Fastidious Gram-negative bacteria

Bacterial vaginosis, HACEK infections, Legionella

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Gram-negative, intracellular pathogens, acquired from the environment

- **Gardenerella vaginalis**
  - associated with bacterial vaginosis
- **Mobiluncus spp.**
- **HACEK (including Actinobacillus)**
- **Legionella spp.**
  - (L. pneumophila)
  - rare causes of endocarditis; (juvenile periodontitis)
  - severe pneumonia (Legionnaires’ disease)
Case: vaginitis

• A 32 year old woman has a vaginal discharge. She has had no pain, vaginal bleeding, or excessive weight gain. She is G2P2. LMP was two weeks ago and was normal and on time. She is monogamous with her husband.

• On examination, there is a malodorous, light gray discharge at the vaginal introitus.

• Application of a pH strip to vaginal wall = 7.0.

• Addition of 10% KOH to a sample of the discharge on a slide produces an intense amine odor (“fishy”). A saline preparation of the discharge shows the following:
“Clue” cells
Clue cell Gram stain
Questions to consider

• Why is the discharge malodorous? What is the “fishy” odor?
• What is the significance of the vaginal pH?
• What is the significance of bacteria-coated cells (“clue” cells)?
• Should her husband be examined and treated?
Bacterial vaginosis (BV)

- Overgrowth of vaginal flora with *G. vaginalis*, *Mobiluncus*, *Prevotella*, *Peptostreptococcus*, and many other anaerobic species
- Displaces normal lactobacilli (responsible for vaginal acid production; pH<4.5)
- Anaerobic bacteria produce amines which release ammonia in 10% KOH
- Resolves with oral or topical metronidazole
**Gardenerella vaginalis**

- facultatively anaerobic, nonsporulating, nonencapsulated, nonmotile, pleomorphic, gram-variable rod
- found in 15% - 69% of women without BV and in 13.5% of girls.
- found in all cases of BV
- risk of bacteremia in pregnant women, post-abortion, and post-hysterectomy
Mobiluncus spp.

- anaerobic, slowly growing, motile, Gram-variable, curved bacilli
- found in 97% of women with BV but in a minority of healthy controls
- susceptible to most antibiotics, but resistant to metronidazole
  - ? role in BV
What is the role of these bacteria in BV?

- unclear...
- Koch’s postulates not satisfied by any single agent
- Note: BV is not sexually-transmitted disease
- When is it necessary to treat?
  - HIV-infected
  - Pregnant
  - Before GYN surgery
  - At risk for other STDs
Questions to consider

- Why is the discharge malodorous? What is the “fishy” odor?
- What is the significance of the vaginal pH?
- What is the significance of bacteria-coated cells (“clue” cells)?
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The HACEK group

H: *Haemophilus aphrophilus* and *H. paraphrophilus*
A: *Actinobacillus actinomycetemcomitans*
C: *Cardiobacterium hominis*
E: *Eikenella corroden*
K: *Kingella kingii*
Characteristics of HACEK

- all are Gram-negative, pleomorphic rods
- all are normal flora of the human mouth
- rare cause of “culture-negative” endocarditis (particularly after dental work)
- fastidious; very slow to grow in culture (require 5-10% CO$_2$)
- most have beta-lactamase enzymes
Actinobacillus

• Found in 20% of adult/teenage mouths
• Cause of juvenile and adult periodontitis
  – with *Porphyromonas gingivalis*
  – In 90% localized, aggressive periodontal infections with loss of teeth and bone
• Can cause infections mimicking Actinomycosis (usually neck, face, lungs and chest wall) - rare
“Juvenile periodontitis”

Lokalisierte, juvenile Parodontitis
Treatment of HACEK organisms

- **Endocarditis**: ceftriaxone, *not* ampicillin or penicillin
- **Human bites**: amoxicillin-clavulanate, fluoroquinolone
- **Severe periodontitis**: tetracycline
Legionella
(primarily *L. pneumophila*)

- Legionnaires’ disease
- Pontiac Fever
Case: pneumonia after travel

- A 55-year-old male automobile dealer was hospitalized with high fever and cough.
- Seven days earlier, he developed symptoms of fever (38.5°C), headache, and generalized muscle ache. The following day, he developed a hacking cough with minimal sputum production.
- He was evaluated 4 days before admission, and a diagnosis of community-acquired pneumonia was made after a chest x-ray which a left lower lobe infiltrate.
Case (continued)

- He was treated with an oral cefuroxime. However, his fever increased, and he developed watery diarrhea.
- His past medical history was unremarkable, but he is a cigarette smoker. He returned from a 2-week vacation with his wife, three children, 75 year old mother in the Florida Keys (Hawthorne Suites) 2 days before onset. None of his family or co-workers were also ill.
- On admission, temp=39.8°C, heart rate =90/minute. O₂ saturation=81% on room air
- WBC=13,700/mm³.
- Sputum Gram stain: numerous PMNs, no bacteria.
- A chest x-ray showed extension of the right lower lobe infiltrate and an extensive new left lower and left upper lobe infiltrate.
Case (conclusion)

- Azithromycin was added to the patient’s antibiotic regimen to treat Legionnaires’ disease. This diagnosis was confirmed by a positive *Legionella* urine antigen test.
- The patient began to improve 48 hours later and recovered. Respiratory secretions obtained from his endotracheal tube grew *L. pneumophila* SG1 after 3 days.
- A call to the Florida Department of Public Health confirmed that 5 other recent patrons of the Hawthorne Suites had also developed severe pneumonia, and a hot tub at the hotel was positive for *L. pneumophila* SG1.
Questions to consider

• Was the hot tub the source of the illness? Why?
• Why were none of the patient’s family members or co-workers affected?
• Why was the sputum Gram stain negative?
• Why was cefuroxime ineffective?
• Why was the diagnosis made by a urine antigen test instead of a culture?
The Bellevue-Stratford Hotel, Philadelphia

(where the first recognized outbreak of *Legionella* infection occurred)
Scope and consequences of the 1976 Bellevue-Stratford outbreak

• 182 American Legionnaires become ill
• 146 were hospitalized
• 29 died (associated with respiratory failure)
• There were no secondary cases
Fibrinopurulent pneumonia
*Legionella*: difficult to distinguish from the background
Laboratory investigation

• Etiologic agent was unknown for months!
• Eventually, the infection was passed from the lung tissues of deceased patients to guinea pigs.
• Then, from guinea pigs to highly-enriched liquid media
• Then, a specialized agar medium
Buffered charcoal yeast extract agar

Supplemented with cysteine and iron pyrophosphate
## Outbreaks of legionellosis that preceded the 1976 Philadelphia outbreak

<table>
<thead>
<tr>
<th>LOCATION</th>
<th>Year</th>
<th>n</th>
<th>Est. attack rate</th>
<th>Case-fatality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Elizabeth’s Hosp., D.C.</td>
<td>1965</td>
<td>81</td>
<td>1.4%</td>
<td>17%</td>
</tr>
<tr>
<td>Health Dept., Pontiac, Michigan</td>
<td>1968</td>
<td>144</td>
<td>95%</td>
<td>0</td>
</tr>
<tr>
<td>James River, Virginia</td>
<td>1973</td>
<td>10</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Benidorm, Spain</td>
<td>1973</td>
<td>89</td>
<td>--</td>
<td>3.4%</td>
</tr>
<tr>
<td>Odd Fellow’s Conv., Phila. PA</td>
<td>1974</td>
<td>11</td>
<td>2.9%</td>
<td>10%</td>
</tr>
<tr>
<td>American Legion Convention, Philadelphia, PA</td>
<td>1976</td>
<td>182</td>
<td>4.0%</td>
<td>17%</td>
</tr>
</tbody>
</table>
Identification and taxonomy

- ~50 *Legionella* spp. (19 have caused human disease)
- *L. pneumophila* causes most of human disease
  - 16 distinct serogroups
  - Most disease due to serogroup 1 (SG1) - ~60%
- Features:
  - All are flagellate GNRs, catalase-positive
  - Survive major temperature extremes (up to 55°C)
  - Identified with group-specific antisera
- LLAPs (cultivable only in amoebae)
Biofilms within residential and institutional water systems

Surface water source *Legionella* + protozoa

Water treatment plant chlorination

Evaporative cooling towers

Fountains

Showers

Faucets

Infectious aerosols

Microaspiration

Bacterial growth in hot water heaters

Aqueous sources of Legionellae
Evidence that Intracellular Infection is Essential for Legionnaires’ Disease

• **From animal models**
  – Max. growth ~ bacteria associated with cells.
  – Susceptibility of an animal species ~ susceptibility of its macrophages to infection *in vitro*.
  – Mutants with poor macrophage growth ~ avirulent.

• **From human infection**
  – Intracellular bacteria are seen in lung sections.
  – Antibiotic efficacy ~ penetration of agent into cells.
Human lung is a dead end for *Legionella*.
Protozoa are the natural hosts for *Legionella*. 

Inhalation of *Legionella* leads to infection of alveolar macrophages, which then lead to Legionnaires’ disease.
Motile, flagellate rods

Association with ER

Delayed phagolysosomal fusion

Expression of flagella & release
The Dot/Icm Complex

- Effector
- Macrophage cytoplasm
- Macrophage membrane
- Bacterial OM
- Periplasm
- Bacterial IM
- Bacterial cytoplasm
Legionella killed by the lysosomes

Dot/icm mutants
Growth-phase regulation of virulence

In vitro

Optical Density

Time

Exponential
“REPLICATIVE”

Postexponential
“TRANSMISSIVE”

In vivo

In vivo
## Clinical diagnostic methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sputum (with acid)</td>
<td>20-80%</td>
<td>100%</td>
</tr>
<tr>
<td>BAL</td>
<td>80-90%</td>
<td>100%</td>
</tr>
<tr>
<td>lung tissue</td>
<td>90-99%</td>
<td>100%</td>
</tr>
<tr>
<td>DFA</td>
<td>25-75%</td>
<td>95-99%</td>
</tr>
<tr>
<td>Serology SG1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>seroconversion</td>
<td>70-80%</td>
<td>95-99%</td>
</tr>
<tr>
<td>single titer &gt; 256</td>
<td>10%</td>
<td>50-70%</td>
</tr>
<tr>
<td>Urine Ag-RIA *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3 to &gt;300 days)</td>
<td>80-98% (SG1)</td>
<td>99%</td>
</tr>
</tbody>
</table>

*accurately diagnoses only SG1 = 60% of cases
## Mortality from Legionnaires’ disease in 3 outbreaks, by antibiotic therapy*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins</td>
<td>46%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>36%</td>
<td>19%</td>
<td>25% (80%)§</td>
</tr>
<tr>
<td>Penicillins</td>
<td>23%</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>11%</td>
<td>4%</td>
<td>7% (24%)</td>
</tr>
</tbody>
</table>

* Tsai *et al.* Ann Intern Med 1979; 90: 509  
Broome *et al.* Ann Intern Med 1979; 90: 573  
Kirby *et al.* Medicine 1980; 59:188  

§ parentheses indicate immunosuppressed patients
### Uptake of Antibiotics by Pulmonary Alveolar Macrophages

taken from Johnson et al. J. Lab Clin Med 1980; 95: 429-39

<table>
<thead>
<tr>
<th>ANTIBIOTIC</th>
<th>C:E ratio at 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>20.6 ± 3.1</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>0.07 ± 0.06</td>
</tr>
</tbody>
</table>
Antibiotic rx of Legionnaires’ Disease

LONGSTANDING CHOICE:

High-dose Erythromycin + Rifampin

TRADITIONAL ALTERNATIVE:

Doxycycline

BETTER ALTERNATIVES:

Azithromycin and newer macrolides

Fluoroquinolones
Questions to consider

• Was the hot tub the source of the illness? Why?
• Why were none of the patient’s family members or co-workers affected?
• Why was the sputum Gram stain negative?
• Why was cefuroxime ineffective?
• Why was the diagnosis made by a urine antigen test instead of a culture?
What should be done in Ghana?

• Frequency of this infection is not known
• Rapid diagnostic tests unlikely to be available
• Treat severe pneumonia on suspicion:
  – Patients on steroids or other immunosuppressive medications
  – Recent overnight travelers
  – People with exposure to heated water or water aerosols
• Therapy includes azithromycin, a fluoroquinolone, or erythromycin + rifampin
Additional Source Information
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Slide 6:  M. Rein, CDC/Public Health Image Library, Clue cells, #3719, http://phil.cdc.gov/phil

Slide 7: Gram stain of a clue cells, source undetermined


Slide 21: X-ray, source undetermined

Slide 23: X-ray, source undetermined


Slide 28: Fibrinopurulent pneumonia, source undetermined

Slide 29: Legionella Gram stain, source undetermined

Slide 31: source undetermined

Slide 40: source undetermined