Bacterial typing in cystic fibrosis patients: When and Why?

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Disclosures

• Nil to declare
Bacterial typing in cystic fibrosis patients

• WHY
  – Acquisition and cross-infection
  – *P. aeruginosa* eradication
  – Organism identification

• WHEN
  – Standards of CF care & Consensus guidelines
  – Recommendations

• OTHER CONSIDERATIONS
CF and bacterial infection

- Defective CFTR gene
  - dehydrated mucous buildup
  - impaired mucociliary clearance
  - bacterial infection
Non tuberculous mycobacteria = 12% prevalence (2015)
- *M. abscessus* & *M. avium* complex (MAC) most common
Most CF pathogens come from the environment. So why type them at a subspecies level?
Environmental acquisition occurs frequently, but so does cross-infection

- **B. cepacia complex**
  - 20 species described
  - Reported outbreaks:
    - *B. cenocepacia*
    - *B. dolosa*
    - *B. multivorans*
  - Patient segregation
  - Climatic factors also play a role in ENV acquisition
TABLE 4 Correlation of yearly incidence rates of nonepidemic strains of *B. cepacia* complex infection in Brisbane and Townsville with local weather conditions, 2001 to 2011

<table>
<thead>
<tr>
<th>Meteorological variable</th>
<th>Brisbane</th>
<th></th>
<th>Townsville</th>
<th></th>
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<tbody>
<tr>
<td>Rainfall</td>
<td>0.65</td>
<td>0.031</td>
<td>0.82</td>
<td>0.002</td>
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<tr>
<td>Dew point</td>
<td>0.47</td>
<td>0.147</td>
<td>0.25</td>
<td>0.453</td>
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<tr>
<td>Temperature</td>
<td>0.04</td>
<td>0.911</td>
<td>-0.51</td>
<td>0.113</td>
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</tbody>
</table>
**P. aeruginosa** cross-infection also occurs frequently

- **Shared CF *P. aeruginosa* strains detected worldwide**
  - UK & Europe  
  
  Fothergill et al, ERJ 2012
  - North America  
  
  Aaron et al, JAMA 2010
  - Australia  
  
  Kidd et al, ERJ 2013
  
  - national prevalence study (n=983)
  
  - ~60% had ≥1 shared strain

- **Assoc. with:**
  - ↑ treatment
  
  Aaron et al, JAMA 2010; Kidd et al, ERJ 2012
  - ↓ clinical outcomes
  
  - Patient mobility between CF centres

- **Patient segregation**

**Strain**    | **No. of patients (% prevalence)** | **No. of CF centres (n=18)**
--- | --- | ---
AUST-01 | 220 (22) | 17
AUST-02 | 173 (18) | 16
AUST-04 | 47 (5) | 12
AUST-05 | 37 (4) | 6
AUST-06 | 31 (3) | 4
M. abscessus complex epidemiology also suggests person-to-person transmission

- **Subspecies:**
  - *M. a. abscessus*
  - *M. a. massiliense*
Through what mechanisms does cross-infection occur?

- Environmental ‘reservoirs’ not found
  

- Short-term air & surface contamination of inpatient rooms
  
  → Contact and droplet transmission

  Jones et al, Thorax 2003
  Panagea et al, J Hosp Infect 2005

- Viable organisms also present in airborne droplet nuclei
  - can travel 4 mts & survive 45 mins after coughing
  
  → Airborne transmission

  Clifton et al, BMC Micro 2008
  Knibbs et al, Thorax 2014
  Bryant et al, Science 2016
Other reasons for genotyping CF pathogens

- **Assessment of* P. aeruginosa* eradication success**
  - Early infection aggressively treated to delay chronic infection
  - There is no definitive definition of “eradication”
  - Genotyping pre- & post-treatment rarely assessed
    - But critical for differentiating failure from re-infection

Adapted from Kidd et al, J Cyst Fibros 2015

![Graph showing patient age vs. treatment type and genotype](image)
Reinfection with different genotype $\rightarrow$ Eradication success

+ve BAL $\rightarrow$ Eradication failure

+ve OP: same genotype

LRT re-infection same genotype

\[ \Delta = \text{BAL} \]
\[ \square = \text{OP} \]

Different colour = different genotype
Genotypic methods are also useful for identification

- **Multilocus sequence typing (MLST)**
  - *Burkholderia* spp.
  - *B. cepacia* complex
  - ID of non-*Bcc* species
  - Novel species characterisation

- **Achromobacter spp.**
  - ~50% of *A. xylosoxidans* misidentified
  - Biochem. & MALDI-TOF inadequate

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<table>
<thead>
<tr>
<th>Isolate fields</th>
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<td>species</td>
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<tr>
<td><em>Burkholderia cepacia</em></td>
<td>4</td>
</tr>
</tbody>
</table>

*Spilker et al, J Clin Micro 2009*
*De Smet et al, IJSEM 2015*
*Ridderberg et al, J Clin Micro 2012*
*Rodrigues et al, J Clin Micro 2015*
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7.3. Clinical microbiology services and the CF MDT

The following items should be agreed between the CF MDT and the clinical microbiology service.

- Typing methods and frequency of typing (i.e. how often the CF MDT should send samples for routine surveillance and when additional typing should be done due to suspicion of cross infection)
Trump May Have given North Korea a Warning. Did Pyongyang Hear It?

European Divorce: Uncertainty in the Wake of “Brexit”

CF Microbiologists still unsure when to undertake genotyping!
Cystic Fibrosis Trust

Standards for the Clinical Care of Children and Adults with cystic fibrosis in the UK

Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis.
First edition. September 2010

Mycobacterium abscessus
Suggestions for infection prevention and control (Interim guidance – October 2013)
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Suggestions for infection prevention and control (Interim guidance – October 2013)

• Regular monitoring for cross-infection and epidemiological surveillance should take place..

• P. aeruginosa:
  – Surveillance on all new isolates
  – Annually on a relevant proportion of patients

• B. cepacia complex:
  – All confirmed B. cenocepacia isolates

• M. abscessus:
  – Annual (at least) screening
  – Knowledge of incidence & prevalence
  – All +ve patients must have an isolate typed

Note: US CFF & ECFS consensus NTM recommendations do not mention strain typing

Floto et al, Thorax 2016
• **B. cepacia complex:**
  - All initial isolates
  - At least 1 isolate/year
  - Any suspected outbreak isolates

• **Surveillance:**
  - Quarterly assessment
  - Incidence & prevalence
  - Local lab &/or Data Registry

• **Other organisms (e.g. P. aeruginosa, NTM)**
  - When epidemiologically indicated
Underpinned by knowing what's in & what might be in your backyard

An unwelcome backyard visitor during the recent Cyclone Debbie floods
WHEN to genotype?: Consensus of the consensuses

- **Surveillance**
  - Regular review of local incidence & prevalence rates for **all CF pathogens**
    - Targeted genotyping when outbreaks are suspected

- **P. aeruginosa**
  - New infection
  - Annually on a relevant proportion of patients

- **B. cepacia complex & NTM**
  - New infection
  - Annually on all patients

- Close relationship between clinical & lab teams is **IMPERATIVE!!**

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Know your bug: Population structure & evolutionary divergence require careful consideration

CLONAL: ↑ discrimination

NON-CLONAL: ↓ discrimination
Identifying outbreaks is often problematic

• Chronic infection (months to years)

• Initial point of infection with individual strains often unknown

• Epidemiological relationships often poorly (or not) defined

• Highlights the need for:
  – Regular surveillance
  – Capacity to identify emerging strains
Reproducibility & comparability is paramount

- **Reproducibility**
  - Longitudinal surveillance

- **Comparability**
  - Within and between labs
  - Local, nationally and globally
  - Curated data sharing tools
Moving with the times

- **Indirect methods**
  - PFGE & PCR fingerprinting (e.g. RAPD, ERIC)
  - Modest reproducibility
  - Difficult to compare

- **Direct methods**
  - MLST, SNP-based assays (ArrayTube, MALDI-TOF), VNTR
  - Whole genome sequencing
  - High reproducibility
  - Database friendly
Whole Genome Sequence Data

Akin to drinking from a firehose!
Help is on the way

• User friendly software
  – CLC Genomics Workbench
  – Nullarbor

• Data quality checks
  – Coverage
  – Contamination
  – Speciation

• MLST
• Resistome
• Core genome SNP Phylogeny
Summary

• WHY
  – Acquisition & cross-infection
  – *P. aeruginosa* eradication efficacy
  – Organism identification

• WHEN
  – Regular surveillance
  – New infections
  – Suspected outbreaks

• OTHER CONSIDERATIONS
  – Reproducible & comparable
  – Emergent strain detection
Acknowledgements

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Patients and their families
Many thanks for your attention!

Any questions?