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Characterization of clinical isolates of *Pseudomonas aeruginosa* in a rat model of respiratory infection

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Pseudomonas aeruginosa (P.a) contributes significantly to the pathogenesis of chronic lung infections in cystic fibrosis (CF) patients. Here, clinical isolates with differing MICs to tobramycin are profiled in a rat lung model of chronic infection. By assessing their growth kinetics, antibiotic sensitivity and associated inflammatory responses the suitability of these strains to model CF is examined.

P.a. was mixed with molten agar Nobel and spun into heated mineral oil with 0.01% $_{v/v}$ SPAN 80 to produce agar beads. Male Wistar rats (195-245g, n=5-8 per group) were inoculated, via a left lung specific intra-tracheal infection, under isoflurane anesthesia, with $9x10^4$ - $3x10^6$ colony forming units (cfu) of PACF1 1 or PACF 2 (courtesy of Stuart Elborn, Queens University Belfast) or $2x10^5$-$5x10^5$ cfu PAO1V (courtesy Mike Vasil, University of Colorado). Sham animals were inoculated with sterile beads. On days 1, 2, 5, 7, 9 and 14 post infection (p.i.) animals were euthanized with CO_2 asphyxiation and the lungs removed aseptically, for bacterial quantification. At 2 days p.i. cell counts were measured in broncheoalveolar lavage fluid (BALF). To assess antibiotic efficacy, animals were treated with vehicle or tobramycin 24 hours post infection (p.i.) via the subcutaneous (s.c.) or intra-tracheal (i.t.) route of administration BID. Cfu was determined after 24 hours of treatment. Data are expressed as the mean ± SD or mean ± SEM and analysed using a Kruskal-Wallis ANOVA with a Dunn's post-test.

For both clinical isolates, infection was present in the lungs 14 days p.i.. Tobramycin reduced bacterial load of PACF 1 (MIC = 0.25 µg/ml) following BID s.c. or i.t. treatment (log_{10} 5.52+0.4 and log_{10} 5.21+0.35 cfu at 400 and 60 mg/kg/day, respectively) versus vehicle treatment (log 8.58+0.5). For PACF2, Tobramycin (MIC = 4 µg/ml) treatment proved less effective with BID s.c (log_{10} 6.78±0.36 at 400mg/kg/day) and i.t. treatment (log_{10} 6.27±0.39 at 60mg/kg/day) versus vehicle (log_{10} 7.4±0.19). Total cell counts in BALF for both isolates were significantly higher versus sham animals (Table 1), with neutrophils being the most prominent inflammatory cell. PAO1V did not significantly increase cells present in BALF.

	Total Cell Counts ($x10^5$)	**Neutrophil Counts ($x10^5$)**
Sham	2.57 ± 0.69	0.08 ± 0.05
PAO1V	6.13 ± 1.84	4.77 ± 1.37
PACF 1	37.85 ± 8.27 ****	34.26 ± 7.97 ****
PACF 2	17.43 ± 3.74 **	13.54 ± 3.53 **

Table 1: BALF total inflammatory cell number and total neutrophil number for control and infected animals. Results are mean ± SEM. ** P<0.01, **** P<0.0001 versus control.

This study demonstrates that clinical isolate strains of *P.a* can be used to readily infect rats and model the inflammation an infection associated with CF. The response of these strains to antibiotic therapy demonstrates the difficulty in treating such infections in a clinical setting.