Bordetella

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Bordetella species

- B. pertussis----------human
- B. parapertussis------human/sheep
- B. bronchiseptica-----animals/human
- B. holmesii-------------human
- B. avium---------------birds
- B. hinzii--------------poultry
- B. trematum-----------animals
- B. petrii--------------environmental

Bordetella

- Small gram-negative coccobacillus
- Strictly aerobic (except B. petrii)
- Oxidize amino acids
  - None ferment carbohydrates
- Catalase positive
- Optimal growth at 35°C, ambient air with humidity
- B. pertussis most fastidious of the Bordetella
  - Inhibited by constituents in media
    - Fatty acids
    - Metal ions
    - Sulfides
    - Peroxides

B. Pertussis Gram Stain

Some virulence factors of B. pertussis

- Non-invasive bacterium—does not normally invade cells
- Attachment
  - Filamentous hemagglutinin (FHA) and pertactin
  - Pertussis toxin
- Toxins
  - Tracheal cytotoxin — cell death
  - Endotoxin — cytokines, fever
  - Adenylate cyclase/hemolysin — inhibits lymphocyte function
  - Lipopolysaccharide (endotoxin)
  - Pertussis toxin (PT)* — inhibits immune cells
  - Chemotaxis and acts as an adhesin
- * = difference between pertussis and parapertussis

Whooping Cough

- Bordetella pertussis
- Classical whooping cough
  - Cold-like illness (Catarrhal phase)
  - Runny nose, sneezing, low-grade fever, tired
  - Dry non-productive cough for >2 weeks
  - “Whooping” cough (Paroxysmal phase)
  - Severe cough with vomiting
  - Severe disease in infants
  - 7-10 day incubation (range 5-21)
  - Epidemic disease every 2-5 years
Parapertussis

- Symptoms are similar to pertussis but typically milder.
  - Cough that occurs in sudden, uncontrollable bursts
  - Shorter duration than pertussis (Avg 14 days)
  - High-pitched whooping sounds when breathing in after a coughing episode
  - Posttussive vomiting
- Incubation & Transmission Periods similar to pertussis
- All age groups can be infected.
  - Young infants (e.g., <6 months of age) may have a more severe course
- Rarely, death can occur in infants with underlying health problems or those that are co-infected with *B. pertussis*.

Pertussis CSTE Case Definition

- Clinical case definition
  - Cough ≥ 2 weeks and at least one symptom: paroxysms, whoop, posttussive vomiting
- Case classification
  - Confirmed cases
    - Culture positive
    - Clinical case and PCR positive
    - Clinical case and epi-linked to confirmed case
  - Probable case
    - Only meets the clinical case definition

Transmission

- Respiratory droplets
- Highly infectious
  - 80-90% of household contacts

Bordetella pertussis

- 20-40 million cases/year worldwide
- 200,000-400,000 deaths
  - Primarily among children
- Cases/yr in the US
  - 2002----------9,771
  - 2003----------11,647
  - 2004----------25,827
  - 2005----------25,616
  - 2006----------15,632
  - 2007----------10,454
  - 2008----------13,278
Number of Specimens Positive for Whooping Cough (6/1/03 - 10/22/05)

- Number of positive specimens ranges from 0 to 75.
- Data points show fluctuations over the specified period.

Number of Specimens Tested, Positive and Percent Positive for Bordetella by PCR at Wisconsin Laboratories

- X-axis represents weeks.
- Y-axis indicates the number of specimens tested.
- Bars show positive and percent positive results.

Pertussis Vaccine

- Included in childhood vaccines:
  - DTaP at 2, 4, 6, and 15-18 months, 4-6 years.
  - Recent addition: Tdap at 11-12 years.
  - "Catch up" for 13-18 yr olds.
- Issue of "waning immunity".

Treatment and Prophylaxis

- Macrolides:
  - Azithromycin
  - Erythromycin
  - Clarithromycin.
- Second choice = sulfa drugs (SXT)
  - Not for infants <2mo.

NOTE: Treatment should be administered during the catarrhal stage to lessen illness. If cough develops, does not affect illness course but may lessen transmission possibility.
Specimen Collection

- Nasopharyngeal specimen
  - Want ciliated epithelial cells
  - Timing critical
- NP aspirates, washes, or swabs
  - Dacron or rayon swabs
    - NO cotton or calcium alginate swaps
- NO throat, sputum, or mouth specimens

Transport

- *B. pertussis* extremely labile
- Direct culture optimal, not feasible
- Transport media
  - If <2hr-----0.5-1.0% Casamino Acid Sol’n at room temp
  - 2hr -- 24hr----Amies with charcoal, room temp
  - >24hr----Regan-Lowe or Jones-Kendrick at 4 C

Laboratory Diagnosis

- Culture
- DFA
- Real-time Polymerase Chain Reaction
  - More rapid than culture
  - More sensitive than culture

Culture

- “Gold Standard”
  - Essential for public health labs
- 100% specific, but low sensitivity
  - (12-60%)
- Highest yield
  - Young children
  - Unvaccinated
  - Early in cough illness prior to antibiotics
Culture Media

- **Bordet-Gengou**
  - Potato infusion, glycerol, sheep/horse blood
- **Regan-Lowe**
  - Charcoal agar, 10% horse blood
- **Jones-Kendrick**
  - Charcoal agar, yeast extract, starch
- **Stainer-Scholte** synthetic medium
- **Legionella buffered charcoal yeast-extract**
- Incorporate antibiotics to suppress normal flora—cephalexin or methicillin
- Incubate 35-36°C with high humidity for a minimum of 7 days

Other Bordetella species

- **B. parapertussis**
  - Colonies within 2-3 days
  - On Regan-Lowe appear grey
  - On BG have brown pigmentation
- **B. holmesii**
  - Colonies similar to B. pertussis
  - Inhibited by cephalaxin
- **B. bronchiseptica**
  - Colonies within 1 day
  - On Regan-Lowe, large, slight brown

Culture Sensitivity

- Considered no more than 60% sensitive
- Factors effecting sensitivity
  - Type and quality of specimen
  - Time specimen obtained in the course of illness
  - Age of the patient
  - Appropriate transport
  - Choice of culture media
  - Length of time cultures incubate

Culture for Public Health

- Important if an outbreak is suspected
- Isolation of the organism confirms pertussis
  - Other agents can cause pertussis-like illness
  - Co-infection with other pathogens occurs
  - Culture helps identify other *Bordetella* spp.
- Necessary for AST and subtyping

Identification

- **B. pertussis**
  - Catalase +
  - Oxidase +
  - Urease -
  - Motility -
  - Nitrate -
  - Blood Agar -
  - MacConkey -
  - Fluor. Ab
- **B. parapertussis**
  - Catalase +
  - Oxidase -
  - Urease +
  - Motility -
  - Nitrate -
  - Blood Agar +
  - MacConkey V
  - Fluor. Ab
Direct Fluorescent Antibody
• In use since 1960
• Direct detection and ID of isolates

DFA
• Problems
  – Poor sensitivity (18-78%)
  – Requires large numbers of organisms (>10⁴ /ml)
  – Best when test early in course of illness
  – Requires skilled and experienced microscopist
  – Antibiotic therapy can affect binding of DFA reagent to cell wall
  – Poor specificity (7-44% false positives)
• Advantage
  – More rapid than culture
  – No longer recommended

Pertussis PCR
• Optimal diagnostic test
  – 70 – 90% Sensitivity
• No prolonged asymptomatic “carrier state”
  – If positive, considered diagnostic
• Recognized by CSTE as official laboratory confirmation of pertussis in addition to culture

Pertussis PCR (cont.)
• Advantages
  – Rapid
  – Extremely sensitive
    • ≤1 CFU (5ul sample)
  – Does not require viable organism
    • Transport delays and antibiotics do not prevent laboratory diagnosis
  – Positive longer than culture
  – Specific
    • Except for detection of B. holmesii if using IS481

Pertussis PCR (cont.)
• Disadvantages
  – Stringent requirements to perform PCR
  – Not presently standardized
  – More expensive than culture or DFA
  – PCR inhibitors
  – Cross reaction with B. holmesii

WSLH Pertussis Data
<table>
<thead>
<tr>
<th>July 2003-July 2004</th>
<th>%Pos of Total</th>
<th>% of Positives</th>
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</thead>
<tbody>
<tr>
<td>3330 Tested by Culture and PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>450 Pos by Cult and/or PCR</td>
<td>13.5%</td>
<td>100%</td>
</tr>
<tr>
<td>167 Pos by BOTH Cult and PCR</td>
<td>5.0%</td>
<td>37.1%</td>
</tr>
<tr>
<td>27 Pos by Culture ONLY</td>
<td>0.8%</td>
<td>6.0%</td>
</tr>
<tr>
<td>256 Pos by PCR ONLY</td>
<td>7.7%</td>
<td>56.9%</td>
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</tbody>
</table>

20 culture positive for B. parapertussis
**Bordetella spp. PCR at WSLH**

- Multi-target RT-PCR
  - B. pertussis
  - B. parapertussis
  - B. holmesii
- Targets
  - IS481
  - plS1001
  - hlS1001
  - ptxS1

**Species Identification**

<table>
<thead>
<tr>
<th>Species</th>
<th>ptxS1</th>
<th>IS481</th>
<th>hlS1001</th>
<th>plS1001</th>
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</thead>
<tbody>
<tr>
<td>B. pertussis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. parapertussis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B. pertussis and B. parapertussis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B. holmesii</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Targets**

- IS481
  - B. pertussis (80-200 copies/cell)
- hlS1001
  - B. holmesii (3-5 copies/cell)
- plS1001
  - B. parapertussis
- RNP
  - Inhibition detection
- ptxS1---Not part of multi-plex
  - Single copy in B. pertussis and B. parapertussis

**Pertussis Serology**

- Require acute and convalescent specimens
- Infection in vaccinated individuals confounds interpretation
- Not included in CSTE case definition
- Can have epidemiologic value
- No standardized assays
- No FDA approved assays
- New CDC developed EIA may provide a useful serologic tool
**CDC IgG Anti-PT ELISA**
- Microwell ELISA format
- Allows for diagnosis of recent infections with a single specimen
- Useful in later stages of the disease (>2 weeks from onset)
- Post-vaccination antibody levels do not interfere with diagnosis
- Can be qualitative

**Bordetella holmesii**
- First identified in 1995
- Associated with pertussis-like respiratory disease and pneumonia
  - 0.6% of patients
  - Role in respiratory disease still unclear
- Bacteremia in asplenic children and sickle-cell patients
  - Endocarditis

**Bordetella holmesii (cont.)**
- Gram negative coccobacillus
- Grows well on SBA in 5% CO₂ after 24hr
  - Inhibited by cephalaxin
- Diffusible brown pigment
  - Can be mistaken for alpha hemolysis
- Poor or no growth on Mac

**Bordetella holmesii (cont.)**
- Negative for catalase (variable), oxidase, NO₃ reductase, urease, indole, motility
- Misidentified as *Acinetobacter lwoffii* on Vitek 2

**Bordetella bronchiseptica**
**Bordetella bronchiseptica (cont.)**

- Rarely isolated from respiratory specimens
- Pertussis-like disease and other respiratory symptoms
  - Infectious bronchitis
- Most frequently in immunocompromised
- Rare cases of bacteremia and septicemia

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**Bordetella bronchiseptica (cont.)**

- Has genes for pertussis toxin
  - Not expressed
- Positive for catalase, oxidase, nitrate, urease, motility
- Grows on Mac, SBA
  - On SBA, small to medium gray colonies with beta hemolysis under the colonies

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**Bordetella bronchiseptica (cont.)**

![Image of growth on Mac with beta hemolysis](image)

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**Bordetella bronchiseptica (cont.)**

- Susceptibility pattern
  - Beta-lactamase producer
  - R to many penicillins and cephalosporins
  - Anti-pseudomonal penicillins usually sensitive
  - Mostly resistant to SXT
  - Most S to amoxicillin-clavulanic acid, tetracycline, gentamicin, and quinolones

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**Thank You**

Questions???