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Lung infection microbiota in cystic fibrosis

Prof Chris van der Gast

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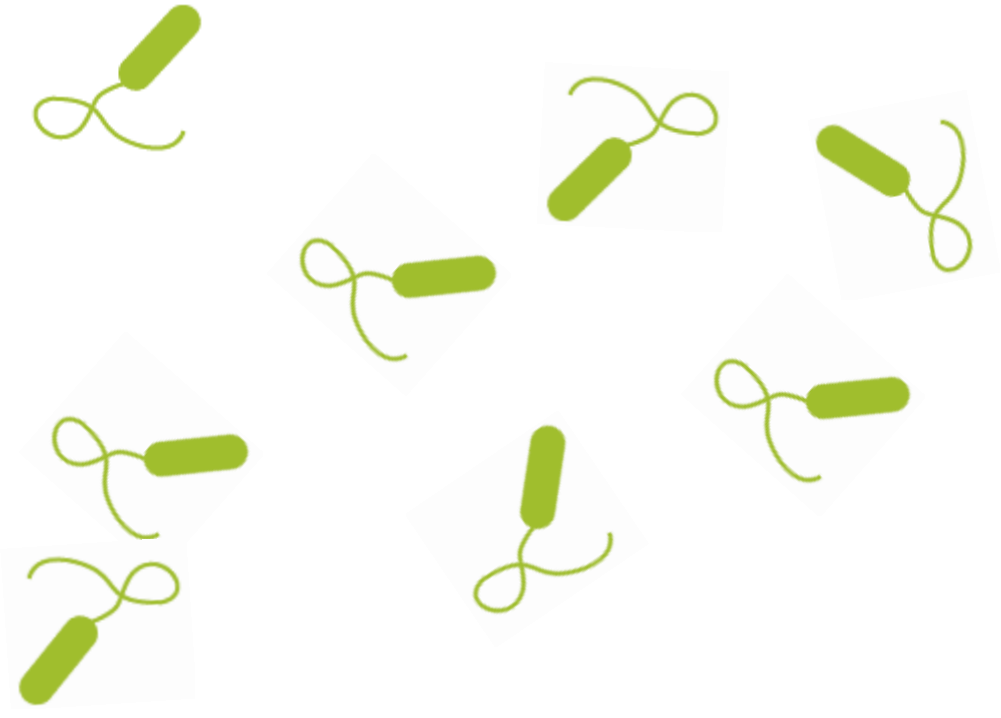
The old microbiology of CF: one microbe, one disease



For the past 40 years, the approach to studying infections in the airways of persons with cystic fibrosis (CF) has largely parallelled that taken in the study of other human infectious diseases. A microorganism (the causative agent) recovered in culture from an infected site is studied in isolation using a variety of in vitro and in vivo models intended to approximate some facet of the human infection.

LiPuma J. 2012 *Thorax* 67: 851

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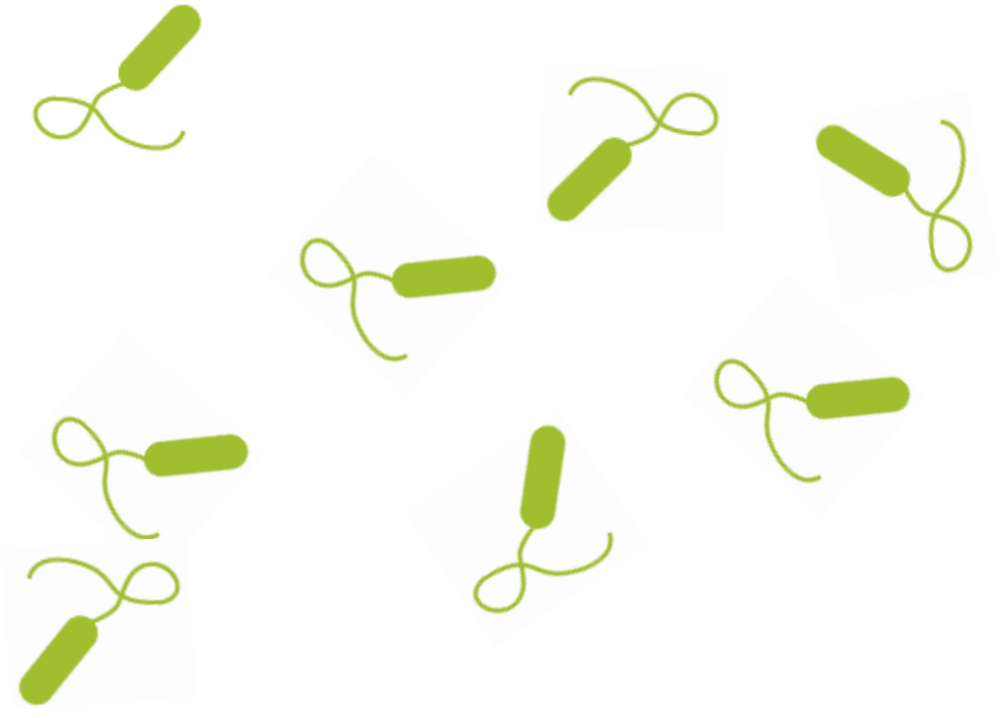

For the past 40 years, the approach to studying infections in the airways of persons with cystic fibrosis (CF) has largely parallelled that taken in the study of other human infectious diseases. A microorganism (the causative agent) recovered in culture from an infected site is studied in isolation using a variety of in vitro and in vivo models intended to approximate some facet of the human infection.

Although this strategy has yielded a wealth of information regarding microbial virulence factors and pathogenic mechanisms for many human infections, its limitations, when applied to the chronic, polymicrobial infections that typify CF, are becoming increasingly obvious.

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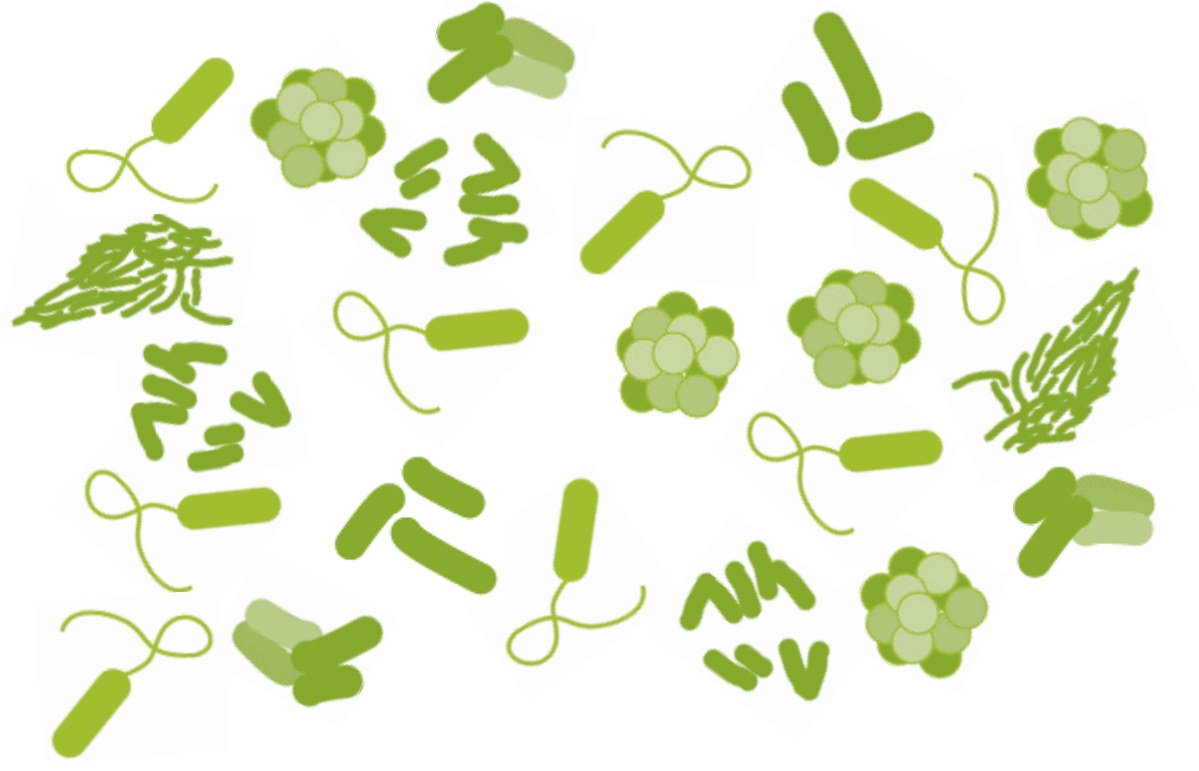
The new microbiology of CF: it takes a community



We now appreciate that respiratory tract infection in CF most often involves diverse communities of opportunistic bacterial species that are well adapted to the peculiarities of this niche.

We have, furthermore, come to understand that species within this community are not merely living unaffected by their microbial neighbours, but rather, are actively engaged with each other. And we are steadily decoding the rules and mechanisms that govern this microbial concert.

LiPuma J. 2012 *Thorax* 67: 851

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The new microbiology of CF: it takes a community

Given this expanding understanding of infection in CF, it seems reasonable to shift our attention towards a conceptual framework that considers the airway microbial community as the 'pathogenic unit.'

Lung microbiome as the 'pathogenic unit'.

LiPuma J. 2012 *Thorax* 67: 851
O'Toole G.A. 2018 *J Bacteriol* 200: e00561-17

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Microbiomics to define CF management

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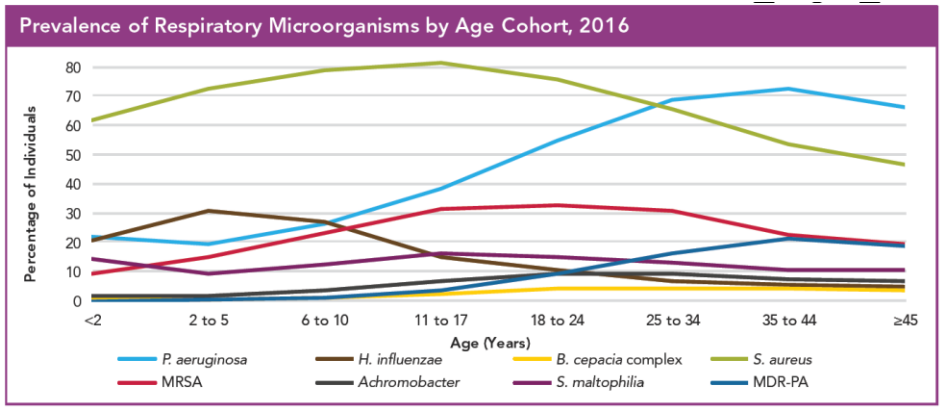
Translating Microbiomics to CF management

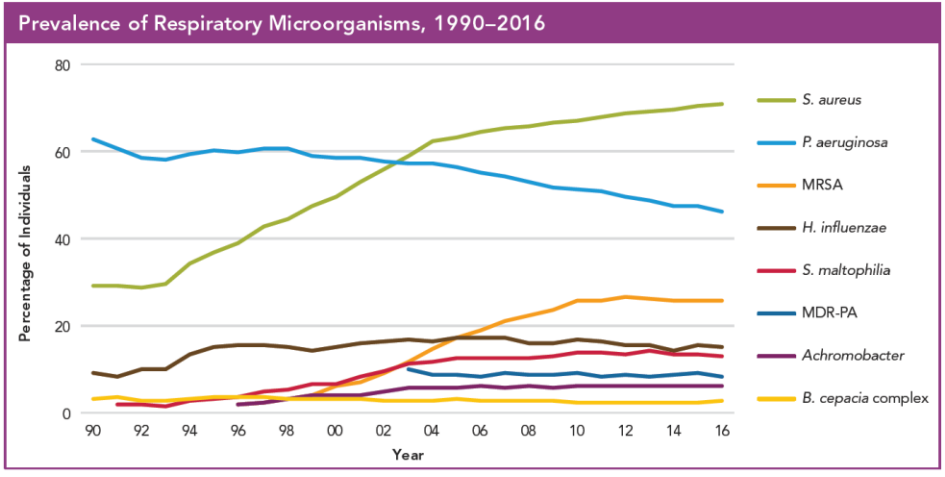
1. Towards microbiome-driven epidemiology of CF infection
2. Antibiotic susceptibility in a microbiome context
3. Personalised models of infection
4. Development of the CF gut microbiome in early life

1. Towards microbiome-driven epidemiology of CF infection

CF Trust and CFF registry data, have provided a wealth of information on the epidemiology of emblematic CF pathogens – by age and through years.

With emergent pathogens of concern such as NTMs and *Aspergillus* now being incorporated – certainly by the CF Trust UK registry.



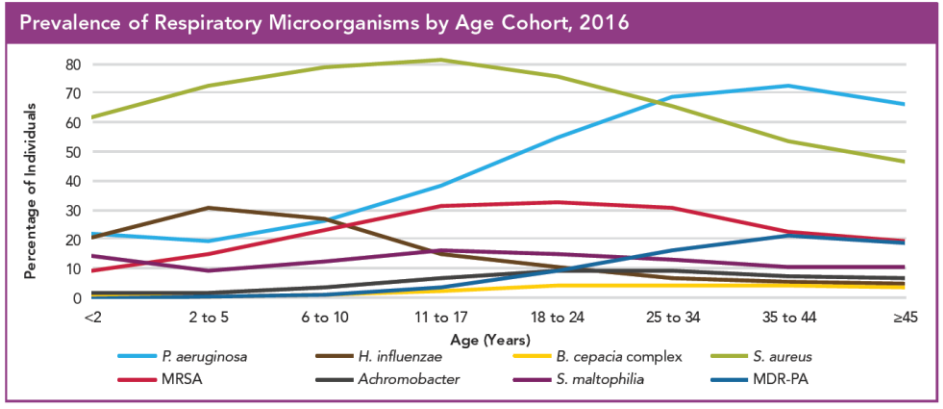


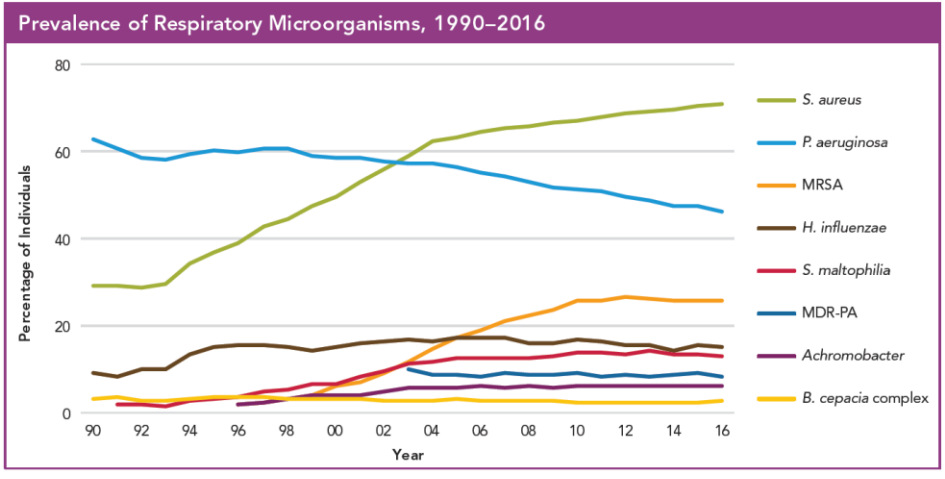
CFF patient registry annual data report 2016

1. Towards microbiome-driven epidemiology of CF infection

This approach cannot provide retrospective examination of emergent pathogens (e.g. *Pandoreara* spp.) or enigmatic anaerobes (e.g. *Prevotella* spp, *Veillonella* spp. and *Rothia*)

But high-throughput sequencing approaches can account for the whole microbiome by targeting the bacterial microbiota (16S rRNA gene) and fungal microbiota (Internal Transcribed Spacer [ITS] region).





CFF patient registry annual data report 2016

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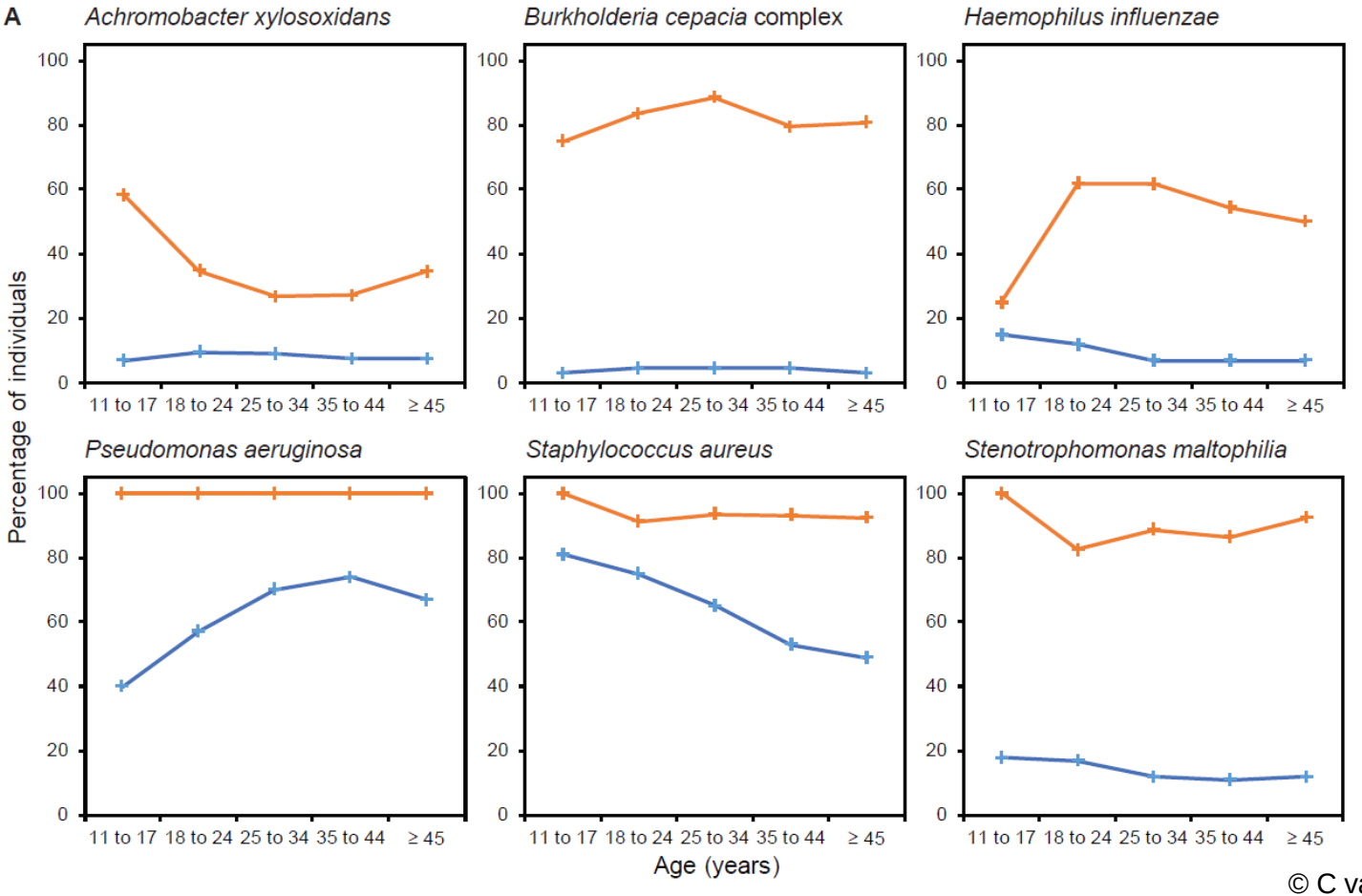
Pathogen prevalence



Registry

Our study

Samples from 297 CF patients (11 to 71)

From 12 UK & US CF Centres

16S rRNA gene targeted amplicon sequencing

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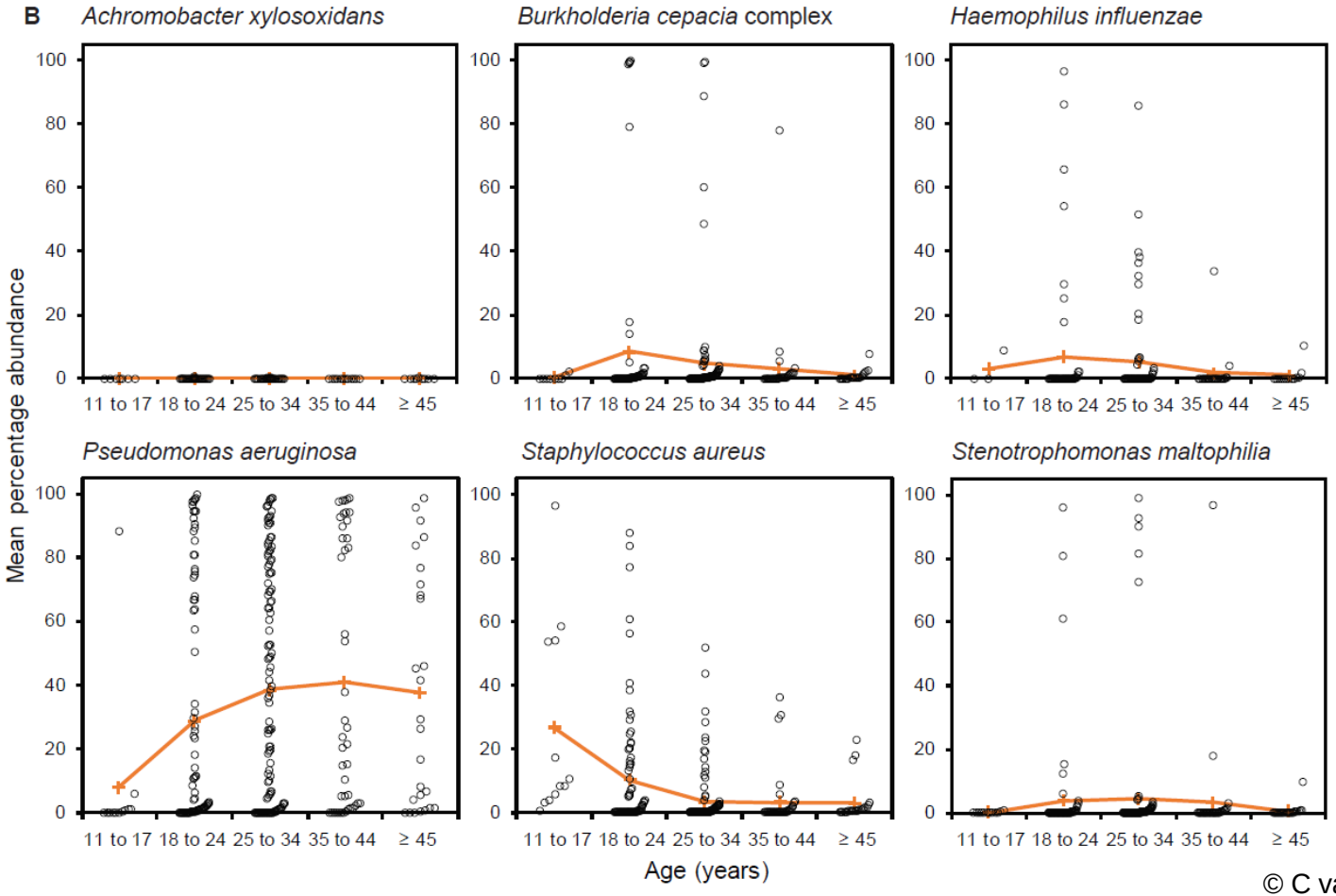
Pathogen abundance



Mean abundance

○ **Abundance in an individual patient**

No registry captures abundance / infection load measures!

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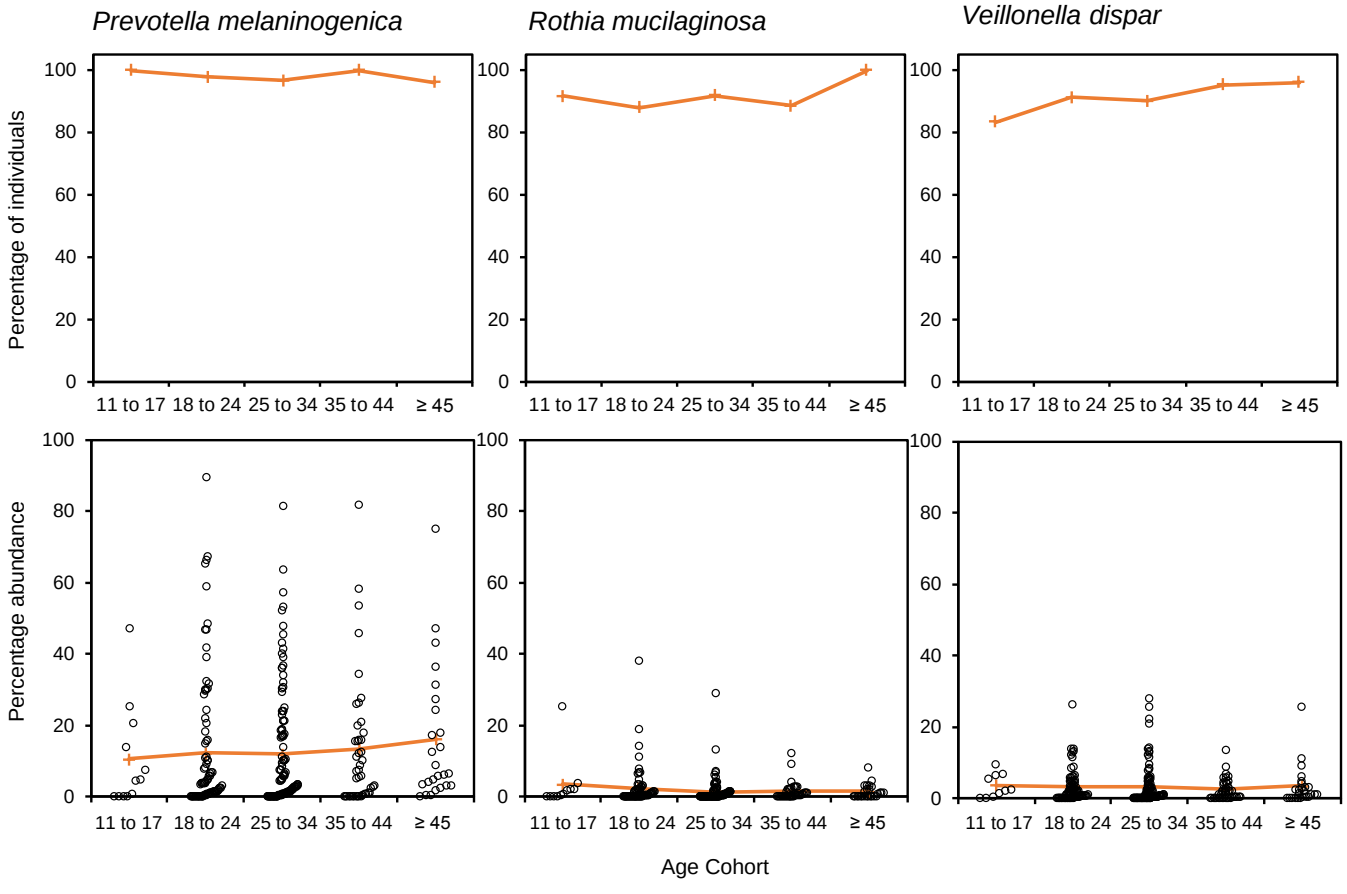
Utility to look at other microbes (even retrospectively)



And combinations of microbes.

Relate back to clinical data

The utility is vast.

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Towards microbiome-driven epidemiology of CF infection

- Start with Manchester Paediatric (RMCH *n* = 355) and Adult (MACFC *n* = 431) Centres
- Bacterial and fungal microbiota through NGS
- Capturing all bacterial and fungal taxa
- Sample on annual review each year
- Allows to refine and improve this longitudinal CF population study approach, with a view to expanding across UK.
- This would be a first globally

Back to abundance - A treatment decision scenario

Two patients: Patients A & B
Similar clinical characteristics

Pathogen '*X*' is detected

Would those two patients be
treated the same / similar?

A treatment decision scenario

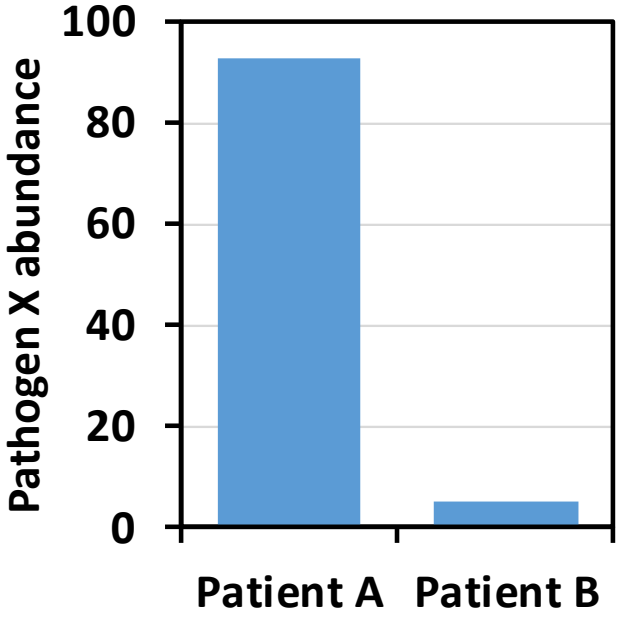
Two patients: Patients A & B
Similar clinical characteristics

Pathogen '*X*' is detected

Would those two patients be treated the same / similar?

Knowing there are different 'amounts' of the pathogen infecting the two patients

Would that change the way these two patients are treated for this pathogen?



Exacerbation study

Cuthbertson et al, 2016 *ISME J* 10: 1081-1091

Table 1 Summary of clinical characteristics for individual patients

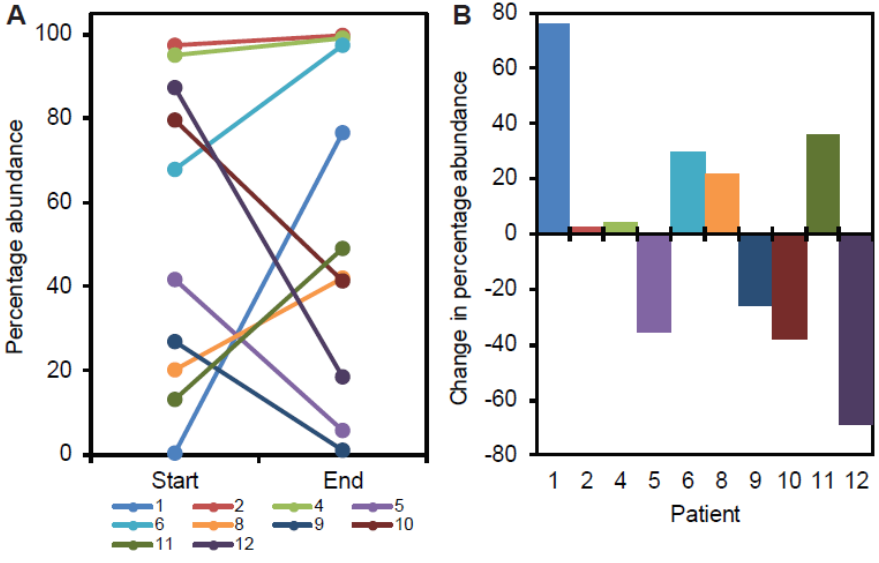
Patient	*Age (years)*	*Gender*	*CFTR genotype*	*BMI*	*CF diabetes*	*CFPE antibiotics*[a]
1	30	Male	ΔF508/NK	29	No	Ciprofloxacin p.o.
2	45	Female	ΔF508/NK	18.2	Yes	Colomycin i.v.+Tobramycin i.v.
3	47	Male	ΔF508/NK	19.9	Yes	
4	22	Female	ΔF508/ΔF508	18	No	Cirprofloxacin p.o., then, Meropenem i.v.+Amakacin i.v.
5	55	Male	ΔF508/G58E	23.9	No	Ceftazidime i.v.+Gentamicin i.v.
6	21	Female	ΔF508/ΔF508	20.3	No	Ciprofloxacin p.o.
7	40	Male	ΔF508/ΔF508	19.4	Yes	
8	22	Male	ΔF508/ΔF508	18.4	Yes	Meropenem i.v.+Colomycin i.v.
9	17	Female	ΔF508/ΔF508	22.5	No	Ceftazidime i.v.+Gentamicin i.v.
10	24	Female	ΔF508/G542X	21	No	Clarithromycin p.o.
11	20	Male	ΔF508/ΔF508	20.4	No	Ciprofloxacin p.o.+Metronidazole
12	20	Male	ΔF508/ΔF508	28.5	No	Ceftazidime i.v.+Gentamicin i.v.

Abbreviations: BMI, body mass index (kg m^2); CFTR, cystic fibrosis transmembrane conductance regulator; NK, genotype not known (the clinical and functional translation of CFTR (CFTR2); http://cftr2.org).
[a]Antibiotics administered as intervention for a clinically defined CFPE: p.o., oral; i.v., intravenous.

All patients chronically colonised with *Ps. aeruginosa* ∴ treatment for exacerbation based on eradication of this pathogen.

☐ *denotes patients that experienced exacerbation in study*

Treatment effects by individual patient – *Ps. aeruginosa*



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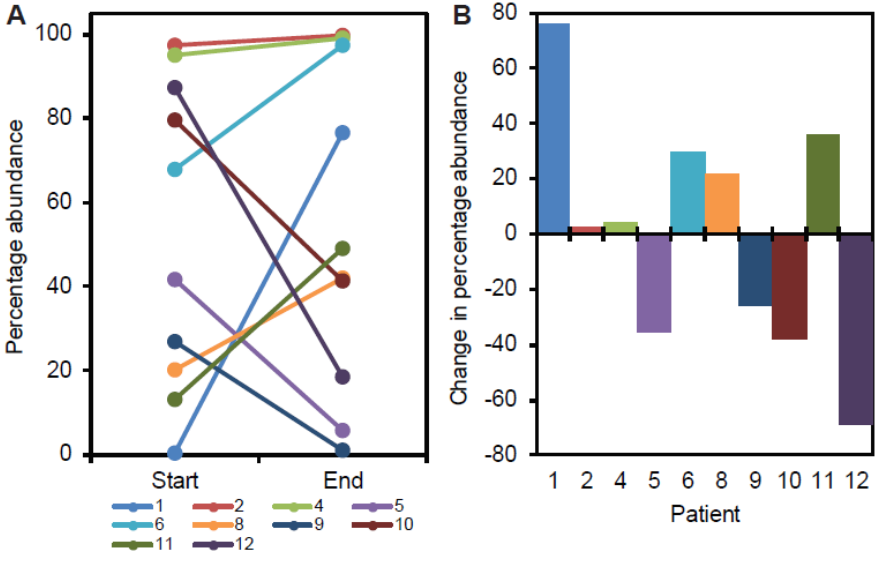
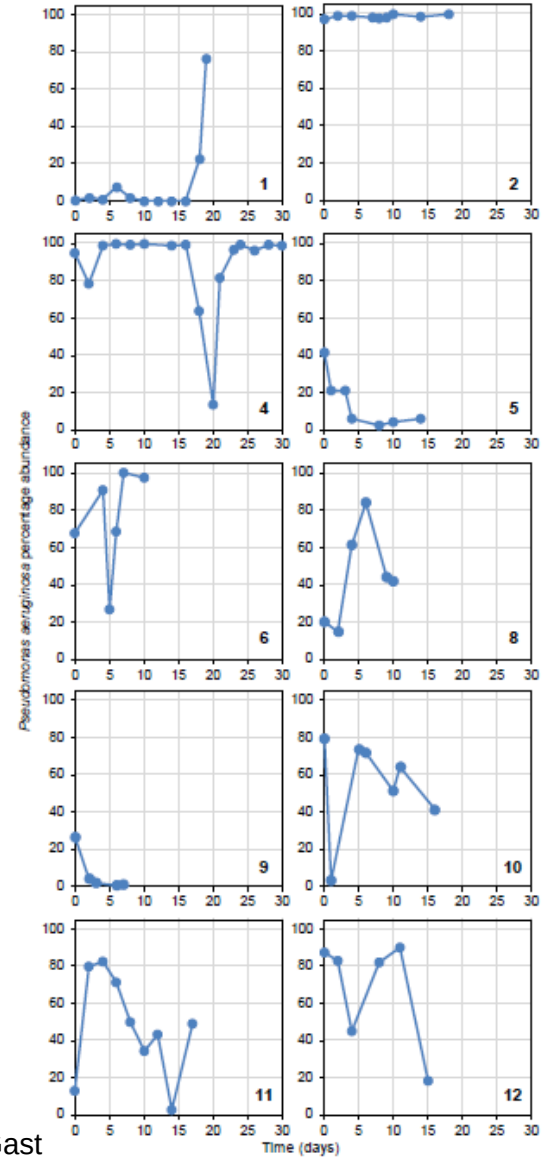
Treatment effects by individual patier





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Translating Microbiomics to CF management

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2. Antibiotic susceptibility in a microbiome context

Clinical relevance / accuracy of susceptibility testing has been scrutinized and questioned – not optimal.

For example in CF, susceptibility profiles from last visit to clinic are used to inform treatment regimen for a current exacerbation (the last visit could've be from months ago).

A patient's lung microbiome as the 'pathogenic unit'

2. Antibiotic susceptibility in a microbiome context

- Use the lung microbiome as the 'pathogenic unit'*
- Patient's sputum (intact microbiome) onto a CF lung epithelium cell line
- Importantly, allowing replication of the pathogen-host environment
- Test with and without antibiotics using Quantitative-PCR detection of all bacteria (16S rRNA gene) and all fungi (Internal Transcribed Spacer [ITS]) to measure growth, so indicating susceptibility or resistance – incorporates abundance
- More rapid, accurate (and cost-effective) than existing methods
- Can also be targeted at pathogens of concern, e.g. *Ps. aeruginosa*, NTM species, …

Li Puma, J. 2012 *Thorax* 67: 851

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3. Personalised models of infection

- At MMU we are able to generate CF patient-specific induced Pluripotent Stem Cells (iPSCs) that can be directed to become lung epithelium cells.
- Incorporate the patient's own CFTR mutation and their underlying genetic factors.
- Crucially, (1) Reproducible. (2) iPSCs derived from blood sample ∴ not infected / contaminated. (3) iPSCs can be used indefinitely / lung cell lines 3-4 months. (4) can induce and measure inflammation on lung cell lines.

McCauley, K.B. *et al* 2017 *Cell Stem Cell* 20: 844-857

3. Personalised models of infection

- Critically, we can (replicated) recreate host-pathogen interactions by directly introducing the patient's own sputum (intact microbiome) or strains of their pathogens to the cell lines.
- The power of this approach could, for example, direct optimisation of personalised exacerbation treatment, personalised host-pathogen responses to existing and new CFTR modulators, to helping direct treatment for pathogens that would prevent being on transplant lists….

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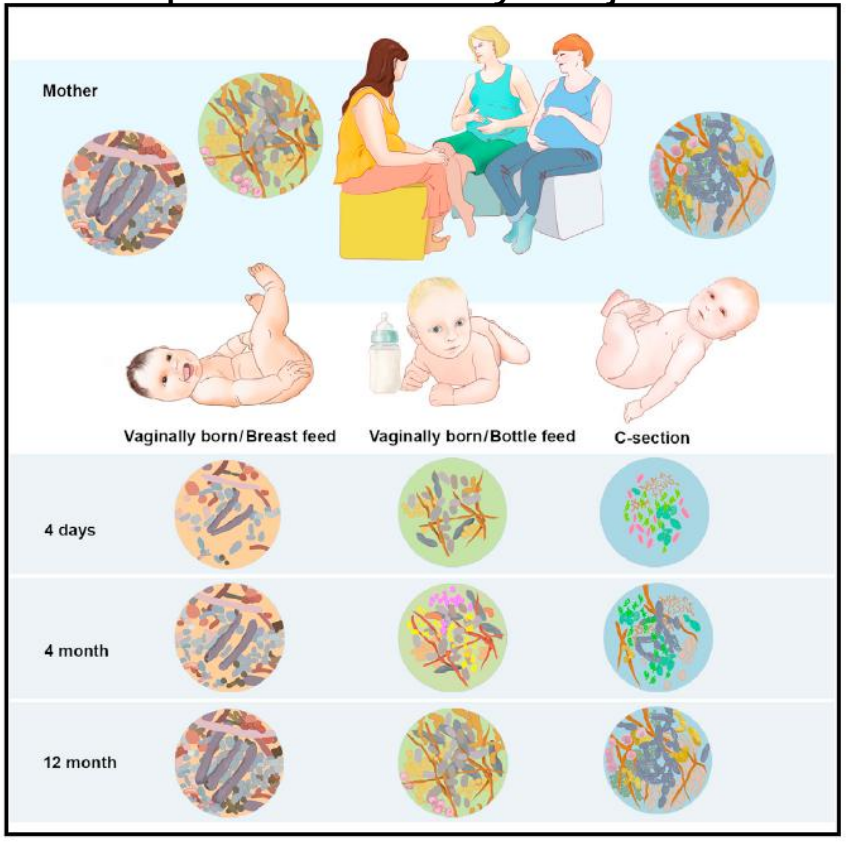
4. Development of the CF gut microbiome in early life

Development in healthy subjects*



Dysbiosis in both the respiratory system and gut contribute to undernutrition, growth failure, and long-term respiratory and systemic morbidity in infants and children with CF.

Understanding the role that the respiratory AND gut microbiomes play in health or disease progression in CF will afford opportunities to better identify interventions to affect clinical changes

There is a critical need to quantitatively and functionally examine the establishment of the CF gut microbiome in early life.

Bäckhed *et al* 2015 *Cell Host & Microbe* 17: 690-703

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