**Objectives**

- Describe the current epidemiology of pertussis infections in the U.S.
- Discuss the most appropriate test methods for diagnosis of pertussis.
- Understand the public health importance of rapidly and accurately identifying cases of pertussis.

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**Pertussis**

- Highly contagious bacterial infection
  - 80% secondary attack rate among susceptible household contacts
- Spread easily via aerosolized droplets from coughing or sneezing
- Reservoir: untreated, symptomatic persons
  - Particularly adolescents & adults
- Immunity from natural infection is NOT lifelong

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**Pertussis**

- Affects all ages – young infants most vulnerable
- Typical Symptoms
  - coryza (no pharyngitis)
  - paroxysmal cough
  - post-tussive vomiting
  - post-tussive whoop
  - lack of fever
  - no systemic illness

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**Pertussis: from Latin meaning “intense cough”**

- Communicable Period
  - Incubation period (max 21 days)
  - Catarhal stage (1-2 weeks)
  - Paroxysmal stage (1-4 weeks)
  - Convalescent stage (weeks to months)
- Onset
  - paroxysms may occur several times a day
  - increasing coughing
  - decreasing coughing
  - days to weeks

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**Pertussis Diagnosis**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sens</th>
<th>Spec</th>
<th>Optimal Timing</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>12-60%</td>
<td>~100%</td>
<td>&lt;2 wks onset</td>
<td>specificity</td>
<td>sensitivity &amp; long TAT</td>
</tr>
<tr>
<td>PCR</td>
<td>70-99%</td>
<td>86-100%</td>
<td>&lt;4 wks cough</td>
<td>rapid &amp; sensitive</td>
<td>2 FDA-app, false positivity</td>
</tr>
<tr>
<td>DFA</td>
<td>11-68%</td>
<td>76-99%</td>
<td>&lt;2 wks onset</td>
<td>rapid</td>
<td>sensitivity &amp; specificity</td>
</tr>
<tr>
<td>Paired Sera</td>
<td>90-92%</td>
<td>72-100%</td>
<td>Onset &amp; 4-6 wks</td>
<td>sensitivity</td>
<td>No FDA-app, too long, vaccination</td>
</tr>
</tbody>
</table>
CSTE Case Definition

- **Clinical Case Definition**
  - Cough ≥2 wks & at least 1 symptom: paroxysms, whoop, posttussive vomiting
- **Case Definition**
  - **Confirmed Cases**
    - Culture Positive
    - Clinical Case + PCR Positive
    - Clinical Case + Epi-linked to confirmed case
  - **Probable Case**
    - Only meets the clinical case definition

Molecular Diagnostics

![Graph showing number of confirmed pertussis cases over time](image)

- 12%
- 44%

**Pertussis Real-Time PCR**

- **Target Sequence**
  - IS481: 50 to >200 copies per genome
  - 114 bp amplicon
  - Cross-reacts with B. holmesii, B. bronchiseptica

**Bordetella holmesii**

- 1st reported case 1983 – bacteremia in asplenia
- CDC NO-2 (non-oxidizer group 2) until 1995
- found in 0 to 29% of NP specimens from patients with pertussis-like illness in several countries
- rare diseases: pneumonia, endocarditis, meningitis, septic arthritis
- in mice, whole cell or acellular vaccines do not protect against B. holmesii infection

**Insertion Sequence Targets**

<table>
<thead>
<tr>
<th>Insertion Sequence Targets</th>
<th>Pertussis, cell or acellular vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS481</td>
<td>50 to &gt;200 copies per genome</td>
</tr>
<tr>
<td>114 bp amplicon</td>
<td>Cross-reacts with B. holmesii, B. bronchiseptica</td>
</tr>
</tbody>
</table>
Targets with Single-Copy Genes

<table>
<thead>
<tr>
<th>Species</th>
<th>ptxS1</th>
<th>IS481</th>
<th>hbl1001</th>
<th>psl1001</th>
</tr>
</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. holmesii</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*With the exception of the psl1001 target, PCR assays targeting the genes listed above are constructed using multiple primer sets and are not listed individually.

CDC Multi-Target PCR Approach

- **Species**
- **ptxS1**
- **IS481**
- **hbl1001**
- **psl1001**

- **B. pertussis**
- **B. parapertussis**
- **B. holmesii**

Illumigene Pertussis

- **Sensitivity**
- **Specificity**

98.6%

FilmArray Respiratory Panel

- **Sensitivity**
- **Specificity**

94.1-100%

99.9%

- **FilmArray Panel including B. pertussis**
  - compared to LDT PCR and/or culture from retrospectively positive NP swab specimens
  - 38/39 positive by FilmArray
  - noted cross-reactivity: B. bronchiseptica & B. parapertussis since both have PtxP

- **Illumigene Pertussis**
  - prospectively collected NP swabs (n=94)
  - compared to B. pertussis PCR (ASR, Cepheid)
  - sens = 94.4% (17 of 18) and spec = 98.6% (73 of 74)
PH Importance of Culture

- Important if outbreak is suspected
- Isolation confirms pertussis (100% specific)
  - Other pathogens w/ similar clinical presentation
  - Co-infections can occur (e.g. RSV)
  - Can identify other Bordetella species
- Needed for susceptibility testing and typing

Post-Treatment

Unvaccinated Infants < 3 Months of Age

- Culture
  - may be positive up to 5-7 days after start of therapy
- PCR
  - positive up to at least 10-13 days after start of therapy
  - No clear association with duration of cough

Pertussis: the last 100 years

- Early 1900’s: 1 death per 10 cases
- 1922: made a notifiable disease
- 1934: >265,000 cases
- 1940: whole cell vaccine available
- 1943: AAP recommends pertussis vaccine
- 1976: 1,010 reported cases

Pertussis: the last 100 years

- 1990’s: acellular vaccine replaces whole cell
- 2000’s: emergence of disease among older children & adolescents (most vaccinated)
- 2005: recommendation for Tdap at age 11 or 12
- 2012: 48,000 reported cases
- 2013: 29,000 reported cases
Recent Pertussis Outbreaks

- 2010, California: >9,000 cases, 800 hospitalizations, 10 deaths (all <3 months of age)
- 2012, Wisconsin: >6,400 cases
- 2012, Washington: 2,500 cases

In all outbreaks, high rates of disease in fully vaccinated children.
Why is Adaptive Immunity Short-Lived

- Mixed Th1/Th2 response (acellular) vs. mostly Th1 (whole cell or natural infection)
- Potentially missing important antigens
- Insufficient concentration or balance of antigens
- Poor match between vaccine Ags and current circulating strains
  - Allelic variation of current strains vs. vaccine strains

Evolution of B. pertussis Strains

- whole genome sequencing of 343 strains from 19 countries isolated between 1920 and 2010, including vaccine strains
- lineages and antigenic genotypes of vaccine strains are not commonly seen in recent isolates
- changes in genes in acellular vaccine components started occurring after introduction of whole cell vaccine but before the switch to acellular

Pertussis Acellular Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Name</th>
<th>Licensed</th>
<th>PT</th>
<th>FHA</th>
<th>PRN</th>
<th>FIM 2/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTap</td>
<td>Pediarix 2002</td>
<td>25.0</td>
<td>25.0</td>
<td>8.0</td>
<td>-</td>
<td></td>
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<tr>
<td></td>
<td>Daptacel 2002</td>
<td>10.0</td>
<td>5.0</td>
<td>3.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tdap 2005</td>
<td>2.5</td>
<td>5.0</td>
<td>3.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boostrix 2005</td>
<td>8.0</td>
<td>8.0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pertactin-Deficient B. pertussis

- Recent emergence since 2009 of B. pertussis isolates lacking pertactin protein
  - Component of acellular vaccine
  - Clinically does not appear to alter disease severity
  - Not clear if it’s due to vaccine selection pressure
Summary

- Pertussis is a highly contagious respiratory infection that has re-emerged in recent years due to waning immunity, changes in VE, and/or changes in circulating strains.

- The specific diagnosis of pertussis is best accomplished by multi-target PCR to rule out other *Bordetella* species.

- In outbreak situations, culture is recommended for confirmation and characterization of isolates.