GRAM POSITIVE BACILLI

Mahon, Lehman, & Manuselis, 4th edition
Bacillus sp. pages 369-373, *Listeria monocytogenes* pages 359-361,
Erysipelothrix rhusiopathiae pages 362-363, *Corynebacterium* sp. pages 353-359,
Gardnerella vaginalis pages 364-365, 510, 907, *Lactobacillus* sp. pages 510,
Nocardia sp., *Actinomyces* sp., & *Streptomyces* sp. pages 365-369

Differentiation of major gram-positive rod genera

- Based upon Gram stain morphology, formation of spores, and catalase reaction
  - Spore-forming, catalase positive (aerobic): *Bacillus* sp.
  - Regularly-shaped, non-spore forming
    - Catalase positive: *Listeria monocytogenes*
    - Catalase negative: *Erysipelothrix rhusiopathiae*, *Lactobacillus* sp., *Gardnerella vaginalis*
  - Irregularly-shaped, non-spore forming, catalase positive: *Corynebacterium* sp. ("diphtheroids")
  - Branching: *Nocardia* sp., *Actinomyces* sp., & *Streptomyces* sp.

Spore-forming, non-branching, aerobic or facultative anaerobic bacilli, catalase positive

A. *Bacillus* species

1. **Epidemiology** - Widely distributed in nature (soil, water, airborne dust)

2. **Virulence factors**
   a. Production of endospores
      - Spores produced when bacteria is stressed
   b. Some species produce capsules and exotoxins

3. **Clinical Significance**
   a. Usually considered environmental contaminants
   b. *B. anthracis* is etiologic agent of anthrax
   c. Opportunistic infections - serious infections can occur in immunocompromised hosts

4. **Laboratory Identification**
   a. Gram stain – Large aerobic or facultative anaerobic gram-positive rods that form endospores (may or not exhibit spores)
      - Spores produced when bacteria are stressed such as drying conditions, unfavorable temperatures
        - Appear as clear areas within bacterial cell on gram stain
        - Spores do not distend (change shape or swell) the cell wall
        - Aerobic production of spores only
        - Organism can be heat shocked to form spores; heat suspension in 56˚C water bath
      - May stain gram variable or negative
      - Perform 3% KOH test to establish gram reaction if needed
        - Place a drop of 3% KOH on slide, with a loop emulsify in KOH on slide, gently raise the loop up approximately 0.5 to 1cm:
          - Viscous string (bacterial DNA) follows loop = GNR
          - No viscous string follows loop = GPR
   b. Colony morphology
      - Growth on SBA or CHOC with 24 hours at 35˚C in ambient air or 5% CO2
      - Colonies are usually large, flat with frequent hemolysis

c. Presumptive Identification – to rule out pathogen *B. anthracis*
    - Usually catalase positive
    - Most species are:
        - Beta-hemolytic on SBA (*B. anthracis* is non-hemolytic)
        - Motile (*B. anthracis* is non-motile)

B. *Bacillus anthracis*

1. **Epidemiology**
    a. Widely distributed in nature
    b. Cause disease in animals and man
        - Animals infected by feeding on plants contaminated with spores
        - Humans primarily infected as a result of contact with animals or animal products
            - Animal hides, fibers, or other animal products
            - Inhalation or traumatic introduction

2. **Virulence factors**
    a. Antiphagocytic capsule
    b. Exotoxins that synergistically mediate cell and tissue destruction (edema factor, lethal factor, and protective antigen)

3. **Clinical Significance**
    a. One of the most virulent microorganisms for humans
        - Bioterrorism agent
    b. **Causative agent of anthrax**
        - Cutaneous anthrax – site of spore penetration, ulceration to formation of **black eschar,** may lead to fatal toxemia (approximately 20% mortality)
        - Inhalation anthrax (woolsorter's disease) – inhalation of spores, respiratory distress, chest edema, cyanosis and death (100% fatal if not treated very early)
        - Gastrointestinal anthrax – ingestion of spores, most patients die from toxemia and overwhelming sepsis

4. **Laboratory Identification – Lab safety is critical when suspecting *B. anthracis***
    a. Gram stain – large, square-ended, gram-positive or gram-variable rods in singles or chains
        - Central spores (do not distend cell wall) are generally not present in clinical samples
        - Presence of capsule (clear zones around cells) strongly presumptive ID
    b. Colony morphology – non-hemolytic, large, gray flat colonies with irregular margins (filamentous projections – Medusa head) on SBA
        - Bicarbonate agar in 5% CO2 will induce capsule formation (mucoid colony)
    c. Preliminary Identification
        - **Non-hemolytic, non-motile** (either wet prep or motility media may be used)
        - **Suspected colonies should be sent to reference laboratory of state health laboratory for confirmatory identification (cases reported to state and CDC)**
        - Penicillin - Sensitive

5. **Treatment/Antibiotic therapy/Prevention**
    a. Most isolates are susceptible to penicillin
    b. CDC recommends treatment with ciprofloxacin or doxycycline
    c. Animal vaccine is responsible for reducing incidence
    d. Human vaccine is available (military and health care workers)

C. *Bacillus cereus*

1. **Epidemiology**
   a. Widely distributed in nature, human GI tract

2. **Virulence factors**
   a. Toxins (enterotoxin or emetic)

3. **Clinical Significance**
   a. Food poisoning – food contaminated with organism or toxins formed by organism
      - Diarrheal type – abdominal pain and watery diarrhea caused by enterotoxin
        - Associated with poultry, meats, soups, vegetables and desserts, symptoms usually 8-16 hours after ingestion, recover 12-24 hours from onset
      - Emetic type – vomiting caused by emetic toxin
        - Associated with fried or boiled rice, symptoms usually 1-5 hours after ingestion, recover 6-24 hours after onset
      - Both diarrheal and emetic forms are usually mild and self-limiting

   b. Serious infections in immunocompromised host
      - Traumatic eye wounds, endocarditis, bacteremia and wounds

4. **Laboratory Identification**
   a. ***B. cereus* is normal stool flora, to diagnose food poisoning must culture suspected food NOT stool**
   b. Gram stain – large, gram-positive rods with spores, can stain gram-variable or gram-negative
   c. Colony morphology – beta-hemolytic; large, feathery, spreading on SBA
   d. Preliminary Identification
      - Beta-hemolytic
      - Motile
      - Penicillin – Resistant

Non-spore-forming bacilli, non-branching, catalase positive

A. Corynebacterium species

1. *Corynebacterium* species – "diphtheroids"

a. General characteristics and morphology

i. Widely distributed in nature
   - Many species normal flora of skin, mucous membranes
   - Most species are non-pathogenic (referred to collectively as "diphtheroids")

ii. Gram stain morphology
   - **Club-shaped and beaded with irregularly staining granules, pleomorphic (many sizes and shapes), palisading (Chinese letters) gram-positive rods**
   - Non-spore forming

iii. Characteristics
   - **Catalase positive**
   - Albert's stain (Loeffler's methylene blue stain) – Babst Ernst granules or metachromatic granules are seen in the organism cells (specific for *Corynebacterium* sp.). Granules stain dark blue/black within greenish rods
   - Non-motile
   - Bile esculin hydrolysis: negative
   - Glucose fermentation/oxidation: variable
   - Sucrose fermentation/oxidation: variable
   - Urease: variable
   - Nitrate reduction: variable

2. *Corynebacterium diphtheriae* – diphtheria

a. Diphtheria
- Disease of respiratory tract
- Pseudomembrane – should be cultured, if not present culture nose, throat, or wound
- Toxigenic versus non-toxigenic **(Exotoxin – toxin)**
  - o Toxin producing – infected by beta-bacteriophage (virus)
  - o Toxin blocks protein synthesis

b. Pathogenesis
i. Found primarily on the epithelial cells of the respiratory tract of persons with the disease or in carriers
ii. Infection occurs by droplets or contact to susceptible (no or low antitoxin) individuals
iii. During infection the organism localizes in upper respiratory tract and produces exotoxin that causes necrosis forming a grayish pseudomembrane (WBCs and organism)
iv. Toxin is absorbed into the blood and affects the myocardium and peripheral nervous system. Death is usually due to congestive heart failure.

c. Treatment and Prevention
i. Treatment - antitoxin is given in the form of a toxoid
ii. Prevention: DPT immunization

d. Isolation & identification
i. Gram stain morphology
- Irregularly staining, pleomorphic gram-positive rods

ii. Colony morphology
- BAP: 24-48 hours at 35°C in ambient or 5% CO2: small, gray, translucent colonies to medium, white, opaque colonies, may be beta-hemolytic

iii. Identification (Mahon pages 414-415)
- **Loeffler's media:** used for isolation of *Corynebacterium* species, enhances the granule formation as seen on Albert's stain and characteristic cellular morphology of *C. diphtheriae*
- **Cystine-Tellurite Blood agar - CTBA** (modified Tinsdale agar): selective for coryneform bacteria and differential; tellurite is reduced to metallic tellurium by *Corynebacterium* species causing colonies to appear grayish-black. *Corynebacterium diphtheriae* can be differentiated from other diphtheroids by it having a **brown halo around the colony**.
- **Nonlipophilic**
- **Glucose and Maltose = "F"**
- Sucrose = negative
- **Urea = negative**
- Nitrate reduction = positive
- **Toxigenicity tests** (Mahon, pg. 413, 415)
  - o **Definitive identification of *C. diphtheriae* as a true pathogen requires demonstration of toxin production by the isolate**
  - o In vivo method
  - o Immunodiffusion – Elek test
  - o ELISA
  - o PCR

3. *Corynebacterium jekeium*

a. Disease states
- Immunosuppressed patients – septicemia, meningitis, pulmonary disease
- Most common cause of diptheroid prosthetic valve endocarditis in adults

b. Isolation & Identification
i. BAP: 48-72 hours at 35°C in ambient air or 5% CO_2 - small, gray to white colony, non-hemolytic
ii. Gram stain
- Pleomorphic; occasionally, club-shaped gram-positive rods arranged in V forms or palisades

iii. Identification
- **Lipophilic** (growth in enhanced with lipid added to media such as Tween 80)
- **Glucose = "O"**
- **Sucrose = negative**
- **Urea = negative**
- **Nitrate reduction = negative**

c. Susceptibility testing
- **Exhibits resistance to multiple antibiotics usually used to treat gram-positive infections, to date all isolates have been susceptible to vancomycin**

4. *Corynebacterium urealyticum*

a. Disease States
- Urinary pathogen
- One of the most frequently isolated clinically significant crynebacteria

b. Isolation & Identification
i. BAP: pinpoint, nonhemolytic, white colonies
ii. Gram stain
- Pleomorphic; occasionally, club-shaped gram-positive rods arranged in V forms or palisades

iii. Identification
- Lipophilic (growth is enhanced with lipid added to media such as Tween 80)
- Catalase = positive
- **Urea = rapidly positive (within minutes following incubation on urea slant)**

c. Susceptibility testing
- **Exhibits resistance to multiple antibiotics usually used to treat gram-positive infections, to date all isolates have been susceptible to vancomycin**

B. Listeria species

1. *Listeria monocytogenes*

a. Epidemiology
i. Widespread in the environment – soil, water, vegetation, and animal products

b. Pathogenesis
i. Bacteremia and meningitis in immunosuppressed hosts
ii. Pregnant females may pass organism onto fetus causing systemic infection and stillbirth
iii. Neonate infections: early-onset (intrauterine infection, sepsis) or late-onset (usually meningitis)
iii. Ingestion of contaminated food: meat and dairy products
- Cheese, chicken, ice cream, luncheon meats, hot dogs

c. Isolation and identification:
   i. Gram stain
      - Small gram positive rod (almost coccal), may be in pairs or short chains
      - Non-sporulating
   ii. BAP 24-48 hours:
      - **beta-hemolytic (small zone);** small, round, smooth, and translucent, 30-35°C in ambient air or 5% CO2
      - Facultative
   iii. Identification
      - **Catalase = positive**
      - **Tumbling motility** on wet prep and **"umbrella-shaped" motility** in semi-solid media at room temperature
      - **Esculin hydrolysis= positive**
      - **Sodium hippurate hydrolysis = positive**
      - Ferments glucose
      - Cold enrichment (will grow at 4°C)

Non-spore-forming bacilli, non-branching, catalase negative

A. Erysipelothrix species

1. *Erysipelothrix rhusiopathiae*
   a. Morphology and characteristics
      i. Colonial morphology
         - Microaerophilic
         - Non-hemolytic or alpha-hemolytic on blood agar

      ii. Gram stain
         - Both short gram-positive rods and long filamentous rods corresponding to 2 colony types (decolorizes easily so may appear gram variable)
         - Non-sporulating

      ii. Identification
         - **Catalase = negative**
         - Non-motile
         - **"Test tube brush" growth pattern in semisolid motility tube after 48 hours incubation at room temperature**
         - **H_2S on TSI or KIA = positive** (the only GPR that is H_2S positive)
         - Sucrose = non-"F"

   b. Disease states
      i. Erysipelas
         - Zoonotic - in swine, it produces an important economic disease called swine erysipelas that is generally fatal
         - Man becomes infected by coming in direct contact with an infected animal
         - Organism enters abraded skin (often finger or hand)
         - A skin disease that is characterized by intense pain and is usually self-limited
         - Rare cases become serious, disseminating to septicemia with arthritis or endocarditis
         - There is no permanent immunity and relapses are common

B. Lactobacilli

1. *Lactobacillus* species
   a. Normal flora – mouth, gastrointestinal tract, female genital tract
      i. Produces lactic acid from glycogen which lowers vaginal pH and suppresses overgrowth of organisms that can be involved in bacterial vaginosis

   b. Disease states
      i. Rarely pathogenic – implicated in rare cases of endocarditis and meningitis

   c. Morphology and characteristics
      i. Colonial morphology
         - Aerotolerant anaerobes – incubate in 5-10% CO_2
         - SBA – pinpoint alpha-hemolytic colonies to medium rough gray colonies

      ii. Gram stain
         - Very pleomorphic - long slender GPR's in chains, or short coccobacilli
         - Non-sporulating

      ii. Identification
         - **Catalase = negative**
         - Sucrose = "F"
         - Resistant to vancomycin – aid in identification

C. Gardnerella species

1. *Gardnerella vaginalis*
   a. Morphology and characteristics
      i. Colonial morphology
         - Pinpoint, nonhemolytic colonies on SBA
         - **Growth on Human Blood agar (V, HBT) shows beta-hemolysis**

      ii. Gram stain
         - Pleomorphic, gram variable (gram positive) rod or coccobacillus
         - Non-sporulating
         - Specimen gram stain: **clue cells:** epithelial cells covered with gram positive and gram negative bacilli clustered around the edge

      ii. Identification
         - **Catalase = negative**
         - Sodium hippurate = usually positive
         - SPS = sensitive

   b. Disease states
      i. Bacterial vaginosis (a polymicrobic infection with *Gardnerella vaginalis*, *Porphyromonas*, *Mobiluncus*, *Prevotella, Mycoplasma hominis*)
         - **Diagnosis – 1) presence of watery non-inflammatory exudate, 2) clue cells seen on Gram stain or wet mount, 3) foul amine or fishy odor when 1 drop of 10% KOH added to discharge on a slide, 4) increased pH (>4.5) of vaginal fluid, 5) lack of *Lactobacillus***
         - **Culture is not recommended for diagnosis**

Branching Nocardioform Bacilli

A. Nocardia species

1. *Nocardia asteroides, N. braziliensis, N. farcinica, M. nova*
    a. Habitat
        i. Soil and on plant material

    b. Disease states
        i. Mycetoma (actinomycetoma) – a chronic, localized, painless, subcutaneous infection
            - Tissue swelling
            - Draining sinus tracts
            - Presence of sulfur granules
        ii. Lymphocutaneous infections
        iii. Skin abscesses or cellulitis
        iv. Immunocompromised patients – pulmonary and disseminated infections

    c. Morphology and characteristics
        i. Colonial morphology
            - **Aerobic growth** appears in 3-30 days
            - Chalky, matte, velvety or dry and crumbly forms, chalky-white to orange-tan
            - Will grown on SBA, mycology media and LJ media
        ii. Gram stain
            - Intertwining, branching, fine, delicate filaments with fragmentation, gram-positive rods that are often beaded in appearance
        ii. Identification
            - **Partially acid-fast positive**

B. Streptomyces species

1. *Streptomyces somaliensis, S. anulatus*
    a. Habitat
        i. Soil and decaying vegetation

    b. Disease states
        i. Mycetoma (actinomycetoma) – a chronic, localized, painless, subcutaneous infection

    c. Morphology and characteristics
        i. Colonial morphology
            - **Aerobic growth** appears in 3-30 days
            - Waxy, bumpy or velvety rugose forms, cream to brown-black
            - Will grown on SBA, mycology media and LJ media
        ii. Gram stain
            - Gram positive rods with extensive branching, chains and spores; does not fragment easily
        iii. Identification
            - **Acid-fast = negative**

Mycobacterium sp. – Brief introduction

Mahon, Lehman & Manuselis, 4th edition, chapter 26, pages 576-602

Examples:
Mycobacterium tuberculosis, Mycobacterium avium complex ("MAC attack" in HIV+ individuals)

Distinguishing Characteristics

A. Aerobic, non-spore forming rod-shaped

B. Organism does not stain readily because of **high lipid content in the cell wal**l. With the Gram stain procedure, the organism appears gram-positive and stains irregularly giving it a "beaded" appearance.

C. Acid-fast: **the organism retains stain even after attempts to decolorize with acid-alcohol, acids or acid-acetone solutions**. This is due to a unique fatty acid in the cell wall – mycolic acid.

   Carbolfuschin stains (Ziehl-Neelsen/Kinyoun) – also known as acid-fast stains: acid fast organisms stain red against a blue background
   Fuchsin = primary stain (red or magenta)
   Methylene blue = counter stain (blue)

D. Growth requirements
   1. 5-10% CO_2
   2. 35-37ºC
   3. 3-8 weeks for growth on solid media (i.e. Lowenstein-Jensen), 10-11 days liquid media systems

Safety – Biosafety Level 3 Procedures

A. Control aerosols
   1. Biological safety hood – Level II
   2. Centrifuges with self-contained carriers
   3. Wear mask, gloves, and lab coat or gown

B. Use appropriate germicide
   1. Amphyl (phenol-soap mixture)
   2. 10% bleach
   3. 70% ethanol
   4. 5% phenol

C. Ultraviolet (UV) light