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Original Article

Impact of antibiotic treatment for pulmonary exacerbations on bacterial diversity in cystic fibrosis

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Abstract

Background: A diverse array of bacterial species is present in the CF airways, in addition to those recognised as clinically important. Here, we investigated the relative impact of antibiotics, used predominantly to target *Pseudomonas aeruginosa* during acute exacerbations, on other non-pseudomonal species.
Methods: The relative abundance of viable *P. aeruginosa* and non-pseudomonal species was determined in sputa from 12 adult CF subjects 21, 14, and 7 days prior to antibiotics, day 3 of treatment, the final day of treatment, and 10–14 days afterwards, by T-RFLP profiling.
Results: Overall, relative *P. aeruginosa* abundance increased during antibiotic therapy compared to other bacterial species; mean abundance pre-antibiotic 51.0±36.0% increasing to 71.3±30.4% during antibiotic (ANOVA: $F_{1,54}=5.16$; $P<0.027$). Further, the number of non-pseudomonal species detected fell; pre-antibiotic 6.0±3.3 decreasing to 3.7±3.3 during treatment (ANOVA: $F_{1,66}=5.11$; $P<0.027$).
Conclusions: Antibiotic treatment directed at *P. aeruginosa* has an additional significant impact on non-pseudomonal, co-colonising species.

Keywords: Antibiotics; Pulmonary exacerbation; Bacterial diversity; T-RFLP; Relative abundance

1. Introduction

Pseudomonas aeruginosa is commonly cultured from the adult CF lung and chronic infection with this organism is also associated with reduced life expectancy [1]. In patients who are chronically infected, *P. aeruginosa* is commonly the primary target for antibiotic therapy in response to the onset of CF pulmonary exacerbation (CFPE) [2]. In such cases, it is common practise to administer two anti-pseudomonal antibiotics with different modes of action; for example, a β-lactam and aminoglycoside [2].

Whilst often referred to as anti-pseudomonal, antibiotics such as ciprofloxacin, ceftazidime, and tobramycin, are broad spectrum, capable of affecting a wide array of Gram positive and Gram negative bacteria. This is potentially important given the many other bacterial species have been reported in adult CF lower airway secretions in addition to *P. aeruginosa* [3-10].

In the most part, this wider bacterial diversity has only come to light with the recent application of culture-independent, molecular profiling techniques that avoid the often substantial challenge of growing bacteria *in vitro*. As such, with the exception of a relatively small group of species recognised as CF pathogens, the clinical significance of these species is not yet known. It has,

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however, been shown that a substantial proportion of these wider species are capable of altering the virulence of *P. aeruginosa* [11,12]. As such, the impact of antibiotics used to target *P. aeruginosa* infections on non-pseudomonal species might be a contributory factor in treatment outcome.

The culture-independent approach of terminal restriction fragment length polymorphism (T-RFLP) analysis has been shown recently to be informative in tracking changes in the presence or absence of species over periods of antibiotic treatment [9]. By differentiating between bacterial species based on their 16S ribosomal RNA gene sequences, T-RFLP profiling allows *P. aeruginosa* populations to be distinguished from populations of other bacterial species present in the same sample, and their relative abundances assessed. Such analysis alone cannot however report accurately changes in relative bacterial population sizes in response antibiotic treatment. This is because DNA present in bacteria killed by action of antibiotics, or subsequently released into the extracellular environment, will contribute to the signal derived. To prevent this, a photochemical strategy of pre-treating samples prior to DNA extraction with propidium monoazide (PMA) can be used to limit signal to only that from viable bacterial cells [13,14]. The principle is based on membrane integrity as a common sign of viability: PMA does not enter cells with intact membranes, whereas it readily does so in cells with compromised membranes [15]. In this way, the combination of sample pre-treatment and T-RFLP profiling allows shifts in the relative abundance of viable bacteria from different species to be determined.

Here, we applied such a strategy to the analysis of sputum samples from twelve adult CF patients, prior to, during, and following, antibiotic treatment for pulmonary exacerbation.

2. Materials and methods

2.1. Subjects and clinical samples

This study was undertaken with local ethical approval from Southampton and South West Hampshire Research Ethics Committee (06/Q1704/26). Sputum samples were collected prospectively from 14 adult CF subjects at Southampton General Hospital, Southampton, UK. Subjects were selected that were persistently productive of sputum. All subjects provided sputum samples and clinical monitoring information three times per week for at least 12 months.

All subjects had a previous history of CFPE. During the study period only 12 of 14 patients experienced at least one exacerbation, and only data from these are presented. The start of a CFPE was defined by the clinician's decision to initiate antibiotic therapy for deteriorating clinical status, broadly based on a range of factors described previously [17]. In turn, the end of CFPE was defined by the decision to cease antibiotic therapy due to stabilisation or improvement in signs and symptoms.

Clinical information and details of antibiotic treatment given during CFPE for these twelve patients are shown in Table 1. A range of antibiotic selections was administered. In addition to antibiotics selected as anti-pseudomonal treatments (ciprofloxacin, colomycin, tobramycin, meropenem, amikacin, gentamicin), antibiotics used included those that potentially would be active against *P. aeruginosa*, but which were not selected for their anti-pseudomonal properties (doxycycline - Patient 3, and clarithromycin - Patient 9), and agents that would not be expected to have an anti-pseudomonal impact (metronidazole, used in conjunction with ciprofloxacin – Patient 10).

Samples were selected retrospectively for analysis that fell approximately 21, 14, 7 days prior to the start of antibiotic treatment for pulmonary exacerbation, and then at day 3 of treatment, on the final day of treatment, and 10–14 days after the end of treatment.

2.2. Clinical assessment

Prospective monitoring of clinically relevant symptoms and spirometry was undertaken at the time of each sputum sample. Lung function (FEV_1) was assessed using a Koko PeakPro home spirometer (Ferraris Cardiorespiratory, Louisville, CO, USA). In addition, four respiratory symptoms were measured using visual analogue scales (VAS), with patients asked to assess levels of breathlessness, cough, sputum production, and general well-being, scored from 0 to 100, representing worst and best respectively.

Table 1
Patient information.

Subject	Age	Gender	Genotype I	Genotype II	FEV_1 (% predicted)	BMI	Diabetic	Antibiotics given for CFPE
1	30	Male	phe508del	Unknown	54.9	29	No	Ciprofloxacin PO
2	45	Female	phe508del	phe508del	40.2	18.5	Yes	Colomycin IV + tobramycin IV
3	30	Female	phe508del	711 + 3A7G	38.2	25	No	Doxycycline PO
4	22	Female	phe508del	phe508del	36.2	19	No	Ciprofloxacin PO, then meropenem IV + amikacin IV
5	55	Male	phe508del	G85E	52.2	24.5	No	Ceftazidime IV + gentamicin IV
6	21	Female	phe508del	phe508del	56.6	19	No	Ciprofloxacin PO
7	22	Male	phe508del	phe508del	16.5	17.9	Yes	Meropenem IV + colomycin IV
8	18	Female	phe508del	phe508del	84	22.5	No	Ceftazidime IV + gentamycin IV
9	24	Female	phe508del	G542X	72.5	23.4	No	Clarithromycin PO
10	20	Male	phe508del	phe508del	26.8	30.4	No	Ciprofloxacin PO + metronidazole PO
11	20	Male	phe508del	phe508del	54.4	21	No	Ceftazidime IV + gentamycin IV
12	23	Male	phe508del	phe508del	53.6	20.7	Yes	Meropenem IV + tobramycin IV