Chlamydia, Mycoplasma, Legionella

Margaret Fitzpatrick, MD, MS
Division of Infectious Diseases
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Chlamydia, Mycoplasma, Legionella

• Do not Gram stain well
  – Intracellular (Chlamydia, Legionella)
  – No cell wall (Mycoplasma)
• Difficult to culture
• Frequently cause respiratory infection
  – Upper respiratory tract infection
  – Atypical community-acquired pneumonia

Learning Objectives

• Distinguish the unique life cycle and structure of Chlamydia from those of other bacteria
• Identify the primary mode of transmission of Chlamydia trachomatis in the U.S.
Chlamydiaceae

- Obligate intracellular bacteria with unique life cycle
  - "Energy parasites": need host cell for energy
    - produce no ATP; derive energy in endosome (exchange ADP for ATP)
  - Can only be cultured within live cells (difficult)

Life Cycle of Chlamydiaceae

EB = Elementary Body (infectious but can't replicate)
RB = Reticulate Body (non-infectious but can replicate)

Chlamydiaceae

- **Structure**
  - Rigid cell membrane without peptidoglycan but high lipid content
  - Inclusion bodies stain purple/blue with Giemsa
  - Cell wall contains penicillin binding proteins (PBPs), but penicillins not clinically effective

- **Clinically relevant species**
  - Chlamydia trachomatis, Chlamydophila psittaci, Chlamydophila pneumoniae

**C. trachomatis**: pathogenesis

- Cell wall contains LPS but it’s a weak form of endotoxin
- Major outer membrane protein (MOMP) = serovars
  - Different serovars associated with different clinical infections
- Infects non-ciliated epithelial cells found on mucous membranes
  - Minute abrasions or lacerations > robust inflammatory response
- No long-lasting immunity

**C. trachomatis**: epidemiology

- Human pathogen
- Transmission mostly via direct contact across mucosal surfaces
- Most common sexually transmitted infection in U.S.
  - > 1.3 million infections/year reported to CDC
- Ocular trachoma endemic in Africa, the Middle East, South Asia, and South America
C. trachomatis: epidemiology

Learning Objectives

- Recognize the four major clinical syndromes caused by infection with C. trachomatis
- Choose the best laboratory test for the diagnosis of Chlamydia trachomatis
- Distinguish the epidemiologic and clinical manifestations of Chlamydia pneumoniae and C. psittaci infection from C. trachomatis

C. trachomatis: clinical syndromes

1. Urogenital tract infection (serovars D-K)
2. Eye disease
   - Ocular chlamydia (serovar A, B, C)
   - Adult inclusion conjunctivitis
3. Lymphogranuloma venereum (LGV) (serovars L1, L2, L3)
4. Congenital infection
   - Infant inclusion conjunctivitis
   - Pneumonitis
**C. trachomatis: urogenital tract infection**

- Sexually active, mostly teens and young adults
  - high rate of transmission
- Clinical
  - Men (up to 25% asymptomatic)
    - urethritis, epididymitis, prostatitis
  - Women (up to 80% asymptomatic)
    - cervicitis, PID (endometritis, salpingitis, tubo-ovarian abscess)
  - Both: rectum (proctocolitis)
  - Adult Inclusion conjunctivitis (age 18-30)

**C. trachomatis: trachoma**

- Chronic inflammatory granulomatous disease of eye
- Follicular conjunctivitis > eyelid cutting and scarring > corneal ulceration, scarring, and blindness
- Mainly seen in poor rural areas of developing countries and commonly affects young children
- Prevention – prompt treatment of disease and improved sanitation
**C. trachomatis**: congenital infections

- Passage through infected birth canal
- Inclusion conjunctivitis
  - Most common cause of neonatal conjunctivitis in U.S.
  - Develops in ~25% of infants whose mothers have genital infections
  - Eyelid swelling, mucopurulent eye discharge
- Infant pneumonia
  - 2-3 weeks after birth; symptoms can last for weeks
  - Rhinitis followed by distinctive staccato cough

**C. trachomatis**: LGV

- Sporadic in U.S. but highly prevalent in Africa, Asia, Central and S. America
- Incubation 1-4 weeks, primary lesion
- Suppurative multilocular inguinal lymph nodes (buboes)
  - Gradually enlarge and form draining fistula
  - Fever/chills, myalgias, headache
  - May resolve or lead to chronic ulcers
- Can also cause proctocolitis
**C. trachomatis:** diagnosis

- Culture of the organism from epithelial cell tissue
- Antigen detection:
  - Direct immunofluorescence stain with monoclonal antibodies
  - Enzyme-linked immunosorbent assays (ELISA)
- Nucleic acid amplification tests (NAAT) - PCR
  - Test of choice due to best sensitivity and specificity (> 90%)
- Serology may be helpful for LGV in right clinical context

**C. trachomatis:** treatment

- Urogenital tract infection and ocular infection
  - Azithromycin 1g x 1 dose
  - Doxycycline x 7d
  - Ophthalmologic surgery for trachoma
- Newborns
  - Erythromycin x 14d
- LGV
  - Doxycycline x 21d
  - May need to drain lesions
**Chlamyphila pneumoniae**

- Atypical community-acquired pneumonia, bronchitis, sinusitis, pharyngitis
  - Usually fairly mild symptoms
  - Person-person spread by respiratory droplets
  - Most common in school age children but any age at risk
- Diagnosis can be difficult
  - Serology; NAATs are best but more variability than for C. trachomatis
- Treatment: Azithromycin, doxycycline, respiratory fluoroquinolone

**Chlamyphila psittaci**

- Psittacosis (zoonosis)
  - Inhale respiratory secretions or droppings of infected birds
  - Psittacine birds (parrots, parakeets)
  - Lower respiratory tract infection
    - Headache, fever/chills, muscle aches, dry cough, bilateral pneumonia
  - Rare but true prevalence unknown
  - Diagnosis is difficult: serology can be used
    - Four fold increase or high titer IgM
  - Treatment = doxycycline x 10-14d, azithromycin is alternative
A 22-year-old previously healthy man presented to clinic with a three-day history of painful urination and occasional scant white discharge from the tip of his penis. He admits to recent unprotected sex with two different female partners in the past month. He denies fever, back or flank pain, blood in his urine, or any penile ulcers or lesions. His physical examination is normal. Recent blood testing for syphilis and HIV is negative. Gram stain from a urethral swab is negative.

Which of the following tests will be most helpful in establishing a diagnosis?

A. Culture from a urethral swab  
B. Acute and convalescent serology  
C. Direct fluorescent antibody stain from a urethral swab  
D. Nucleic acid amplification test (e.g., PCR) from voided urine

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**Mycoplasma**

- *Mycoplasma pneumoniae*
- *Mycoplasma hominis*
- *Mycoplasma genitalium*
- *Ureaplasma urealyticum*
Learning Objectives

- Distinguish the unique structural and microscopic characteristics of Mycoplasma and how they affect antibiotic treatment
- Recognize the unique epidemiology and clinical syndromes caused by Mycoplasma pneumoniae including secondary complications

**Mycoplasma: structure and culture**

- Smallest free living bacteria (0.6 µm)
- **No cell wall**, single triple layered cell membrane (sterols)
  - Require cholesterol for growth
  - Antibiotics active at the cell wall (i.e. beta-lactams) are not effective
- Culture difficult - Eaton agar used, "fried egg" appearance

**Mycoplasma: pathogenesis**

- Human pathogen causes respiratory and urogenital tract diseases
- *M. hominis* and *M. genitalium* bind oligosaccharide receptors on mucosal epithelial cells
- *M. pneumoniae* attaches to cilia of respiratory epithelium via attachment structure (P1 adhesin protein)
- Acts like superantigen – stimulates cytokines TNF, IL-1, IL-6
**M. pneumoniae: epidemiology**
- Strict human pathogen
  - Spread via large respiratory droplets
- Epidemics every 4 - 7 years
- 5-15% of community acquired pneumonia
- Peak incidence in school age and teens
- Military recruits and prisons

**M. pneumoniae: clinical manifestations**
- Headache, fever, chills, malaise, myalgias
- Pharyngitis (25-50%)
- Tracheobronchitis
  - Initially severe dry, non-productive cough > later, white / clear sputum
- Pneumonia
- Symptoms can last weeks or longer
**M. pneumoniae: clinical manifestations**

- Secondary complications:
  - Cold agglutinin (IgM) hemolytic anemia
  - Meningoencephalitis
  - Transverse myelitis and paralysis
  - Pericarditis
  - Arthritis
  - Mucocutaneous lesions and rashes

*M. pneumoniae: diagnosis and treatment*

- **Diagnosis**
  - Culture on Eaton agar (10-14 days)
  - Paired IgG serology or single high titer IgM
  - NAAT - PCR from respiratory tract samples
- **Treatment**
  - Azithromycin, doxycycline, respiratory fluoroquinolones
Learning Objectives

• Identify two *Mycoplasmas* that cause urogenital tract infection and their clinical manifestations
• Choose the best laboratory test for the diagnosis of urogenital *Mycoplasma* infections.

Urogenital *Mycoplasma*

• *M. hominis*, *M. genitalium*
• Both may colonize urogenital tract
• Both implicated in true infection and are associated with other STIs
• *M. hominis* more common in women
• *M. genitalium* more common in men

*Mycoplasma hominis*

• Postpartum fever, chorioamnionitis
• Commonly isolated in bacterial vaginosis
• Pyelonephritis
• Endometritis, PID
• Systemic infections in immunocompromised
**Mycoplasma genitalium**

- Urethritis in men (strong link)
- Cervicitis and PID in women (moderate link)
- Tubal infertility, spontaneous abortions (weak link)
- May see > 5 WBC / hpf on a urethral or cervical swab and negative GC/Chlamydia

**Urogenital Mycoplasmas: diagnosis and treatment**

- **Diagnosis:**
  - *M. hominis* can be cultured but very difficult
  - NAAT (PCR) testing is preferred due to greater sensitivity
  - No FDA-approved PCR assays approved or sold commercially
- **Treatment:**
  - *M. hominis*: doxycycline
  - *M. genitalium*: azithromycin (increasing resistance), doxycycline, moxifloxacin

An 19 year old man is seen in the clinic due to persistent painful urination and penile discharge. He has had unprotected sex with three different partners in the last 3 months. He was treated for a presumed sexually transmitted urethritis 2 weeks ago with ceftriaxone and azithromycin. He reports no improvement in his symptoms and no new sexual partners. NAAT testing of urinalysis was negative for N. gonorrhoeae or C. trachomatis. HIV and syphilis testing is negative. Urinalysis shows 8 WBC / hpf.

Which of the following is the most likely diagnosis?

A. Antibiotic resistant C. trachomatis
B. Antibiotic resistant N. gonorrhoeae
C. Mycoplasma genitalium
D. Mycoplasma hominis
E. Non-infectious urethritis
**Legionella**

**Learning Objectives**

- Distinguish the unique structural and microscopic characteristics of *Legionella* from those of other bacteria
- Identify the requirements for and limitations of laboratory culture of *Legionella*.

**Legionella: structure**

- Slender, pleomorphic Gram-negative bacilli
- Gram stains poorly in clinical specimens
- Seen in tissue with Dieterle’s silver stain

**Legionella: taxonomy**

- One genus: *Legionella*
- Ubiquitous aquatic organism
- 39 species and 60 serogroups
- 90% of all infections are caused by *L. pneumophila*
  - serotype 1 and 6 are the most common

**Legionella: culture**

- Obligate aerobe
- Non-fermentative
  - Derive energy from amino acids, not carbohydrates
- Fastidious growth requirements
  - Buffered charcoal yeast extract agar (BCYE)
  - Growth enhanced with addition of cysteine and iron salts

Buffered Charcoal Yeast Extract Agar - BCYE
Legionella: pathogenesis

- Inhale infectious aerosols
  - Shower, sink tap, cooling tower, humidifier
- Bacterial OMP (porin) binds C3b complement receptor on mononuclear phagocytes
- Endocytosis into phagosome > inhibit phagolysosome fusion > hijack intracellular organelles > multiplication > bacteria kill cell with lysis of vacuole
- Immune response is primarily cell mediated

Learning Objectives

- Identify the reservoir, mode of transmission and unique epidemiologic characteristics of *Legionella pneumophila*.
- From a clinical description and laboratory tests, diagnose *L. pneumophila* infection
Legionella: epidemiology

- Found worldwide in bodies of water
  - Lakes and streams
  - Bacteria proliferate in man made water reservoirs
    - Air conditioning cooling towers, whirlpool spas, showers, hot tubs, water misters
    - Growth enhanced by warm temperature, stagnation, scale, sediment and commensal algae, protozoa and bacteria
- 1200-2000 cases/year reported (estimated 10,000-12,000)

Legionella: epidemiology

- Community acquired
  - Sporadic
  - Outbreaks: cooling towers, showers, grocery store mist machines, fountains, high-pressure cleaners, whirlpools
  - Travel associated – hotels
- Healthcare-associated (~25%)
- Host risk factors
  - Recipient of a transplanted organ
  - Cigarette smoking
  - Chronic lung disease
  - Older age, men

Former lobby fountain, J.W. Marriott Hotel, Michigan Ave.
**Legionella: clinical manifestations**

- **Pontiac Fever** – febrile influenza-like illness without pneumonia
  - Short incubation – 1-2 days
  - Self-limited, resolves without treatment
- **Legionellosis or ‘Legionnaires disease’**
  - Longer incubation – 2-10 days
  - Fever, chills, cough, HA; severe pneumonia
  - Multi-system disease: GI, CNS, liver, kidney
  - Hyponatremia
  - 15-20% mortality

**Legionella: diagnosis**

- **Microscopy**
  - Gram stain not helpful; Silver stain or DFA in tissue
- **Culture** – BCYE media with iron and cysteine
  - BAL, lung biopsy, pleural fluid, other body fluid/tissue
- **Urinary antigen test**
  - ELISA detects L. pneumophila serogroup 1
  - 60-95% sensitive; >99% specific
- **Serology** – 4 fold titer rise
- **NAATs** – not well standardized or available yet
**Legionella: treatment and prevention**

- **Treatment:**
  - Pontiac fever – no treatment
  - Legionellosis -- Azithromycin or respiratory fluoroquinolone

- **Prevention:**
  - Eliminate or reduce bacterial numbers in water supply
  - Clean water reservoirs (air-conditioning cooling towers)
  - Remove from potable water systems
    - Superheating, hyperchlorination, copper/silver ionization

A 72 yo man with a history of a kidney transplant in 2011 and tobacco abuse presented with fever to 103°F, confusion, cough productive of thick sputum, and diarrhea. It is summer, and the patient has spent time outside working in his garden and installing a new water fountain feature. On physical exam, the patient has a dense R basilar infiltrate on CXR. His WBC is mildly elevated at 12.7 and his sodium level is low at 123. Other labs are normal. Gram stain and routine culture of expectorated sputum are negative.

Which of the following special culture media could be used to grow the organism responsible for this patient’s infection?

A. Eaton agar with added sterols
B. Sheep’s blood agar
C. Buffered charcoal yeast extract agar (BCYE)
D. Chocolate agar
E. Thayer-Martin agar