Firmicutes: The Low G + C Gram-Positive Bacteria
Genus *Clostridium*...

- Great practical importance
  - food spoilage through the Strickland reaction (oxidation and reduction of amino acids to organic acids)
  - industrial production of butanol
  - toxin production (botulinum toxin)
  - endospore formers
Important Species of *Clostridium*

- **C. botulinum**
  - food spoilage (especially canned foods); botulism
- **C. tetani** – tetanus
- **C. perfringens**
  - gas gangrene, foodborne illness
- **C. difficile** – important nosocomial infection
  - post antibiotic infection (CDAD), pseudomembranous colitis
- **C. acetobutylicum** - manufacture of butanol
Botulism

• Caused by *Clostridium botulinum*, an obligately anaerobic, endospore-forming, Gram-positive rod
  – on Select Agent List (agents that pose a severe threat – CDC)

• Most common source of infection is insufficiently heated home-canned food
  – endospores not killed, then germinate and produce toxin
  – if food inadequately cooked, toxin remains and causes disease
Botulism

- Botulinum toxin
  - neurotoxin that binds to synapses of motor neurons
- Diagnosis
  - restricted to reference laboratories
  - demonstration of toxin in patient’s serum, stool, or vomitus or *C. botulinum* in stool cultures
Botulism

• Infant botulism (most common form)
  – endospores ingested, germinate, reproduce, and produce exotoxin (stomach not acidic)
  – constipation, listlessness, general weakness, and poor appetite; death may result from respiratory failure

• Treatment, prevention and control
  – symptomatic/supportive therapy and administration of antitoxin
  – safe food processing practices and not feeding honey to babies under one year of age
Tetanus

• Caused by *Clostridium tetani*
  – anaerobic, Gram-positive spore-former
    • endospores found in soil, dust, hospital environments, and mammalian feces
  – produces tetanospasmin in low oxygen tension environments
    • causes prolonged muscle spasms
  – also produces tetanolysin, a hemolysin

• Portal of entry – skin wounds (puncture wounds most susceptible)
Tetanus

• Clinical manifestations
  – early in disease – tension or cramping and twisting of skeletal muscles and tightness of jaw muscle
  – advanced disease – trismus (“lockjaw”), characteristic facial expressions, board-like rigidity of trunk, tonic convulsions, and backward bowing of back
  – death usually results from spasms of diaphragm and inter-costal respiratory muscles
Tetanus

• Diagnosis
  – clinical history of wound infection and muscle stiffness

• Treatment, prevention, and control
  – antibiotic therapy and treatment with antitoxin
  – active immunization with toxoid (DPT vaccine), and proper care of wounds contaminated with soil, prophylactic use of antitoxin
Gas Gangrene or Clostridial Myonecrosis

- Most commonly caused by *Clostridium perfringens*
  - Gram-positive, spore-forming rod
  - produce gas gangrene, a necrotizing infection of skeletal muscle or clostridial myonecrosis
  - secretes toxin and tissue damaging enzymes
- Transmitted by contamination of injured tissue by spores from soil or bowel microbiota
Gas Gangrene

• Clinical manifestations
  – severe pain, edema, drainage, muscle necrosis

• Diagnosis
  – recovery of appropriate clostridial species and characteristic disease symptoms

• Treatment, prevention, and control
  – surgical debridement, administration of antitoxin, antibiotic therapy, and hyperbaric oxygen therapy
  – prompt treatment of all wound infections and amputation of limbs
Antibiotic-Associated Colitis (Pseudomembranous Colitis)

- *Clostridium difficile* (*C. difficile* associated diarrhea – CDAD)
  - uncomplicated diarrhea
  - pseudomembranous colitis
    - viscous collection of inflammatory cells, dead cells, necrotic tissue, and fibrin that obliterates the intestine
  - toxic megacolon
    - inflammation resulting in intestinal tissue death
Clostridium difficile

• Anaerobic spore forming bacillus found in the intestines of some healthy people
  – numbers are kept in check by other normal intestinal microbiota
  – excessive antibiotic use eliminates normal microbiota and allows *C. difficile* to overgrow
    • most common are amoxicillin, ampicillin, clindamycin, cephalosporins
  – increasing due to use of hand sanitizers
C. difficile Virulence Factors

- C. difficile multiplies and produces toxins
  - toxin A (enterotoxin causing diarrhea)
  - toxin B (cytotoxin kills cells)
- Inflammation, diarrhea, fever, nausea, cramping
- Most common cause of diarrhea in hospitalized patients
- Treatment is with antibiotics
Genus Bacillus

- Motile, peritrichous flagella, usually aerobic, catalase positive
- Various species produce antibiotics
- *Bacillus subtilis* is type species
  - Gram-positive, facultative anaerobe
  - soil-dwelling, spore forming
  - may develop biofilms
Bacillus subtilis

• Used as model organism for
  – gene regulation, cell division, quorum sensing, cellular differentiation

• Genome was one of first to be sequenced
  – families of genes expanded by gene duplication
  – 18 genes for sigma factors
  – ≥10 integrated prophages or remnants of prophages
Other Important Species of Bacillus

- **B. cereus** – food poisoning
- **B. anthracis** – anthrax
- **B. thuringiensis** and **B. sphaericus** – used as insecticide for years
Anthrax

• Caused by *Bacillus anthracis* (Select Agent)
  – Gram-positive, aerobic, endospore-forming
  – endospores viable in soil and animal products for decades
  – plasmid encodes genes for anthrax toxin

• Transmitted by direct contact with infected animals or their products
  – portal of entry determines form of disease

• Potential bioterrorism agent
Anthrax Virulence

- *B. anthracis* evades immune system by
  - capsule which inhibits phagocytosis
  - synthesis of complex exotoxin
    - protective antigen – forms hole for entry of other toxins
    - edema factor – fluid release and edema
    - lethal factor – inhibits cytokine production
  - macrophages die, release toxic contents leading to septic shock, death
Cutaneous Anthrax

• Infection through cut or abrasion of skin

• Clinical manifestations
  – 1 to 15 day incubation
  – skin papule that ulcerates (eschar), headache, fever, and nausea

• Antibiotic therapy
Pulmonary and Gastrointestinal Anthrax

• Pulmonary anthrax
  – woolsorter’s disease
  – inhalation of endospores
  – resembles influenza
  – if bacteria reach the bloodstream, usually fatal

• Gastrointestinal anthrax
  – ingestion of endospores
Anthrax

• Diagnosis
  – presumptive ID in sentinel labs of Laboratory Response Network (LRN)
    • Gram-stained smear of skin lesion, cerebrospinal fluid or blood; also growth and biochemical characteristics of culture
  – confirmatory diagnosis by PCR and serology
• Treatment, prevention, and control
  – antibiotic therapy and symptomatic/supportive therapy
  – immunization of animals and persons at high risk
Genus *Sporosarcina*

- Only known endospore - former that has coccoid and not rod shape
- Tolerates pH up to 10
  - degrades urea to ammonia and carbon dioxide
- Isolated from agricultural soils where animals urinate
Family *Staphylococcaceae*

- 5 genera, includes *Staphylococcus*
- Facultatively anaerobic, nonmotile, Gram-positive cocci
- Usually form irregular clusters
- Normally associated with warm-blooded animals in skin, skin glands, and mucous membranes
Members of *Staphylococci*

- **S. aureus** – coagulase positive, pathogenic
- **S. epidermidis** – coagulase negative, less pathogenic but nosocomial opportunists
- Many pathogenic strains are slime producers
- Teichoic acid and peptidoglycan contribute to pathogenicity
Staphylococcal Diseases

• Caused by members of the genus *Staphylococcus*
  – Gram-positive cocci, occurring singly, in pairs, tetrads, or grape-like clusters
  – facultative anaerobes and usually catalase positive
  – normal inhabitants of upper respiratory tract, skin, intestines, and vagina
Staphylococcal Diseases

• Harbored by asymptomatic carriers or active carriers (have the disease)
  – spread by hands, inanimate objects or expelled by respiratory tract, or through blood

• May produce disease in almost every organ and tissue

• Immune compromised most at risk
Virulence Factors of Staphylococci

• Exotoxins and enzymes involved in invasiveness

• Toxin genes may reside on plasmids and on chromosome

• Examples
  – enterotoxin – food intoxication
  – bacteremia and abscess formation
<table>
<thead>
<tr>
<th>Product</th>
<th>Physiological Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactamase</td>
<td>Breaks down penicillin</td>
</tr>
<tr>
<td>Catalase</td>
<td>Converts hydrogen peroxide into water and oxygen and reduces killing by phagocytosis</td>
</tr>
<tr>
<td>Coagulase</td>
<td>Reacts with prothrombin to form a complex that can cleave fibrinogen and cause the formation of a fibrin clot; fibrin may also be deposited on the surface of staphylococci, which may protect them from destruction by phagocytic cells; coagulase production is synonymous with invasive pathogenic potential</td>
</tr>
<tr>
<td>DNase</td>
<td>Destroys DNA</td>
</tr>
<tr>
<td>Enterotoxins</td>
<td>Are divided into heat-stable toxins of six known types (A, B, C1, C2, D, E); responsible for the gastrointestinal upset typical of food poisoning</td>
</tr>
<tr>
<td>Exfoliative toxins A and B (superantigens)</td>
<td>Cause loss of the surface layers of the skin in scalded-skin syndrome</td>
</tr>
<tr>
<td>Hemolysins</td>
<td>Alpha hemolysin destroys erythrocytes and causes skin destruction. Beta hemolysin destroys erythrocytes and sphingomyelin around nerves.</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>Also known as spreading factor; breaks down hyaluronic acid located between cells, allowing for penetration and spread of bacteria</td>
</tr>
<tr>
<td>Panton-Valentine leukocidin</td>
<td>Inhibits phagocytosis by granulocytes and can destroy these cells by forming pores in their phagosomal membranes</td>
</tr>
<tr>
<td>Lipases</td>
<td>Break down lipids</td>
</tr>
<tr>
<td>Nuclease</td>
<td>Breaks down nucleic acids</td>
</tr>
<tr>
<td>Protein A</td>
<td>Is antiphagocytic by competing with neutrophils for the Fc portion of specific opsonins</td>
</tr>
<tr>
<td>Proteases</td>
<td>Break down proteins</td>
</tr>
<tr>
<td>Toxic shock syndrome toxin-1 (a superantigen)</td>
<td>Is associated with the fever, shock, and multisystem involvement of toxic shock syndrome</td>
</tr>
</tbody>
</table>
Staphlococcus aureus

• Most important human staphloccoccal pathogen
  – e.g., abscesses, boils, wound infections, pneumonia, toxic shock syndrome
  – major cause of common food poisoning

• Virulence factors
  – coagulase which causes blood plasma to clot
  – the toxin β-hemolysin lyses cells
Staphylococcal Food Poisoning

• Results from ingestion of improperly stored or cooked food (e.g., ham, processed meats, chicken salad, ice cream, and hollandaise sauce) in which *Staphylococcus aureus* has grown and released enterotoxin

• Bacteria produce heat-stable enterotoxins in food
  – properly cooking the food will not destroy toxin; intoxications can result from thoroughly cooked foods

• Symptoms include abdominal pain, cramps, diarrhea, vomiting, and nausea
Staphylococcal Food Poisoning

• Diagnosis
  – based on symptoms or laboratory identification of bacteria from food
  – enterotoxins can be detected in foods by animal toxicity tests

• Treatment, prevention, and control
  – fluid and electrolyte replacement
  – avoidance of food contamination, and control of personnel involved in food preparation and distribution
Staphylococcal Scalded Skin Syndrome (SSSS)

• Caused by strains of *S. aureus* that carry a plasmid-borne gene for exfoliative toxin (exfoliatin)

• Epidermis peels off revealing red area underneath

• Diagnosis
  – isolation/identification of *Staphylococcus* involves commercial kits
Staphylococcal Scalded Skin Syndrome (SSSS)

- Treatment, prevention, and control
  - isolation and identification based on catalase test, coagulase test, serology, DNA fingerprinting, and phage typing
  - antibiotic therapy
    - many drug-resistant strains
  - personal hygiene, food handling, and aseptic management of lesions
Toxic Shock Syndrome (TSS)

• Caused by *S. aureus* strains that release toxic shock syndrome toxin and other toxins

• Some cases occur in females who use superabsorbent tampons

• Disease results from body’s response to staphylococcal superantigens

• Clinical manifestations
  – low blood pressure, fever, diarrhea, extensive skin rash, and shedding of skin
Staphylococcal Lesions

- Localized abscess
  - *S. aureus* infects a hair follicle, tissue necrosis results
  - coagulase is produced forming a fibrin wall around lesion, limiting spread
  - liquefaction of necrotic tissue in center of lesion occurs; abscess spreads
  - may be a furuncle (boil) or carbuncle
  - bacteria may spread from area via lymphatics or bloodstream
Methicillin-Resistant Staphylococcus aureus (MRSA)

- S. aureus isolates that are resistant to β-lactam antibiotics (penicillins and cephalosporins)
- Community acquired (CA) – MRSA
  - healthy individuals not recently hospitalized
  - associated with serious and fatal infection
  - may also be acquired in health care setting
S. aureus Antibiotic Resistance

• Methicillin-resistant S. aureus (MRSA) and Vancomycin resistant S. aureus (VRSA)
  – among most threatening antibiotic resistant
    • VRSA may have no treatment
  – obtained from genetic elements received from other organisms
  – virulence factors also acquired from mobile genetic elements
Slime Producers (SP)

- Produced by pathogenic strains of *Staphylococcus*
  - a viscous extracellular glycoconjugate
  - allows bacteria to adhere to smooth surfaces and form biofilms
  - Inhibits neutrophil chemotaxis, phagocytosis, and antimicrobial agents
Staphylococcus epidermidis

- Common skin resident
- Sometimes responsible for endocarditis and for infections of patients with lowered resistance
  - e.g., wound infections, surgical infections, and urinary tract infections
Genus *Listeria*

- Wide distribution in nature - common in decaying matter
- *L. monocytogenes* pathogen of humans and animals
  - listeriosis - food-borne infection
  - especially dangerous to pregnant women, the fetus and infant, and compromised individuals (90% of cases)
  - 1600 cases/yr in US, 3rd leading cause of fatalities associated with food-borne illnesses
  - grows at refrigeration temperatures
  - associated with many foods that are not cooked, i.e. lunch meats, cheeses, sprouts, fruits
Order *Lactobacillales*

- Also called lactic acid bacteria (LAB)
- Morphologically diverse
  - nonsporing
  - usually nonmotile
- Ferment sugars for energy
  - lack cytochromes
  - fastidious
- Contains several important genera
Genus *Lactobacillus*

- Widely distributed in nature
  - on plant surfaces
  - in dairy products, meat, water, sewage, beer, fruits, and other materials
  - normal flora of mouth, intestinal tract, and vagina
    - usually not pathogenic
Importance of Lactobacilli

- Fermented products
  - vegetable products (sauerkraut, pickles, and silage)
  - beverages (beer, wine, juices, milk)
- Sour dough bread
- Swiss cheese and other hard cheeses, yogurt
- Sausages
- *L. acidophilus* – sold as probiotic agent
- Food spoilage – beer, wine, milk, meat
Importance of *Leuconostoc*

- Wine production
- Production of sauerkraut and pickles
- Production of buttermilk, butter, and cheese
- Synthesis of dextrans (*L. mesenteroides*)
- Involved in food spoilage
  - tolerate high sugar concentrations
  - grow in heavy syrup
Families *Streptococcaceae* and *Enterococcaceae*

- Chemoheterotrophic, mesophilic, nonsporing cocci, usually nonmotile
- Fermentative only
- Aerotolerant and anaerobic
- Groups
  - enterococci
  - lactococci
  - streptococci
Three Groups of Streptococci

• Pyogenic (pus producing) streptococci
  – e.g., S. pyogenes – streptococcal sore throat, acute glomerulonephritis, and rheumatic fever

• Oral streptococci
  – e.g., S. mutans – dental caries

• Other streptococci
  – e.g., S. pneumoniae – lobar pneumonia and otitis media
Streptococcal Diseases

• Caused by strep, group of Gram-positive bacteria
  – *Streptococcus pyogenes*
    • one of most important pathogens
    • group A β-hemolytic streptococci (GAS)
  – virulence factors
    • extracellular enzymes that break down host molecules
    • streptokinases – dissolve clots
    • streptolysin O and S – kill host leukocytes
    • capsules and M protein for attachment
Streptococcal Diseases

• *Streptococcus pyogenes*
  – widely distributed, some carriers
  – common infection “Strept throat”
  – transmission
    • respiratory droplets, direct or indirect contact

• Diagnosis
  – based on clinical and laboratory findings
  – rapid diagnostic tests available
Streptococcal Pharyngitis

- Common infection called strep throat
- Spread by droplets of saliva or nasal secretions
- Infection in throat (pharyngitis) or tonsils (tonsillitis)
- Signs and symptoms of disease not diagnostic because many viral infections have similar presentation
- Physical manifestations
  - redness, edema, exudate in 50% and lymph node enlargement in throat
Additional Streptococcal Diseases

• Contact superficial cutaneous diseases
  – include cellulitis, impetigo, and erysipelas

• Invasive diseases
  – may reach underlying muscle
Superficial Cutaneous

• Cellulitis
  – diffuse, spreading infection of subcutaneous tissue
  – redness and swelling

• Impetigo
  – also caused by *Staphylococcus aureus*
  – superficial cutaneous infection commonly seen in children
  – crusty lesions and vesicles surrounded by red border

• Erysipelas
  – acute infection of dermal layer of skin
  – red patches that may occur periodically at same site for years
Invasive Streptococcal Infections

• Caused by certain virulent strains of *S. pyogenes*

• Rapidly progressive
  – carry genes for exotoxins
    • superantigens (Select Agent)
    • tissue-destroying protease
Invasive infections

- Clinical manifestations
  - necrotizing fasciitis
    (“flesh eating”)
    - destruction of sheath covering skeletal muscle
  - myositis
    - inflammation and destruction of skeletal muscle and fat tissue
  - toxic shock-like syndrome (TSLS)
    - precipitous drop of blood pressure, failure of multiple organs, and high fever
Streptococcal Pneumonia

• Opportunistic pathogen
  – caused by one’s own normal microbiota
• Caused by *Streptococcus pneumoniae*
  – produces polysaccharide capsule and a toxin
  – rapidly multiplies in alveolar spaces
• Disease only occurs in individuals with predisposing condition
Poststreptococcal Diseases

- Glomerulonephritis (Bright’s disease) and rheumatic fever
- 1–4 weeks after an acute streptococcal infection
- Nonsupportive (nonpus-producing)
- Most serious problems associated with streptococcal infections in U.S.
Streptococcal Pneumonia

• Primary virulence factor
  – capsule of hyaluronic acid that is anti-phagocytic
  – allows rapid multiplication of bacteria in alveolar spaces

• Release of pneumolysin
  – destroys host cells
  – alveoli fill with blood cells and fluid
Streptococcal Pneumonia

• Diagnosis
  – chest X-ray, gram stain, culture, and tests for metabolic products

• Clinical manifestations
  – abrupt onset of chills, hard labored breathing, chest pain, and rust-colored sputum

• Treatment, prevention, and control
  – antibiotic therapy
    • resistant strains have appeared
  – immunization and treatment of infected persons
Streptococcal Diseases

• Other diseases are
  – sinusitis, conjunctivitis, otitis media
  – bacteremia, meningitis

• Treatment, prevention, and control
  – most treated by antibiotic therapy
  – Pneumovax capsular vaccine
Glomerulonephritis

• Inflammatory disease of renal glomeruli
  – a type III hypersensitivity

• Clinical manifestations
  – edema, fever, hypertension, and hematuria
  – may spontaneously heal or may become chronic

• Diagnosis
  – clinical history, physical findings, and confirmatory evidence of prior streptococcal infection

• Treatment, prevention, and control
  – antibiotic therapy (to kill residual bacteria), otherwise no specific therapy
Rheumatic Fever

• Autoimmune disease involving heart valves, joints, subcutaneous tissues, and central nervous system

• Clinical manifestations
  – vary widely, making diagnosis difficult

• Treatment, prevention, and control
  – therapy directed at decreasing inflammation and fever, and controlling cardiac failure
  – treatment with salicylates and corticosteroids
Genus *Streptococcus*

- Hemolysis patterns used in Lancefield grouping
  - alpha (α) – hemolysis
    - incomplete lysis of red blood cells
    - seen as greenish zone around colony on blood agar
  - beta (β) – hemolysis
    - complete lysis of red blood cells
    - seen as clear zone around colony on blood agar
Hemolysis on blood agar

Beta hemolytic (complete)

Alpha hemolytic (partial)

Gamma hemolytic (none)
Group B Streptococcal Disease

• Caused by Gram-positive *Streptococcus agalactiae* or Group B streptococcus (GBS)

• Common cause of neonatal and newborn diseases such as sepsis, meningitis, and pneumonia

• Transmitted directly from person-to-person with many people being transient carriers; vagina may be source for newborns
GBS

• Clinical manifestations
  – early onset disease
    • presents within first few hours after birth
    • may be severe meningitis or death
  – late onset disease - rare

• Diagnosis
  – Gram-positive, beta-hemolytic, streptococcal bacteria growth from cultures of otherwise sterile body fluids

• Treatment, prevention, and control
  – detect pregnant carriers
  – antibiotics
Important Enterococci and Lactococci

• *Enterococcus faecalis*
  – normal biotic in gastrointestinal tract
  – opportunistic pathogen (urinary tract infections and endocarditis)
  – Used as an indicator organism in natural waters, ocean, brackish water, frozen food

• *Lactococcus lactis* – production of buttermilk and cheese