL at 24 and 48 h was analysed.

Subsequently, the cells, previously contaminated, were infected for one hour from aliquots of USA300 prepared at an optical density of 0.8 and with a MOI (Multiplicity of Infection) of 5 bacteria per cell. Finally, the infection was stopped using a 1:10 ratio of vancomycin-gentamicin in DMEM. The readings of this assay were performed at 24 h in a fluorescence reader (VICTOR Nivo™, PerkinEkmer).

It is important to mention that the cell line used is transfected with m-Cherry, which allows direct analysis of the results over time without the need to add additional reagents. However, in order to establish a comparison, the same studies have been carried out using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay.

The preliminary results indicate an upward trend in cell viability as the concentration of PM_{10} increases, referred exclusively to exposure to this particulate matter. This finding contrasts with what was obtained after the infection, where a slight decrease in viability is observed at the highest concentrations of the pollutant.

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