Bacillus, Clostridium

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The family Bacillaceae

The feature they all share is formation of endospores

2 clinically important genera:
- Bacillus (aerobic spore-formers)
- Clostridium (anaerobic spore-formers)
Bacillus

- More than 70 species
- B. anthracis: the most important
  - the most feared agent of biological warfare
  - Anthrax (cutaneous, gastrointestinal, inhalation)
- B. cereus: -gastroenteritis, ocular infection, catheter-related sepsis, opportunistic infections
Bacillus anthracis

- Greek: *anthrakis* : coal – the black lesions of the infection
- Large gram-positive
- Arranged as single or paired rods or as long, serpentine chains
- Facultative anaerobe
- Spores (in 2-3-day-old cultures, are not seen in clinical specimen): in the center
Bacillus anthracis’s virulence

- Capsule: coded by a plasmid
- Polypeptide capsule (consisting of poly-D-glutamic acid) in clinical specimen
- This capsule is not produced in vitro unless special growth conditions are used.
Bacillus anthracis’s virulence

- Three exotoxins: a second plasmid
  - Protective antigen (PA)
  - Edema factor (EF)
  - Lethal factor (LF)

- PA + EF = Edema toxin (EdTx)
- PA + LF = Lethal toxin (LeTx)
Bacillus anthracis

- Edema toxin: protective antigen (responsible for binding to the host cell) + edema factor
- Lethal toxin: protective antigen + lethal factor
Bacillus anthracis - Epidemiology

- Anthrax is primarily a disease of herbivores.
- Rarely isolated in developed countries.
- Is prevalent in impoverished areas where vaccination of animals is not practiced.
Bacillus anthracis

- The spores of the organism contaminate soil or animal products and remain infectious for many years.
- Individuals at risk include people in contact with infected animals or contaminated soil.
- Anthrax as a threat of biological warfare.
Bacillus anthracis

- acquired by
  - inoculation (cutaneous anthrax - most common)
  - ingestion - rare
  - inhalation - the most deadly form
B. anthracis: an agent of bioterrorism

- 1979 in Sverdlovsk in the former Soviet Union: 79 cases of anthrax with 68 deaths
- Contamination of employees of US Postal Service with letters containing B. anthracis (22 cases)
**B. anthracis - Lab. diagnosis**

- **Microscopy:** long, thin, gram-positive rods, arranged singly or in long chains.
- Spores are not observed in clinical specimens but only in cultures incubated in a low CO$_2$ atmosphere (special spore stain: malachite green stain).
- In the center of nonmotile bacilli.
B. anthracis - Lab. diagnosis

- The capsule is produced in vivo but is not typically observed in culture.
- Contrasting stain such as India ink (the ink particles are excluded by the capsule so that the background but not the area around bacteria appears black).
- Direkt fluorescent antibody (DFA).
B. anthracis-Lab. diagnosis

- Can grow on most non-selective media
- Grow rapidly and firmly adherent to the agar
- Colonies not hemolytic, nonmotile in motility tests
- Have a dry "ground glass" surface and irregular edges with projection along the lines (medusa head)
Bacillus cereus

- Two enterotoxins:
  - Heat-stable: emetic form
  - Heat-labile: diarrheal form. Is similar to the enterotoxins produced by Escherichia coli and Vibrio cholerae; each stimulates the adenilate cyclase-cyclic adenosine monophosphate system
The pathogenesis of B. cereus ocular infections

- Three toxins:
  - Necrotic toxin (a heat-labile enterotoxin)
  - Cereolysin (a hemolysin)
  - Phospholipase C (a lecithinase)
Bacillus cereus- Diseases

- Food poisoning (rice, meat, vegetables)
- Ocular infection
- Catheter-related sepsis
Bacillus cereus

- Are ubiquitous organisms, present in virtually all environment
- Is responsible for two forms of food poisoning:
  - Vomiting disease (emetic form)
  - Diarrheal disease (diarrheal form)
The emetic form (vomiting disease)

- results from the consumption of contaminated rice
- Most bakteria are killed during the initial cooking of rice, but spores survive.
- The spores germinate and the heat-stable enterotoxin is released
- 1-6 hour incubation period
The diarrheal disease (diarrheal form)

- Consumption of contaminated meat, vegetables or sauces
- A longer incubation period, during which the organism multiplies in the patient’s intestinal tract and produce heat-labile enterotoxin
Ocular infections

- Occur after traumatic, penetrating injuries of the eye with soil-contaminated object
Laboratory diagnosis

- The implicated food (e.g., rice, meat, vegetables) should be cultured.
- Fecal colonization is common: isolation of the organism from the patient should not be attempted.
- Specimens collected from infected eyes, intravenous culture sites: Gram stain and culture.
Clostridium

All anaerobic: Most strict anaerobes
Some aerotolerant

Gram-positive
Forms endospores (except C. perfringens rarely)
The traditional method for classifying an isolate in *Clostridium*

- demonstration of spores
- growth in anaerobic conditions
- a complex set of biochemical tests
- gas chromatography analysis

more than 177 species
Clostridium

- Ubiquitous (everywhere): soil, water, sewage, gastrointestinal flora of animals and humans
- Most are harmless saprophytes
- Some are well known pathogens:
  - tetanus (C. tetani)
  - botulism (C. botulinum,..)
  - gas gangrene (C. perfringens,..)
Clostridia-Diseases

- Skin and soft tissue infection
- Food poisoning
- Antibiotic associated diarrhoea and colitis
The Clostridia

Ability to survive adverse environmental conditions through spore formation
Rapid growth in a nutritionally enriched oxygen deprived environment
Production of numerous histolytic toxins, enterotoxins and neurotoxins.
Pathogenic Clostridia: six important human pathogens

- C. difficile-common
- C. perfringens-common
- C. septicum-uncommon
- C. tertium-uncommon
- C. botulinum-uncommon
- C. tetani-uncommon
Rare clostridia

- C. baratii,
- C. butyricum,
- C. histolyticum,
- C. novyi,
- C. sordellii
Clostridium perfringens

- Gram-positive large rod shaped bacteria
- Spores are rarely observed after in vivo or in vitro cultivation
- Nonmotile, but rapidly spreading growth on laboratory media
Clostridium perfringens—culture

- Rapidly grows in tissue and in culture
- Hemolytic
- Metabolically active
- Lethal toxins (α, β, ε, τ toxins)
- 5 types (A to E)
C. perfringens - Pathogenesis

- Can cause severe life-threatening disease
- Self-limited gastroenteritis
- Soft tissue infections (cellulitis, myositis, myonecrosis or gas gangrene)
C. perfringens - Toxins

- 12 toxins and enzymes
- α toxin: the most important toxin
- A lecithinase (phospholipase C)
- Lyses erythrocytes, platelets, leukocytes and endothelial cells
- It increases vascular permeability, resulting in massive hemolysis, bleeding, tissue destruction, hepatic toxicity and myocardial dysfunction
C. perfringens - Toxins

- $\beta$ toxin: responsible for the necrotic lesions in necrotizing enteritis
- $E$ toxin: a protoxin activated by tripsin and increases the vascular permeability of the gastrointestinal wall
- $\iota$ toxins: the fourth major lethal toxin, has necrotic activity and increases vascular permeability
Clostridium perfringens

Enterotoxin (cytotoxic, enterotoxic):
- Heat-labile;
- Leads to altered membrane permeability in ileum and loss of fluid and ions;
- Neurominidase – promotes capillary thrombosis
C. perfringens - Epidemiology

- Type A commonly inhabits the intestinal tract of humans and animals
- Widely distributed in nature, particularly soil and water contaminated with feces
- Type A is responsible for most human infections
- Type C is responsible for one important infection in humans: enteritis necroticans
C. perfringens - Diseases

- Soft-tissue infections:
  - Cellulitis
  - Fasciitis, myositis
  - Myonecrosis or gas gangrene (gas in tissue)
C. perfringens- Diseases

- Clostridial food poisoning: short incubation period (8-24 hours)
- Necrotizing enteritis (enteritis necroticans or pig-bel)
- Septicemia
C. perfringens-Lab. diagnosis

- Performs a confirmatory diagnosis of soft-tissue diseases because therapy must be initiated immediately.
- The microscopic detection of gram-positive rods in clinical specimens, usually in the absence of leukocytes; characteristic morphology.
- Can be detected on simple media.
C. perfringens - Lab. diagnosis

- Forms spores but rarely in clinical specimen or culture
- Replicates rapidly
- Large spreading colonies within the first day of culture
- Double zone of hemolysis (complete hemolysis and partial hemolysis by different toxins)
Clostridium perfringens

- Produces many toxins and hemolytic enzymes
- White blood cells absent in gram-stained clinical specimen
Clostridium tetani

- Large, motile
- Spore-forming
- Gram-positive bacilli
- Terminal spores (drumstick appearance)
- Strict anaerobe (very sensitive to oxygen)
- Difficult to isolate from clinical specimen
Pathogenesis - Virulence

- The vegetative cells die rapidly when exposed to oxygen, but (!!)
- Spore formation allows the organism to survive in the most adverse conditions
- Produces two toxins:
  - Tetanolysin: an oxygen-labile hemolysin
  - Tetanospasmin: heat-labile neurotoxin
Pathogenesis - Virulence

- Tetanospasmin: heat-labile neurotoxin, blocks the release of neurotransmitters: causes spastic paralysis
- Plasmid-encoded: nonconjugative
- Released when the cell is lysed
- Responsible for the clinical manifestations of tetanus
Virulence - Tetanospasmin

- Is synthesized as a single peptid that is cleaved into:
  - light (A-chain) subunit and
  - heavy (B-chain) subunit by an endogenous protease

- Inactivates proteins that regulate release of the inhibitory neurotransmitters glycine and gamma-aminobutyric (GABA)
C. tetani - Epidemiology

- Ubiquitous
- Spores in soils
- Transiently colonizes the gastrointestinal tracts of humans and animals
- Exposure to spores is frequently
- Disease is uncommon (increased risk where there is a poor access to vaccine and medical care)
Clostridium tetani’s diseases

- **Tetanus:**
  - Generalized
  - Localized
    - Cephalic tetanus
  - Neonatal
C. tetani - Laboratory diagnosis

- The diagnosis of tetanus is made on the basis of the clinical presentation.
- The microscopic detection or culture is useful but frequently unsuccessful.
- Culture are positive in only 30% of patients with tetanus.
Clostridium botulinum

- The etiologic agent of botulism
- Large, spore-forming
- Anaerobic bacilli
- Seven toxins (A to G): A, B, E, F the most common:
  - Neurotoxin subunit + nontoxic subunits which protect them from stomach acids
Clostridium botulinum

- Similar to tetanus toxin
- The target neural cells are different
- Blocks neurotransmission at peripheral cholinergic synapses
- Prevents release of neurotransmitter acetylcholine
- Flaccid paralysis
Clostridium botulinum

- Foodborn botulism
- Infant botulism
- Wound botulism
- Inhalation botulism (a major concern in this era of bioterrorism)
C. botulinum - Lab. diagnosis

- Isolation from specimens contaminated with other organisms:
- Heating the specimen for 10 min at 80°C to kill all non-clostridial cells
- Culture on anaerobic media allows the heat-resistant C. botulinum to germinate
C. botulinum - Lab. diagnosis

- Lipase production: egg-yolk agar is used and iridescent film on colonies
- Digests milk proteins
- Hydrolyzes gelatin
- Ferment glucose
Clostridium botulinum

- Demonstration of toxin: mouse bioassay
- Two aliquots: one with antitoxin and intraperitoneal incubation
- If the antitoxin treatment protects the mice, toxin activity is confirmed
- Sample of implicated food, stool specimen
Clostridium difficile

- Until the mid-1970s the clinical importance of C. difficile was not appreciated
- Toxin A: enterotoxin
- Toxin B: cytotoxin
- A part of normal flora
Clostridium difficile

- Taking antibiotics alter the flora
- The disease develops in people taking antibiotics, because the drugs alter the normal enteric flora
- Occurs if the organisms proliferate in colon and produce their toxins
- Life-threatening pseudomembranous colitis
Clostridium difficile

- The organism isolated on highly selective media
- Cytotoxin detection
- Enterotoxin detection
- In a stool specimen from a patient with compatible clinical symptoms
- Commercial immunoassays