Ajoene, a novel therapeutic that shows enhanced activity in in vivo and in vitro models of *Pseudomonas aeruginosa* infection.

Bricio-Moreno L[1], Waters EM[1], Neill DR[1], Evans G[2], Graz M[2], Fothergill JL[1], Kadioglu A[1]

1. Institute of Infection and Global Health, University of Liverpool, UK.
2. Neem Biotech, Abertillery, UK.

Introduction

Pseudomonas aeruginosa is a major cause of chronic respiratory infection in patients with cystic fibrosis and non-CF bronchiectasis. Once established, these infections can be lifelong and despite intensive antibiotic treatment remain established in the respiratory tract. Over time, the *P. aeruginosa* populations display impressive diversity which both allows adaptation to the respiratory niche and selection of resistant isolates. In this study we assessed the antimicrobial properties of Ajoene, a sulphur-containing compound present in garlic.

Aims

- Assess the ability of Ajoene to inhibit biofilm formation or to disrupt a pre-formed biofilm by *P. aeruginosa* (LESB65) in artificial sputum media (ASM).

- Study the effect of Ajoene on expression of genes involved in Quorum Sensing, biofilm formation, exopolysaccharide production and CFTR inhibition.

- Study the effect of Ajoene on bacterial clearance in a chronic *P. aeruginosa* in vivo model.

Results - In vitro (ASM)

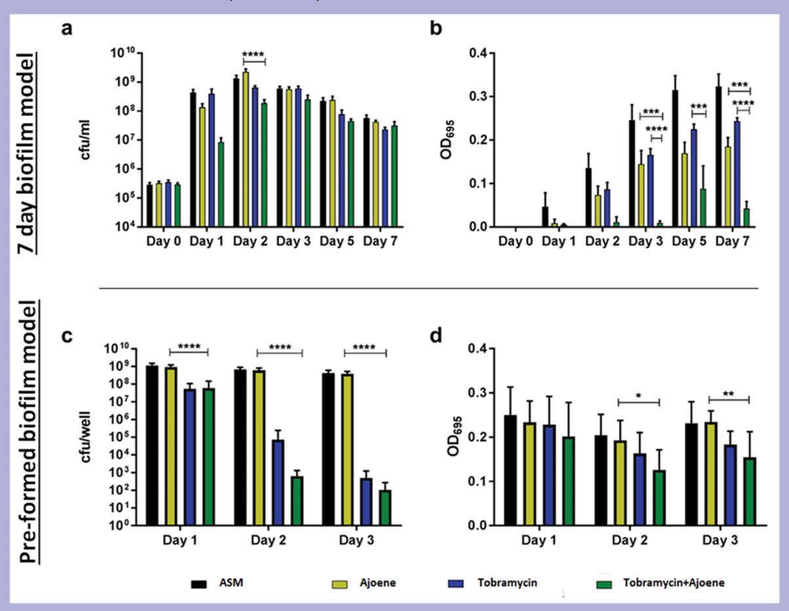

Figure 1. Number of *P. aeruginosa* bacteria (LESB65) forming a biofilm in ASM (a) and pyocyanin production (b) after single treatment with ASM, Ajoene, Tobramycin or Tobramycin+Ajoene. Number of *P. aeruginosa* bacteria remaining in a preformed biofilm in ASM (c) and pyocyanin production (d) after 3 consecutive days of treatment with ASM, Ajoene, Tobramycin or Tobramycin+Ajoene.

Results - In vitro gene expression

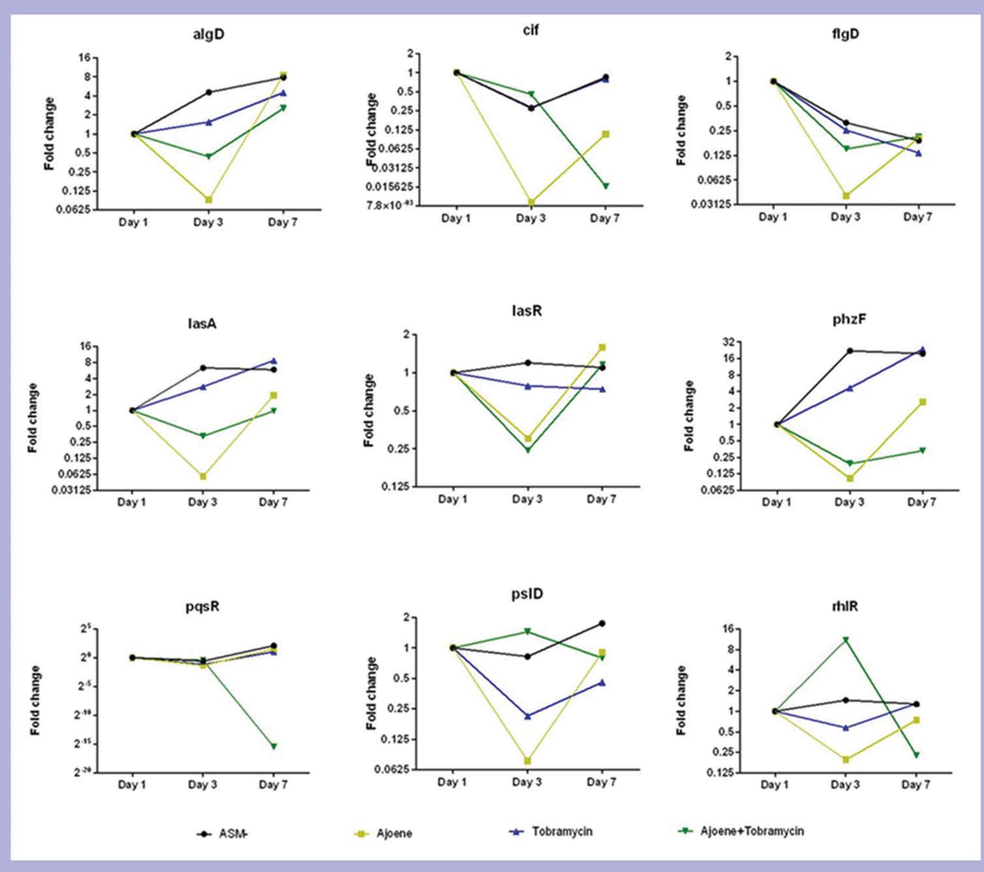

Figure 2.
Gene expression levels in *P. aeruginosa* (LESB65) grown in ASM in the presence of ASM, Ajoene, Tobramycin or Tobramycin+Ajoene. The genes studied were genes involved in Quorum Sensing, biofilm formation, exopolysaccharide production or CFTR inhibition.

Results - In vivo

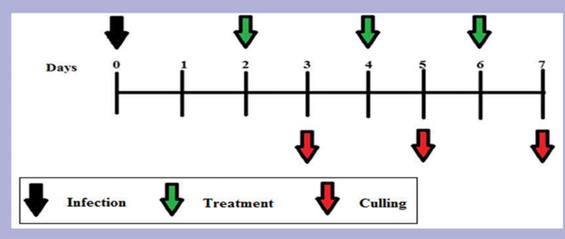

Figure 3. Experimental setting of the in vivo *P. aeruginosa* infection model.

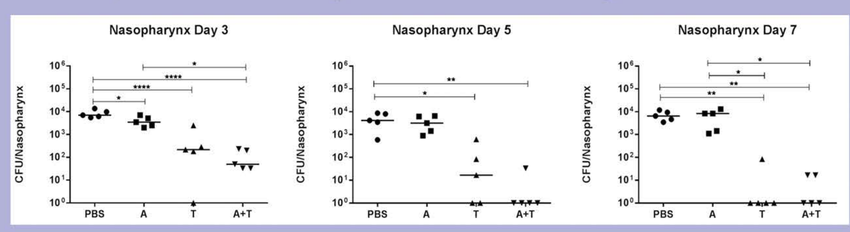

Figure 4. CFU/nasopharynx of Balb/c male mice infected with *P. aeruginosa* (LESB65) and treated on days 2, 4 and 6 post-infection with PBS, Ajoene, Tobramycin or Tobramycin+Ajoene.

Conclusions

- Ajoene enhances the antimicrobial properties of Tobramycin in vitro and significantly inhibits the production of pyocyanin when administered in combination with tobramycin.

- Ajoene induces down-regulation of genes involved in the Quorum Sensing system (lasA, lasR, rhlR, phzF, pqsR), biofilm formation (algD, flgD, pslD) and CFTR inhibition (cif).

- Treatment with the Tobramycin + Ajoene combination in vivo leads to faster clearance from the nasopharynx (reservoir) and lungs (infection site) of Balb/c mice when compared to treatment with Tobramycin alone.