AN ACCREDITED *EX VIVO* PORCINE MUCOSAL MODEL

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Introduction

Patients suffering with pulmonary diseases, ventilator-associated pneumonia or cystic fibrosis (CF) are at higher risk of *Pseudomonas aeruginosa* biofilm colonisation in the lungs (Harrison and Diggle, 2016). These infections can be difficult to treat and can lead to respiratory failure and/or death in humans. Mucous membrane is the moist lining of body cavities that communicate with the exterior. These tissues line the mouth, gastrointestinal (GI) tract, nasal passages, urinary tract and vaginal canal. Prolonged pressure applied to this tissue can render it ischemic and lead to ulceration. Mucosal tissues are especially vulnerable to pressure from medical devices, such as oxygen tubing, endotracheal tubes, bite blocks, orogastric and nasogastric tubes, urinary catheters and faecal containment devices.

It is widely researched that porcine internal anatomy is similar to that of a human (Judge et al. 2014) therefore, replicating how *P. aeruginosa* biofilms develop on porcine mucosal tissue will provide a platform to provide efficacy testing for pulmonary treatments. The purpose of conducting this study was to validate a model to support efficacy testing for treatments intended to be used for pulmonary diseases, such as Cystic Fibrosis.

This study aimed to determine the reproducibility of *P. aeruginosa* biofilms on *ex vivo* porcine mucosal tissue and to develop a platform for topical and aerosolised treatments for infected mucosal wounds, in line with ISO 17025. Both active and inactive antibiotics were tested for their biofilm removal capabilities.

Methodology

Pseudomonas aeruginosa was used to develop biofilms on *ex vivo* porcine mucosal tissue incubated with a protein-based medium over 72 hours. Planktonic organisms were removed by washing prior to treatment. Following washing, individual treatments were applied to the tissue for 24 hours ± 2 hours at 37 °C ± 2 °C. Remaining viable organisms were recovered by sonication and resultant suspensions were plated onto agar.

Results

Following a 24-hour treatment, an average of 6.57 ± 0.14 $Log_{10}CFUmL^{-1}$ was recovered from untreated tissue. Averages of 4.12 ± 0.40 $Log_{10}CFUmL^{-1}$ and 5.82 ± 0.31 $Log_{10}CFUmL^{-1}$ were recovered from the active and inactive antibiotic, respectively. No viable microorganisms were recovered from the positive control or the sterility control samples (Figure 1).

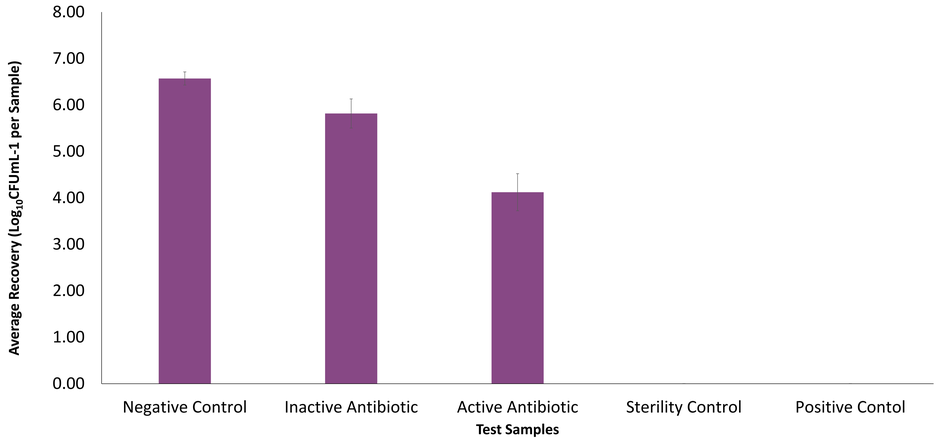

Figure 1. Average quantity of *Pseudomonas aeruginosa* recovered from biofilms grown on *ex vivo* porcine lung tissue samples for 72 hours following a 24-hour treatment time with a range of test products. Error bars indicate standard deviation.

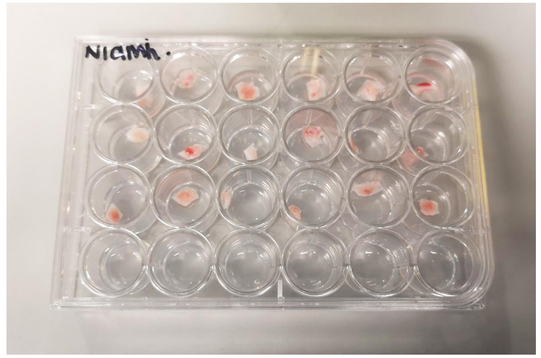

Figure 2. Dissected porcine lung tissue prior to inoculation in a 24-well plate.

Discussion and Conclusion

When compared to the negative control, reductions of 2.45 ± 0.40 and 0.75 ± 0.31 $Log_{10}CFUmL^{-1}$ *P. aeruginosa* were observed from the active and inactive antibiotic, respectively. The sterility control was included to determine the starting bioburden of the tissue. The positive control was included to prove that the biofilms could be removed.

There is an increasing need to design and develop new platforms to provide a real-world challenge for anti-biofilm efficacy test methods to differentiate between antimicrobial products. The use of *ex vivo* porcine mucosal tissue mimics the way microorganisms attach to surfaces in a clinical setting. This model may also apply to other types of wound which are not within the mucosa as wound healing is the same in mucosa as it is in the skin, except for the formation of scar.

References

Harrison, F. and Diggle, S.P., 2016. An *ex vivo* lung model to study bronchioles infected with *Pseudomonas aeruginosa* biofilms. Microbiology, 162.

Judge, E.P., Hughes, J.L., Egan, J.J., Maguire, M., Molloy, E.L. and O'Dea, S., 2014. Anatomy and bronchoscopy of the porcine lung. A model for translational respiratory medicine. American journal of respiratory cell and molecular biology, 51(3), pp.334-343.

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