THE GRAM POSITIVE RODS AND AEROBIC ACTINOMYCETES

- **Reading Assignment:** Murray et al., *Medical Microbiology*, 8th ed. 2016. Chapters 20-22

**OBJECTIVES**

- Describe the key identifying characteristics of *Bacillus anthracis*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Corynebacterium diphtheriae*, *Arcanobacterium hemolyticum* and *Nocardia* species.
- Describe the epidemiology, and clinical presentation of infections caused by: *Bacillus anthracis*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Corynebacterium diphtheriae*, *Arcanobacterium hemolyticum* and *Nocardia* species.
- Describe the three forms of disease caused by *Bacillus anthracis*.
- List three patient populations that are at risk for listeriosis.
- Name an aerobic Gram-positive rod that can cause food poisoning.
- List the diseases associated with *Nocardia*.
Since you've let those bacteria near ya—
All those club-shaped corynebacteria—
There's a membranous coat
On the back of your throat,
And you've likely contracted diphtheria.

“Not every wee microbe is bad,”
Said my father, "Eat up and be glad
That the lacto-bacillus
Will cure us, not kill us.
So finish that yogurt, my lad."
Gram Positive Rods

Bacillus
Listeria
Erysipelothrix
Corynebacteria
Arcanobacterium
Lactobacillus
Nocardia

Gram Positive Rods

Gram Stain Morphology

- Pleomorphic/Coryneform
- Beaded Branching Filamentous
- Straight

Listeria monocytogenes
Corynebacterium sp.
Erysipelothrix rhusiopathiae
Arcanobacterium sp.
Garvenella vaginalis
Lactobacillus sp.

Nocardia sp.
Bacillus sp.
Clostridium sp.

Bacillus, Coryne, Listeria are **catalase +**
Arcano, Erysip, Lacto are **catalase -**
**Bacillus species**

*Key Characteristics*

- Gram positive rods
- Beta- or non-hemolytic colonies
- Spore forming bacillus
- Catalse +

**Characteristics of Bacillus species**

- Spore forming Gram positive rod
- Widespread in nature
- Prefers aerobic conditions
- Most are motile except for *B. anthracis*

**Bacillus Spores**
Case

- 61 y.o. male past medical history including chemical pneumonitis with recurrent pneumonias
- Past three weeks on a road trip out West visiting National Parks, including Yellowstone, Glacier, and the Badlands.
- Began to feel some malaise and generalized fatigue
- Developed fevers, shaking chills, global-type headache, dyspnea and a cough productive of yellow sputum
- Symptoms worsened and he presented to a community hospital and admitted for community acquired pneumonia
- Treatment with antibiotics (ceftriaxone and azithromycin)

Case

- Transferred to an academic medical center with worsening respiratory status and increasing oxygen requirements.
- WBC = 20.3 x 10^3/μL
- Transferred to MICU
- Blood cultures positive within a few hours
- Antibiotic regimen was changed to (Ciprofloxacin, Vancomycin, Meropenem)

Gram Stain Direct from Blood Culture

“Bamboo-like” Rods
Case Diagnosis

*Bacillus anthracis*—confirmed by CDC

- Naturally Acquired Inhalation Anthrax
- Minnesota Department of Health (MDH) released a statement stating it was investigating a case of inhalation anthrax
- Due to environmental exposure and case did not represent an increased risk to the general public.
- Ultimately, the environmental source in this case was not found.

*Bacillus anthracis*

- Disease: Anthrax
  - Inoculation (95%)
    - Contaminated soil or infected animal products
  - Ingestion
  - Inhalation
    - Wool Sorters disease
    - Processing goat hair
    - Biological weapons (Category A Biothreat Agent)

NIAID Emerging Infectious Diseases/Pathogens

- Category A pathogens are those organisms/biological agents that pose highest risk to national security and public health because they:
  - Can be easily disseminated or transmitted from person to person
  - Result in high mortality rates and have potential for major public health impact
  - Might cause public panic and social disruption
  - Require special action for public health preparedness

NIAID Emerging Infectious Diseases/Pathogens

Category A Priority Pathogens

- *Bacillus anthracis* (anthrax)
- *Clostridium botulinum* toxin (botulism)
- *Yersinia pestis* (plague)
- Variola major (smallpox)
- *Francisella tularensis* (tuleremia)
- Viral hemorrhagic fevers
  - Flaviviruses: Dengue
  - Filoviruses: Ebola, Marburg

Inhalation Anthrax

- Prolonged latent period (2 months or more)
- Initial symptoms – nonspecific
  - Fever, SOB, cough, HA, vomiting, chills, chest and abdominal pain
- Second stage
  - Rapidly worsening fever, edema and enlargement of mediastinal lymph nodes (responsible for the widened mediastinum observed on chest X-ray)
- Pulmonary disease rare, meningeal symptoms occur in 50% of patients
- Shock & death occurs within 3 days unless treatment is initiated immediately

Chest Imaging

Mediastinal widening is a classical finding.
Cutaneous Anthrax

- Inoculation
- Painless papule
- Ulcer surrounded by vesicles
- Necrotic eschar
- Anthrax = Greek word for “coal”
- 20% mortality

Gastrointestinal Anthrax

- Ingestion
- Upper GI
  - Ulcers in mouth and esophagus
- Lower GI
  - Terminal ileum most common
  - N/V, malaise, bloody diarrhea
- Mortality approaches 100%

Pathogenesis

- Capsule: inhibits phagocytosis of replicating cells
- Edema toxin: fluid accumulation
- Lethal toxin: stimulates macrophages to release tumor necrosis factor and interleukin 1 beta
- Protective AG: binds specific cell surface receptors that enables ET & LT to enter the cell
**Bacillus anthracis**

Lab Identification
- Culture; skin, blood, sputum, CSF
- Gram + box car shaped bacilli
- Biochemical tests
  - Motility - negative
  - Non-Hemolytic

**Rule Out Bacillus anthracis**

**Bacillus cereus**

Infections
- Food poisoning
  - Caused by exotoxins
  - Emetic form
  - Diarrheal form
- Ocular infections
- Central line infections
  - Contaminated lines must be removed
- Opportunistic infections
Listeria monocytogenes

Key Characteristics

- Gram positive rods
- Beta-hemolytic colonies
- Catalase +

Listeria monocytogenes

Epidemiology

- Found in soil, stream water, sewage
- Part of the fecal flora of many animals
- Plants, meats and dairy are contaminated with the water or animal feces
- Undercooked and unpasteurized foods
- Asymptomatic human fecal carriage (small %)

Listeria monocytogenes

Epidemiology

- Soft cheeses, veggies, and cold cuts at the deli counter become contaminated
- “Ready to eat” foods, smoked seafood
**Listeria monocytogenes**

*Pathogenesis*

- Bacteria invade epithelial cells, M cells, macrophages by internalin protein
- Bacteria are engulfed in vacuole
- Bacteria produce listeriolysin and phospholipases escapes from phagosome
- Encoded by ActA gene, host cell actin utilized to move bacteria into adjacent cells without exposure to immune system

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**Listeria monocytogenes**

*Pathogenesis*

- Humoral immunity relatively unimportant because bacteria move within cells, thus avoiding antibody-mediated clearance
- Patients with defects in cellular immunity but not in humoral immunity are particularly susceptible to severe infections
**Listeria monocytogenes**

**Infections**

- Neonates
  - Early-onset disease: acquired transplacentally in utero, characterized by disseminated abscesses and granulomas in multiple organs
  - Late-onset disease: acquired at or shortly after birth presents as meningitis with sepsis
- Elderly
  - Typically influenza-like illness with or without gastroenteritis
- Pregnant women or patients with cell-mediated immune defects
  - Primary bacteremia or disseminated disease with hypotension and meningitis

**Listeria monocytogenes**

**Lab Identification**

- Culture blood, CSF, fluids
  - Grows in 1-2 days
  - Beta hemolytic (soft-hemolysis similar to Group B Strep)
- Gram Stain:
  - Short Gram-Positive rods
- Biochemical tests
  - Catalase +
  - Tumbling motility
  - Motile at room temp
  - Non-Motile at 37°C
  - Demonstrates “Umbrella” Motility in semi-solid media

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![Image of Listeria monocytogenes](image1)

**Figure 1:** Umbrella Motility in Semi-solid Nutrient Agar at Room Temperature
**Listeria monocytogenes**

**Prevention**

People at high risk should avoid eating:
- raw or partially cooked foods of animal origin
- soft cheeses, and
- unwashed raw vegetables and fruits (cantaloupe)

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**Erysipelothrix rhusiopathiae**

**Key Characteristics**

- Gram positive rods
- Catalase -
- H₂S Production

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**Erysipelothrix rhusiopathiae**

**Epidemiology**

- Ubiquitous in soil and groundwater, distributed worldwide
- Animal disease widely recognized but human disease is uncommon
- Recovered from tonsils and digestive tract of mammals, birds and fish.
- Colonization high in swine and turkeys
- Human infection acquired from animals, primarily occupational: butchers, meat processors, farmers, poultry workers, fish handlers and veterinarians
**Erysipelothrix rhusiopathiae**

Infection

Two forms of human infection

- **Erysipeloid:**
  - Cutaneous infections typically develop after the organism is inoculated subcutaneously
  - Localized skin infection, on fingers or hands and appears violaceous with a raised edge.
  - Slowly spreads peripherally as discoloration fades.
  - Suppuration is uncommon (separates from streptococcal erysipelas)

- **Septicemia:**
  - Uncommon, when present frequently associated with endocarditis

**Lab Identification**

- Pleomorphic Gram positive rods that form long filaments ("hairlike")
- Microaerophilic or facultative anaerobe
- Slow growth, requires 2-3 days incubation
- Small, grayish, α-hemolytic colonies
- Catalase negative, non-motile,
- Produces H2S

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**Corynebacterium species**

Key Characteristics

- Gram positive rods
- Catalase +
Characteristics of *Corynebacterium*

- Pleomorphic Gram positive rods
- Clumps of organisms often referred to as resembling Chinese “letters”
- Aerobic or facultative anaerobic
- Small white non-hemolytic colonies
- Commonly called “diphtheroids”

Gram Stain Morphology

*Corynebacterium* species

**Epidemiology**

- Ubiquitous in plants and animals
- Colonize humans
  - Skin
  - Upper respiratory tract
  - Gastrointestinal tract
  - Urogenital tract
**Corybacterium diphtheriae**

**Epidemiology**
- Infection transmitted by respiratory droplets or direct contact with cutaneous infection
- Etiologic agent of diphtheria
- Rare disease in U.S. due to immunization
  - Ab produced against toxin in natural infection
  - Vaccinate with toxoid = formalin treated toxin
  - Re-vaccinated every 10 years

**Pathogenesis**
- Disease caused by potent exotoxin >> inhibits protein synthesis of eukaryotic cells
- Two subunits of toxin
  - A = shuts off protein synthesis
  - B = binds to cell receptor
- Toxin gene carried in bacteriophage

**Infection**
- Respiratory Form
  - Pharyngitis with patchy exudates on tonsils, uvula, soft palate
  - Tough gray pseudomembrane consists of fibrin, white cells, bacteria, debris >> respiratory obstruction and suffocation
  - Toxin circulates to heart >> injury
  - Toxin circulates to CNS >> reversible paralysis
Diptheria “leathery skin”

**Coryebacterium diphtheriae**

**Infection**

Cutaneous Form
- Chronic, non-healing ulcer
- Often associated with travel history
- Staphylococcus aureus and *Streptococcus pyogenes* often co-isolated in wound culture

**Lab Identification**
- Diagnosed by clinical evaluation
- No rapid lab test
- Toxin testing done through CDC lab (Elek Test)
- Must notify lab to look at throat cultures for *C. diphtheriae*
  - Grow the organism on selective agar
  - Prove presence of toxin
- Treat with antitoxin and antibiotics
**Arcanobacterium hemolyticum**

- Non-spore forming Gram-positive rod producing irregular, club-shaped, curved or “V” formation
- Catalase-negative
- Beta hemolytic - colonies appear similar to beta-hemolytic Group A Strep on blood agar
- Isolated mostly from young adults (15-25 Y.O.)
- Symptomatic pharyngitis, fever, occ. cutaneous rash, some with pseudomembranes pharynx/tonsils, and submandibular lymphadenopathy
- Also isolated from wounds, abscesses and blood of patients with septicemia and endocarditis

**Lactobacillus species**

**Key Characteristics**

- Gram positive rods
- Catalase -
- Non-spore forming Gram positive rod
- Normal flora of oral cavity, vaginal tract
- Found in food products (yogurt)
- Prefers carbon dioxide or anaerobic atmosphere
- Opportunistic pathogen
  - Sepsis
  - Endocarditis
Aerobic Actinomycetes

• Aerobic Gram-positive, catalase-positive rods that can colonize animals and humans and are found commonly in soil and decaying vegetation.
• ≠ Actinomyces sp. (typically reviewed with anaerobes)

Nocardia species
Key Characteristics

- Beaded branching Gram positive rods
- Chalky colonies

Characteristics of Nocardia (11 species)

• Gram-positive, partially acid-fast rods; cell wall with mycolic acid
• Strict aerobe, will grow on most nonselective agars, prolonged incubation (7 days or more may be required)
Nocardia – Gram Stain

Nocardia – Modified Acid Fast Stain

*Nocardia species*

**Epidemiology**

- Worldwide distribution in soil
- Exogenous infections acquired by inhalation (pulmonary) or traumatic introduction (cutaneous)
- Disease most common in immunocompetent pts. with chronic pulmonary disease or immunocompromised pts. with T-cell deficiencies
**Nocardia species**

- Bronchopulmonary disease
- Primary or secondary cutaneous infections
- Secondary CNS infections (e.g., brain abscesses)
- Infections are treated with antibiotic therapy and proper wound care

A 22 year old medical student presents with symptoms of pharyngitis with a fever, and cutaneous rash. A throat culture reveals small beta hemolytic colonies that are catalase-negative and stain as short Gram-positive rods exhibiting irregular morphologies. What is the most likely agent of this student’s pharyngitis?

- A. Arcanobacterium hemolyticum
- B. Corynebacterium diphtheriae
- C. Erysipelothrix rhusiopathiae
- D. Listeria monocytogenes
- E. Streptococcus pyogenes

A knee joint fluid is received in the lab. A small, gray-white colony is growing on blood agar. It produces a narrow zone of β-hemolysis and looks like Streptococcus. However, the Gram stain shows a Gram positive rod and it’s catalase positive. What is the most likely identification of this bacteria?

- A. Streptococcus agalactiae
- B. Arcanobacterium hemolyticum
- C. Staphylococcus aureus
- D. Bacillus anthracis
- E. Listeria monocytogenes