New(er) Pathogens in Pediatric Respiratory Tract Infections

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Objectives

• Discuss emerging pathogens in pediatric respiratory tract infections through case based discussion.
Case #1

• 9 month-old child living in Saudi Arabia with infantile nephrotic syndrome
  – (genetic renal disease with poor outcome)
• Develops *Streptococcus pneumoniae* bacteremia and is admitted to the PICU
  – Vancomycin and Meropenem
  – Mechanical ventilation and inotropes
• Patient gradually improved over the next four days
  – Inotropes discontinued, minimal ventilator support
  – Some ascites and chest wall edema
Case #1

- On day 8 his condition again started to deteriorate – Increased work of breathing and O2 requirement – Chest Xray showed diffuse bilateral haziness
- Switched to high-frequency oscillatory support
- Vitals and Labs – Afebrile – CRP – 24 (0–6) – WBC – 9.6 (5–14.5) – Repeat blood cultures were negative – Tracheal aspirate was sent for real-time PCR and was positive.

Figure 1 - Chest x-ray with bilateral chest infiltration in a 9-month-old child.
Case #1

- Patient continued to receive supportive treatment.
- Decreased urine output
- Renal failure
- Continued to deteriorate and died 4 days later.
- Blood cultures remained negative
- Respiratory cultures were negative
- Only the tracheal aspirate PCR was positive.

What caused this patient’s infection?
Middle East Respiratory Syndrome: MERS

Clinical Presentation

- Affects the respiratory system
  - Severe acute respiratory illness
    - Fever, cough, SOB
- Some have GI symptoms
- ~40% mortality

- Novel coronavirus first reported in Saudi Arabia in September of 2012
- To date – all cases have been linked to the Arabian Peninsula
- Ages of the infected range from <1-99 years
- Person to person spread
  - close contact such as caring for or living with an infected person
  - Precise mechanism not well understood.
- Affects the respiratory system
- Some have GI symptoms
- ~40% mortality
South Korea MERS Outbreak

- S. Korean man visited Saudi Arabia in May.
- Visited multiple healthcare facilities in Seoul before getting diagnosed.
- More than 6,000 people in quarantine
- 24 deaths
- All cases linked to healthcare facilities
MERS in Children

Table 1 - Summary of reported pediatric cases with confirmed Middle East respiratory syndrome-coronavirus in the literature.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Reference</th>
<th>Age</th>
<th>Gender</th>
<th>Symptoms</th>
<th>Co-morbidities</th>
<th>Chest x-ray</th>
<th>Intensive care unit</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Our patient</td>
<td>9-month-old</td>
<td>Male</td>
<td>Severe respiratory distress</td>
<td>Infantile nephrotic syndrome</td>
<td>Bilateral diffuse infiltrate</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>2</td>
<td>Family contact</td>
<td>8-year-old</td>
<td>Male</td>
<td>Mild respiratory symptoms</td>
<td>No</td>
<td>Not documented</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>3</td>
<td>Inpatient</td>
<td>4-year-old</td>
<td>Male</td>
<td>Mild respiratory symptoms</td>
<td>No</td>
<td>Not documented</td>
<td>Not documented</td>
<td>Survived</td>
</tr>
<tr>
<td>4</td>
<td>Inpatient</td>
<td>2-year-old</td>
<td>Male</td>
<td>Fever, severe respiratory distress</td>
<td>Cystic fibrosis</td>
<td>Bilateral diffuse infiltrate</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>5</td>
<td>Inpatient</td>
<td>14-year-old</td>
<td>Female</td>
<td>Fever</td>
<td>Down’s syndrome</td>
<td>Bilateral diffuse infiltrate</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>6-14</td>
<td>Family contact</td>
<td>3-16-year-olds</td>
<td>2 males, 7 females</td>
<td>None</td>
<td>No</td>
<td>Not done</td>
<td>No</td>
<td>Survived</td>
</tr>
</tbody>
</table>

- Only approximately 2% (14 total) of the world’s confirmed MERS cases have been in children.

Thabet et al. Saudi Medical Journal. 2015. 36(4)
Does MERS prefer adults?

• Is pediatric MERS different?
  – Less severe disease?
    • 14% mortality vs. ~40% in adults
    • Similar mortality difference in SARS
  – Atypical presentation?
  – Asymptomatic carrier state?
Laboratory Diagnosis of MERS

Molecular

• Detect active infection
• Real-Time reverse transcription PCR
• Most state labs will perform the CDC EUA PCR test
• Recommended specimens
  – Lower respiratory
    • BAL, Tracheal Aspirate, Sputum
  – Upper respiratory
    • NP/OP Swab
  – Stool (optional)

• Rule/Out
  – Single negative result from recommended specimens
• Evidence of cleared infection
  – Two consecutive negative tests from all specimens
    • Not specified how far apart these must be

http://www.cdc.gov/coronavirus/mers/lab/lab-testing.html#molecular
Laboratory Diagnosis of MERS

Serological

• Detect past infection
  – Antibodies indicate that a person has been previously exposed and developed an immune response

• CDC has a two phase approach
  – Phase 1 – Screen
    • ELISA
  – Phase 2 – Confirmatory
    • IFA and/or microneutralization

• Results
  – Primarily used for epidemiological screening
  – NOT for diagnosis
  – CDC will not release results until primary PCR results are available

http://www.cdc.gov/coronavirus/mers/lab/lab-testing.html#serology
MERS Laboratory Safety

• DO NOT try to culture MERS
• Testing for common respiratory pathogens by methods other than culture is strongly recommended.

Standard Precautions
• Hematology
• Urinalysis
• Chemistry studies
• Microbiology performed on serum, blood, or urine

BSL-2 with BSL-2 Practices
• Molecular analysis of extracted nucleic acid
• Routine examination of bacterial and fungal cultures
  • Routine staining and smear analysis
    Plus a Class II BSC
  • Aliquoting specimens
  • Inoculating specimens
  • Smear preparation
  • Nucleic acid extraction
• Diagnostic tests not involving propagated viral agents

Case #2

- September 2014 - A 7 year old boy living in Richmond, VA develops 2 days of upper respiratory tract symptoms including a cough, rhinorrhea, and fever.
- The patient’s symptoms then worsened to include dyspnea at which point he was brought to the Emergency Department.
- Chest X-ray was abnormal and showed lobar opacification.
Case #2

History

• Asthma
• Otherwise healthy
• Lives with mom, dad and a younger sibling (4 yo)
• No sick contacts
• Favorite Disney movie is Frozen

Labs and Exam

• On admission he was found to be hypoxic and had significant wheezing.
• WBC = 18.8 (5-14.5)
• CRP mildly elevated
Case #2

- Patient admitted to the PICU and worsened to the point where he had to be mechanically ventilated.

- Testing
  - BAL performed
    - Bacterial, fungal and mycobacterial cultures = negative
    - Resp. pathogen PCR Panel = Rhinovirus/Enterovirus
  - Blood and urine culture = negative

Are we missing something?
Enterovirus D68

Aug, 19th, ‘14 – Children’s Mercy in KC, MO reported an increase in severe respiratory illness in patients with Rhino/Entero.

Aug, 23rd ‘14 – U of Chicago Comer Children’s reported the same as Children’s Mercy.

• 2014 outbreak
  – August 2014 to January 2015
  – 1,193 cases in 49 states
    • 14 children died
  – Estimated that there were millions of additional undiagnosed cases.

• Almost all severe cases were in children with asthma

• 30 of 36 specimens sent to CDC had EV D68
• 68% of kids had asthma
• Only 26% were febrile
Enterovirus D68

- Enteroviruses cause many different types of diseases
  - CNS
  - GI
  - Mild URI

- EV D68 is primarily associated with respiratory tract illness

- Laboratory detection
  - Luminex
    - Rhinovirus/Enterovirus
  - FilmArray
    - Rhinovirus/Enterovirus
  - GenMark
    - Rhinovirus only.....

- 49 Rhinovirus positive specimens during EV D68 2014 outbreak
- 33 were confirmed to be EV D68
- Predictably low positive

RESEARCH ARTICLE
Epidemic 2014 Enterovirus D68 Cross-Reacts with Human Rhinovirus on a Respiratory Molecular Diagnostic Platform
Shane C. McAllister¹, Mark R. Schleiss¹, Sophie Arbeleville³, Marie E. Steiner⁴, Ryan S. Hanson¹, Catherine Pollock¹, Patricia Ferrier¹,³
Avian Influenza: H7N9

- First discovered in China 2013
- WHO then described 132 cases in China with 44 deaths
- Incidence of disease then declined by September 2013
  - Closed live bird markets
  - Change in season
- Incidence increased once again during winter
The eight genes of the H7N9 virus are closely related to avian influenza viruses found in domestic ducks, wild birds and domestic poultry in Asia. The virus likely emerged from "reassortment," a process in which two or more influenza viruses co-infect a single host and exchange genes. This can result in the creation of a new influenza virus. Experts think multiple reassortment events led to the creation of the H7N9 virus. These events may have occurred in habitats shared by wild and domestic birds and/or in live bird/poultry markets, where different species of birds are bought and sold for food. As the above diagram shows, the H7N9 virus likely obtained its HA (hemagglutinin) gene from domestic ducks, its NA (neuraminidase) gene from wild birds, and its six remaining genes from multiple related H9N2 influenza viruses in domestic poultry.
H7N9 Transmission and Disease

- No evidence for sustained person-to-person transmission.
  - Some limited person-to-person
- Some mild illness has been reported but most has been severe, life-threatening respiratory illness.
  - 30% mortality
H7N9: Laboratory Diagnosis and Safety

Detection

• Specimen:
  – NP and/or OP swab
  – Lower respiratory preferred if presentation warrants

• Performance of FDA cleared assays unproven
  – PCR Preferred
  – Some evidence that rapid antigen tests have poor performance

• Likely will detect “FluA” but fail to subtype

• State and public health labs can facilitate testing

Safety

• Standard, contact, and airborne precautions recommended for patient management.

• DO NOT try to culture the virus

• Use BSL 3 containment facilities when working with specimens that could contain live virus

http://www.cdc.gov/flu/avianflu/h7n9/specimen-collection.htm
H7N9 in Children

• Average age of infected = 60
• Children appear to experience milder disease
• 7 reported pediatric cases (February 2014)
  – 5/7 had exposure to poultry
  – 4/7 also had family with confirmed H7N9 infection
  – 6/7 sought health care
  – All 7 survived
• Environmental monitoring found 26% of surfaces and samples to be positive
  – Feces
  – Bird cages
  – Chopping blocks

Zheng et al. PIDJ. 2015: 34(1)
The Challenging Microbiology of Cystic Fibrosis Patients

- Culture techniques
- Identification and AST
  - Mucoid *P. aeruginosa*
  - Small colony variants
  - Unusual and emerging pathogens
  - Synergy testing
  - Changing mycological taxonomy
Cystic Fibrosis Epidemiology - 1991

- *Staphylococcus aureus*
  - But NOT MRSA
- *Pseudomonas aeruginosa*
- *Pseudomonas cepacia*
  - Very difficult to recover at this time – no selective media
- *Haemophilus influenzae*
  - Anaerobic incubation of Horse Blood agar
- Glucose non-fermenters considered to be secondary pathogens of minor importance
- Mycobacteria
  - Role unclear
  - Almost impossible to isolate due to *P. aeruginosa* overgrowth
- Speculated that *Legionella* may be a significant CF pathogen
- *Aspergillus fumigatus*

Gilligan PH et al. CMR. 1991. 4(1)
Cystic Fibrosis Epidemiology - Now

Old
• P. aeruginosa
• B. cepacia
• S. aureus
• H. influenzae
• Aspergillus fumigatus

New
• Stenotrophomonas maltophilia
• Achromobacter xylosoxidans
• Burkholderia gladioli
• Ralstonia, Cupriavidus, Pandoraea
• Inquilinus limosus
• Mycobacterium abscessus
• Mycobacterium chelonae
• Fungi
  – Scedosporium
  – Exophiala
  – Geosmithia

LiPuma JJ. CMR. 2010. 23(2)
Case #3

- 17 year old female with Cystic Fibrosis.
- Recent culture history
  - *S. aureus* – MRSA
  - *Pseudomonas aeruginosa*
  - *Aspergillus fumigatus*
- At quarterly pulmonology visit her bacterial sputum cultures grow the following...
  - *S. aureus* – MRSA
  - *P. aeruginosa*
  - **AND**...
Case #3

- Gram negative rod
- Grows on MacConkey agar
  - Non-lactose fermenter
- Grows on *B. cepacia* selective agar
  - Non-mucoid in appearance
  - No odor observed
- Colistin resistance confirmed
- Oxidase negative
- Vitek2 Identification = *Burkholderia cepacia*

Do you believe it?
Burkholderia gladioli

- Not a member of the *B. cepacia* complex
- Accounts for a significant amount of *Burkholderia* infection in CF
- More commonly recovered than most species of *B. cepacia* complex
- Does not appear to spread from patient to patient like other epidemic *B. cepacia* strains

LiPuma JJ. CMR. 2010. 23(2)
**Burkholderia gladioli**

**Identification**
- Will grow on MacConkey, PCA, BCSA
- Oxidase negative
- Non-MALDI commercial systems
  - 83-94% accurate for *B. cepacia*
  - *B. gladioli* frequently misidentified as *B. cepacia*

**Susceptibility**
- Very little systematic evaluation of *B. gladioli* susceptibility
- Appear to be more susceptible than *B. cepacia*
- Segunds et al. (18 clinical isolates)

<table>
<thead>
<tr>
<th>Good Activity</th>
<th>Variable Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin</td>
<td>Ceftazidime</td>
</tr>
<tr>
<td>Imipenem</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td></td>
</tr>
</tbody>
</table>

Shelly DB et al. JCM. 2000. 38(8)
Segunds et al. JCM. 2009. 47(5)
Case #4

• Three months later the same 17 year old girl returns for another sputum culture.

• This culture grows
  – *S. aureus* – MRSA
  – *P. aeruginosa*
  – *B. gladioli*
  – AND.....
Case #4

- Gram negative rod
- Grows on both MacConkey and B. cepacia selective agar as a highly mucoid organism.
- Oxidase positive
- Colistin resistance confirmed
- TSI slant with lead acetate paper demonstrated H2S positivity.
- BD Phoenix ID produced no answer
**Inquilinus limosus: Full of Slime**

- Described in 2002
- Highly mucoid
- CF patients can become chronically infected
  - Mostly adolescent or adult patients
- Clinical significance is unknown
- Source unknown
- No patient to patient spread documented

**Characteristics**
- Gram-negative
- Oxidase positive
- H2S positive by paper strip test, not on a TSI
- Not in commercial systems
  - MALDI ID’s correctly
  - Otherwise 16S sequencing required
- Multidrug resistant
  - Colistin resistant

Bittar F et al. EID. 2008. 14(6)
Fungal Infections in CF

46 BALS from CF patients seen at Stanford 2012-2013
- Resulted in 53 species of Aspergillus
  - 52 were A. fumigatus complex
  - 1 was A. niger complex
- No cryptic species were identified

Other fungal pathogens
- Exophiala dermatitidis
- Scedosporium
- Penicillium emersonii
- Acrophiachophora fusipora
- Rasamsonia

Fungal Infections in Cystic Fibrosis
Rasamsonia: Formerly – *Geosmithia and Penicillium*

- Commonly misidentified as *Penicillium* or *Paecilomyces*
- Significance in CF unknown
- Resistant to azoles
- Causes pneumonia in CGD
- 152 Patients screened by PCR
  - 4 were positive

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>18</td>
<td>37</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>FEVI</td>
<td>41%</td>
<td>30%</td>
<td>63%</td>
<td>80%</td>
</tr>
<tr>
<td>Culture +</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

De Ravin SS et al. CID. 2011. 52(6)
Steinmann J et al. NMNI. 2014. 2:72-78
Acrophialophora fusipora

- Thermotolerant soil fungus
- Prevalence likely underestimated
  - Previously misidentified as *Scopulariopsis* and *Paecilomyces*
- Significance in CF unknown
  - Reports of repeated isolation from CF patients

Identification
- SAB growth – highly variable
  - Gray brown top
    - Range from orange to pale gray
  - Dark brown bottom
- Thermotolerant
  - Good growth at 43°C

Cimon et al. JCM. 2005. 43(3)
Acrophialophora fusipora

Cimon et al. JCM. 2005. 43(3)
Thank you for your attention!

Questions?
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