2013 SEACM Annual Meeting Abstracts

Utilizing New Technologies and Knowledge to Understand Chronic Lung Infections In Cystic fibrosis Patients

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General Overview of Cystic Fibrosis (CF)

- Most common autosomal recessive genetic disease in Caucasian populations but can be seen in other racial groups
    - In Us median life expectancy is approximately 37 years
- Seen primarily in North America, Northern Europe and Australia/New Zealand but foci also seen in Brazil and Argentina
- Mutations in CFTR results in abnormal electrolyte transport causing thick, dry sticky mucus
- Abnormal mucus effects mucocilliary clearance
    - Provides ideal niche for chronic lung infection
    - Series of pulmonary exacerbation cause lung damage which eventually results in death
- Over 85% of premature deaths in CF are due to cardiopulmonary failure secondary to chr9onic lung infection

Cystic Fibrosis Pathogen of Chronic Lung Disease in 2013

Known	**Possible/Likely**	**Unlikely**
Staphylococcus aureus	*Haemophilus influenzae*	*Stenotrophomonas maltophilia*
P. aeruginosa	*MAC*	*Achromobacter*
B. multivorans	*Anaerobic bacteria esp Prevotella*	*Rolstonia spp*
B. cenocepacia	*Streptococcus anginosis group*	*B. gladioli*
B. dolosa	*Viruses*	*Streptococcus pneumoniae*
Aspergillus	**Unknown**	*Candida albicans*
Scedosporium	*Nocardia*	
Mycobacterium abscessus	*Pandoraea*	
	Other members of B. cepacia 17 species	
	Trichosporon	

Impact of Technology of CF Lung transplantation

- Pulse field Gel Electrophoresis (PFGE) *B. cepacia* speciation by specific PCR reactions and then sequencing resulted in the following findings:
    - Transplanted patients with *B. cenocepacia*-poorer outcomes than those with *P. aeruginosa*
    - *B. cenocepacia* spread from CF transplants to other immunocompromised hosts with disastrous consequences
    - Transplantation of CF patients with *B. cenocepacia* discontinued at many centers

Impact of Technology on Understanding *Burkholderia cepacia* impact on CF lung disease

- 1980's-Recognition of *B. cepacia* role in CF lung disease;development of selective media
- 1990's-Lung transplantation developed, PFGE used to understand molecular epidemiology, PCR techniques used for speciating *B. multivorans* and *B. cenocepacia*
- 2000's-Sequencing used to differentiate B. cepacia complex into 17 species, establish that *B. cenocepacia* and *B. multivorans* key CF pathogens.
- 2010's-MALDI-TOF MS will replace sequencing for identification of *B. cepacia* complex organisms.

How B. cepacia and M. abscessus are similar

- Both are environmental organisms-appear to be spread from patient to patient
- Both seem to have a predilection for the lungs of CF patients
- Both are difficult to speciate
- Both are highly resistant
- Both are difficult or impossible to eradicate from CF lungs

Viruses in CF patients

- Influenza is an important CF pathogen
    - Mortality of H1N1-5x general population
- Rhinovirus found in 20-30% of CF patients
    - Higher viral load in exacerbation, can persist for months

Role of Microbiome in CF Lung disease-General Observations

- As lungs disease progressed, diversity becomes less
- *Pseudomonas aeruginosa* predominated in the microbiome in advanced CF lung disease
- Both anaerobes and *Streptococcus anginosus* group are believed to play a role in pulmonary exacerbation.

Eucalypts to Time of Flight:
Current Topics in the Clinical Mycology Laboratory

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Cryptococcus gattii

- Originally known as *C. neoformans* serotypes B and C
- Species only recognized formally since 2002
- Thought to be found only in tropical and subtropical regions
- Thought to be primarily associated with immunocompetent patients
- Emerged in British Columbia in 1999
- Emerged in Pacific Northwest in 2004
    - Most cases in WA and OR

C. gattii surveillance

- The CDC performs passive surveillance for C. gattii in the US
- Vast majority of isolates come from Pacific Northwest and California
- Most are submitted by physicians with patients who are refractory to care or who do not fit the cryptococcosis profile.

Identification of *C. gattii*

- Canavanine-glycine-bromthymol blue (CGB) agar
- DNA sequencing
- MALDI-TOF
- Not Vitek II, API-20C or cryptococcal antigen

Molecular Typing

- Divides isolates into VGI, VGII, VGIII, VGI
- Currently only by DNA-based tests
    - Performed free of charge by CDC
- Multilocus Sequence typing (MLST)
    - Sequence based typing system
    -

***C. gattii* in US**

- Three different Molecular types in Us
    - VGI-throughout US
    - VGIII-primarily in California but found throughout US
    - VGII-almost exclusively in Pacific Northwest US

Why Should We Determine species

- There are differences in the MIC
    - C. gattii MICs are higher
    - C. gattii produces more cryptococcomas
- Do we Need more information than just species
    - VGII strains cause more pulmonary disease and have higher MIC values to fluconazole
    - VGI and VGIII strains cause more meningitis

Do We Need to Identify *C. gattii* in our Hospital?

- Best course of action is to consult with Your ID doctors and medical director
    - Is your area endemic?
    - Do you suspect C. gattii is going undiagnosed?
    - Does your hospital have cases of difficult to manage cryptococcosis or unusual population-healthy people showing up with cryoptococcosis
    - Is it cost effective or do we suspect so few that we can easily send them out

Matrix-Assissted Laser Desorption Ionization-Time of Flight

- Pros
    - Good yeast databases exist and are growing
    - Low daily cost. Rapid
    - Good accuracy for yeasts
- Cons
    - High initial start-up cost
    - No good database for molds yet

Summary

- C. gattii has now been identified across the US
- Molecular typing is available for free from the CDC
- Identification could be beneficial to patient care
- MALDI-TOF is a valid identification system for yeasts
- There is some work to be done for mold identification using MALDI

Back Up Systems-When Automation Fails

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Reasons for Automation Failure

- Disasters-Internal or External
- Instrument Malfunction
- Instrument maintenance
- Organism not in the database
- Drug-bug issue
    - Mucoid
    - Unexpected resistance

Disasters

- Power outage
    - Don't always have warning of outage
    - Completion of testing already in progress
    - Safety of stored microorganisms
- Structural damage: fire, water.flood
- Natural disaster
    - Disruption of electricity and water, supplies, receipt of specimens off site locations
    - Disruption of telephone service
    - Damage to computers, BSC, equipment
- Joint Commission Hospital Emergency Management Guidelines
    - Identify capabilities and establish response in an effort to provide patient care for 96 hours
    - What tests absolutely must be performed
        - Admitted patients and ER patients
    - Laboratory Equipment
        - Essential-Blood culture, ID/AST, BSC's Incubators, refrigerators, freezers
    - Emergency power
    - Environmental factors affecting instrument performance
        - Extreme hear-use of fans or moving equipment to cooler areas
        - Reagent grade water

Blood Culture Systems

- Bottles from other systems-blind subcultures/acridine orange stains
- Disruption of supply
    - Bact/Alert: interchange standard and FAN bottles
    - Bactec: interchange standard and plus bottles
    - Versa Trek: interchange REDOX and EZ draw REDOX
    - Substitute adult bottles for peds bottles
    - Isolater (lysis centrifugation) tubes

Organisms Identification Systems

- The backbone of accuracy is the strength and utility of the database
    - # species in database may be 1 to 100s
    - Software updates provided up to every 4 years
- Problems: unusual microbes or common microbes with atypical phenotypic characteristics
- Result from the most reliable system can be misleading-ID by alternative method
- Desirable system has > 95% accuracy of common microbes and > 90% accuracy for uncommon microbes
- Lab staff must be aware that the accuracy of a system is limited to the claims of the manufacturer for the database version currently in the instrument
    - Database may be outdated
    - Procedure manual must state action to be taken for questionable results
        - Backup ID procedure
        - Reporting-Genus name only/"bacteria present closely resembling"
        - Send to reference lab

Abbreviated Bacterial and Yeast Identification (CLSI M35-A2)

Rapid Bile solubility	Rapid Methylumbelliferyl-B-D-glucuronidase (MUG)
Rapid CAMP	Spot oxidase
Catalase	Rapid porphyrin synthesis (ALA)
Germ tube	Rapid trehalose assimilation (RAT)
Rapid hippurate hydrolysis	Urea and phenylalanine deaminase (PDA)
Spot indole	
Indoxyl acetate disk	

Abbreviated Bacterial Identification (CLSI M35-A2)

- Isolate from ascites fluid: GNB, large colonies on BAP (Beta hem) & chocolate, LF on MAC, oxidase (-) Indole (+) is: ***Escherichia coli***
- Isolate from synovial fluid: tiny GNCB, growth on BAP (nonhem) & choc at 48 hrs, NG on MAC, Oxidase (+), catalase (+), Indole (-): ***Brucella***
- Isolate from blood tiny GNB/CB, growth on chocolate at 28 hr, NG on BAP with Staph streak, Oxidase (-), catalase weak (+): ***Francisella***
- Isolate from blood: GPC in clusters, growth on BAP (non-hem) & choc, cat (+), slide coag iffy, tube coag (-), PYR (+): ***Staphylococcus lugdunensis***
- Isolate from blood: GPC tetrads/ clusters, alpha-hem on BAP, cat (-), PYR (+), LAP (-): ***Aerococcus***
- Isolate from CNS shunt fluid: coryneform GPB, small opaque enamel-white round colonies on anaBAP, Cat (+) (15% H_2O_2), Indole (+): ***Propionibacterium acnes***
- Isolate from blood: GNCB, translucent growth on anaBAP (pits), NG on BBE, cat (-) (15% H_2O_2), Indole (-): ***Bacteroides ureolyticus***
- Isolate from blood: thin, GPB, smoothly swarming colonies on anaBAP, swollen subterminal spores, Indole (-), catalase (-): ***Clostridium septicum***

Abbreviated Yeast Identification (CLSI M35-A2)

- Isolate from ascites fld: budding yeast, “feet” on colonies on BAP or choc**:** ***Candida albicans***
- Isolate from blood: small yeast without hyphae, better growth on BAP than choc, RAT (-): ***Candida glabrata***
- Isolate from blood: spherical pleomorphic budding yeast, nonpigmented colonies on BAP, urea (+), phenol oxid (+): ***Cryptococcus neoformans***