Cystic Fibrosis cultures: What’s clinically needed and practical

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(I have no financial disclosures.)
What is Cystic Fibrosis (CF)?

- Mutation in the Cystic Fibrosis transmembrane conductance gene (CFTR)
- It is the most common and important autosomal recessive genetic disease in the Caucasian population
  - 1 in 3300 Caucasians births
  - 1 in 10,000 -15,000 Hispanic and African-American births
  - 1 in 30,000 Asian births
CF clinical and research structure

- CF Foundation Patient Registry
  - Coordinates information on the health of 27,000 people from CF Foundation accredited care centers.

- UNC Cystic Fibrosis and Pulmonary Research and Treatment Center:
  - Pathogenesis and therapy of CF and other pulmonary diseases
  - Research areas: Genetics, Transplant, Animal models, Virtual Lung, Mucus, Cell Biology, Pharmacology, Ion Transport, and Microbiology

- UNC McLendon Clinical Laboratories
  - CF cultures annually: ~4000 (2014) with ~88% positivity
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Goals of the CF Bench

- Identify known principle pathogens:
  - Contribute to decreased lung function
  - Impact epidemiologic and/or infection control practices
- Select proper media for isolation of principle pathogens
- Perform Susceptibilities
- Stock isolates for future testing and research
Known CF Principle Pathogens

- **Staphylococcus aureus:**
  - Methicillin resistant
  - CF Nursing Coordinators notified of 1st isolation of MRSA
  - Small colony variant (SCV)
  - Vancomycin-intermediate S. aureus (VISA)

- **Pseudomonas aeruginosa:**
  - Mucoid: Chronic inflammatory response that damages the lungs

- **Burkholderia cepacia complex: cenocepacia, dolosa, and multivorans**
  - Cepacia syndrome;
  - *B. cenocepacia* is an absolute contraindicated for lung transplant
  - CF Nursing Coordinators notified of 1st isolation of *Burkholderia cepacia* complex

- **Mycobacteria: M. abscessus group**

- **Mould: Aspergillus sp., Scedosporium sp.**

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Other organisms\textsuperscript{1} we will encounter

- **Likely CF Pathogens**
  - *Haemophilus influenzae*
  - *Mycobacterium avium* complex

- **Unlikely CF Pathogens**
  - *Stenotrophomonas maltophilia*
  - *Achromobacter* sp.
  - *Ralstonia* sp.
  - *Burkholderia gladioli*\textsuperscript{*}
  - *Chryseobacterium* sp.

- **Unknown CF Pathogens**
  - *Pandoraea* sp.
  - *Burkholderia cepacia* complex
    (not *B. cenocepacia*. *B. multivorans*, *B. dolosa*)
  - *Trichosporon* sp.
  - *Inquilinus* sp.
  - *Nocardia* sp.

Getting started – Culture Media

- CF specific culture that differs from routine culture
  - Gram stain screening not needed
- Routine Media: Chocolate, CNA, and MacConkey

1. Specialized Media\(^1\): *Burkholderia cepacia* Selective Agar (BCSA) incubated in Non-CO2 at 35°C
   - CF infection control guideline
   - Gentamicin, Vancomycin, Polymixin B, and Crystal Violet
   - *B. cepacia* will cause a yellowing of the agar around the colony
   - *Burkholderia* sp., *Pandoraea* sp., *Achromobacter* sp., *S. maltophilia*, Rapidly growing mycobacteria

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2. Specialized Media: Chromogenic *S. aureus* agar\(^1\) or Mannitol Salt Agar (MSA) incubated in Non-CO2 at 35\(^\circ\)C for 3 days

- MSA 7.5\% NaCl
- *Staphylococcus aureus* ferments the mannitol producing a yellow halo around the colony

Documentation of patient culture history... Determining what to do.

- **Day One:** Documented patient culture history and in/out patient status.
- **First time principle pathogens**
  - Regardless of quantity: identified and susceptibilities done
- **Outpatients with a positive culture history: >10 colonies**
  - Gram Negative Rods at 3 month intervals
  - <3 months MALDI-TOF identification, susceptibilities by physician request
  - *Staphylococcus aureus* full susceptibilities done annually
  - MSSA: oxacillin and vancomycin screening
  - MRSA: “Probable MRSA, Susceptibilities upon request”
  - Exceptions to outpatient guidelines:
    - <10 colonies if >year: *S. aureus, P. aeruginosa* and *Burkholderia* sp.
- **Inpatients**
  - Gram negative rods only,
  - unless last clinic culture is within 14 days of admission
  - MSSA: oxacillin and vancomycin screening
Day Two Isolation of MacConkey

Smooth *Pseudomonas aeruginosa* (non-mucoid)  

Mucoid and Smooth *Pseudomonas aeruginosa*
Isolation of Pseudomonas aeruginosa

A: mixed

B: Smooth

C: mucoid

D: rough
Day Two Isolation of BCSA

*Burkholderia multivorans*  
*Burkholderia cenocepacia*
Mucoid *P. aeruginosa* on MacConkey and *Burkholderia* sp. on BCSA
Isolation continued

Mucoid *Achromobacter* sp.
48 MacConkey

Mucoid *Achromobacter* sp.
48 CNA
Day Two *Staphylococcus aureus*

- Not necessary to isolate for ID/Sensitivities
  - Staph Latex, Tube-Coagulase, MALDI-TOF MS
  - Caution ~1% fail to ferment mannitol
- Yellow Colony could also be Small Colony Variant (SVC) of *Staphylococcus aureus*
Small Colony Variant
*Staphylococcus aureus*

- Result of treating with trimethoprim-sulfamethoxazole (TMP-SMX) and oral cephalosporins
- Resistant to aminoglycosides
- Nutritionally deficient variants requiring 1% thymidine, or CO2 for susceptibility testing
- 21 patients (6%) (2014)
  - 9 MRSA and 12 MSSA
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## Identification of Gram Negative Rods

<table>
<thead>
<tr>
<th>Organism</th>
<th>Oxidase</th>
<th>Colony Morphology</th>
<th>Manual ID</th>
<th>Instrumentation ID</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>POS</td>
<td>Mucoid, Hemolytic, metallic</td>
<td>TSI, PV+, Colistin-Susceptible</td>
<td>MALDI-TOF, Automated ID systems</td>
</tr>
<tr>
<td><em>S. maltophilia</em></td>
<td>NEG</td>
<td>Non-hemolytic, slight yellow pigment</td>
<td>TSI, OF sugars</td>
<td>MALDI-TOF, Automated ID systems</td>
</tr>
<tr>
<td><em>Achromobacter sp.</em></td>
<td>POS</td>
<td>Small grey non-hemolytic</td>
<td>TSI, OF Sugars, Colistin-variable, Aminoglycoside Resistant</td>
<td>16s Sequencing, MALDI-TOF Automated ID systems</td>
</tr>
<tr>
<td><em>Chyrseobacterium sp.</em></td>
<td>POS</td>
<td>Hemolysis variable, very yellow</td>
<td>N/A</td>
<td>16s Sequencing, Automated ID Systems MALDI-TOF</td>
</tr>
</tbody>
</table>
Other Environmental Gram Negative Rods

<table>
<thead>
<tr>
<th>Organism</th>
<th>Oxidase</th>
<th>Colony Morphology</th>
<th>Manual ID</th>
<th>Instrumentation ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pandoraea sp.</td>
<td>POS/NEG</td>
<td>Non-hemolytic BCSA small pink</td>
<td>N/A</td>
<td>16s Sequencing MALDI-TOF +/-</td>
</tr>
<tr>
<td>Ralstonia sp.</td>
<td>POS</td>
<td>Non-hemolytic BCSA small pink</td>
<td>N/A</td>
<td>16s Sequencing, MALDI-TOF</td>
</tr>
</tbody>
</table>

Identified by 16s Sequencing or MALDI-TOF
- *Bordetella* sp.
- *Comamonas* sp.
- *Herbaspirillum* sp.
- *Elizabethkingia* sp.
- *Cupriavidus* sp.
- *Ochrobactrum* sp.

Reported as:
- Gram Negative Rods NOT *Burkholderia cepacia* complex,
- *Ralstonia* sp. or *Pandoraea* (XBRP)
- ❖ No Susceptibilities done
Burkholderia sp. Identification (pre-MALDI-TOF)

- Oxidase variable, non-hemolytic, foul smelling
  - Colistin and aminoglycoside resistant
- Commercial phenotypic systems\(^1\) have difficulty accurately identify *B. cepacia* complex from other glucose non-fermenters and between the differing *Burkholderia* sp.
- 16S rRNA gene sequencing:
  - *Burkholderia cepacia* complex and *B. gladioli*
  - Turn around time: 7-10 days
  - Further speciation of the *Burkholderia cepacia* complex requires sequencing additional genes (i.e., *recA* gene)
- The US CF Foundation *Burkholderia cepacia* Research Laboratory and Repository at the University of Michigan ran by Dr. John LiPuma
  - Support CF Centers by offering for free identification of *Burkholderia cepacia* complex
  - Simultaneously 16S rRNA gene sequencing and Dr. LiPuma identification

Burkholderia sp.
MALDI-TOF Identification

- ¹Bruker Biotyper (v 3.0) vs Vitek MS (RUO SARAMIS v 3.62)
  - 72.5% vs 80% to species level
- ²Bruker Biotyper(v3.2) vs Vitek MS (RUO SARAMIS v 4.09) vs Vitek MS (IVD v 2.0)
  - B. cepacia complex to species level: 70.3% vs 75.7% vs 59.5%
  - B. gladioli: 95.1 vs 100% vs 100%
- Advantage to using MALDI-TOF
  - Same day TAT identification: B. gladioli and B. cepacia complex

**Burkholderia sp.**
**MALDI-TOF Identification**

- VITEK MS (IVD v.2)
  - Database: *B. gladioli; B. cepacia* (implies complex); *B. multivorans; B. stabilis; B. vietnamensis*
  - Score 90% *B. gladioli* and *B. multivorans*
  - Score 90% *B. cepacia* (complex) sent to Dr. LiPuma lab
  - Future VITEK MS IVD v3 database: *B. cenocepacia* and other complex members
    - ASM 2015 Poster by Dr. Rongpong Plongla et al.
Susceptibility testing

- Vitek 2/MicroScan/Phoenix
  - normal phenotype of *Staphylococcus aureus*
- Kirby Bauer Method
  - Non-*Pseudomonas*, Non-*Burkholderia cepacia complex*, Non-*Stenotrophomonas maltophilia*
    - Utilize *Pseudomonas* CLSI breakpoints
    - Isolate comment: “Nonstandarized susceptibility”
- Growth Method
  - CLSI: Performance Standards for Antimicrobial Disk Susceptibility Tests
- Pan-Resistant: 2 consecutive susceptibility tests
  - “Susceptibilities not performed. Previous isolate Pan Resistant.”

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Mycobacteria

- **M. avium complex** and **M. abscessus**
- **AFB culture**
  - Challenge of AFB culture:
    - 50% – 70% patient have *P. aeruginosa* that are resistant to specimen decontamination process using 0.25% N-acetyl cysteine-1% sodium hydroxide
    - Add 2.5% Oxalic acid, decreased contamination rate to 3% to 5%
    - Caution low number of mycobacteria may not be recovered
- **Identification and Susceptibilities**
  - **M. avium complex**
    - 16S rRNA gene sequence analysis every 6 months
    - Susceptibilities: by physician request to reference lab
  - **M. abscessus group**
    - MALDI-TOF or *hsp65* gene sequencing
    - Susceptibilities: annually in house by Trek Panels
M. abscessus group has earthy, musty smell

Detects lower numbers of rapid growers that otherwise are lost in the decontamination process.

Requested by physician in conjunction with the CF culture

Media: *Burkholderia cepacia* selective agar (BCSA)

1\(^{st}\) 4 days at 35\(^\circ\)C

5 - 14 days at 30\(^\circ\)C

Fungus

- Routine CF culture: *Aspergillus fumigatus* (ASFUM) and *Scedosporium apiospermum*
  - Fungal Culture – Bronchial lavages
- Identification by microscopic means annually
  - < 1 year sight read cultures, presumptive Identification
  - < 1 year unable to sight read, hold for two weeks
    - “Further Identification upon Request”
- *Trichosporon* sp and *Exophiala* sp.
  - pure or predominate amounts
  - Sight read: waxy, wrinkly and cigar shaped on Gram Stain
  - Darkly pigmented or Black

Day 4: ASFUM on BCSA/CNA

Day 4: *Trichosporon* sp. CNA

*Scedosporium* sp

Day 4: *Exophiala* sp.
Stocking of Isolates

- Freeze -70⁰ TSB/Glycerol
- 1st Time:
  - *Burkholderia* sp., mucoid *Pseudomonas aeruginosa*, MRSA, Small Colony Variant *Staphylococcus aureus*, *Trichosporon* sp.
- Annually: MRSA, *Burkholderia* sp.
- Any organism identified by 16S rRNA gene sequencing
- All isolates that get sent to CF Foundation *Burkholderia cepacia* Research Laboratory and Repository at the University of Michigan
- Holding media TSI or Nutrient Agar slants for 3 months
- VISA – genetic testing
Summary/Key Points

- Start with proper culture media, utilizing selective media for *S.aureus*, *B. cepacia* complex and *M. abscessus* group

- Accurate identification of *Burkholderia cepacia* complex to the species level.

- Ability to stock isolates for furthering research and the repository by Dr. John LiPuma
Thank You

McLendon Clinical Laboratories
Microbiology and Molecular Directors:
Dr. Peter Gilligan and Dr. Melissa Miller
Laboratory Specialist: Melissa Jones
Microbiology/Immunology staff