

Bacillus

The Bacillus genus contains numerous species, many of which are not of clinical importance. Two important human pathogens – *Bacillus anthracis* and *Bacillus cereus* cause anthrax and food poisoning, respectively.

Definition

Gram-positive bacilli often arranged in chains; aerobic (some species are obligate aerobes and some facultative anaerobes); spore-forming; most species are motile; usually catalase-positive; some species are capsulate; grow over a wide temperature range on simple media.

B. anthracis

Epidemiology

Anthrax is principally a zoonotic disease and is common in some parts of the developing world.

Human infections can be classified as: non-industrial (direct human contact with infected animals) or industrial (processing of animal products by humans). Spores can survive in the soil for long periods of time and are relatively resistant to chemical disinfectants and heat.

Infection with *B. anthracis* in the UK is rare but is normally associated with handling imported animal products. Recent UK cases have occurred in intravenous drug users, probably as a result of contaminated heroin. Anthrax has been used as a biological weapon.

Morphology and identification

Can grow under anaerobic conditions; its nonmotility allows it to be distinguished from other Bacillus species; virulent strains are capsulate; do not produce a zone of haemolysis on blood nor a zone of precipitation on egg yolk agar (i.e. does not produce lecithinase). Identification is normally confirmed by morphological and biochemical tests.

Pathogenicity

Virulent strains of *B. anthracis* possess a protein capsule, which prevents phagocytosis. This microorganism also produces a plasmid-encoded exotoxin, which is composed of three proteins: protective antigen, oedema factor and lethal factor.

Protective antigen is concerned with receptor binding and therefore the attachment and translocation of oedema factor and lethal factor into the cell. Oedema factor causes impairment of macrophage function and lethal factor lysis of macrophages.

Associated infections

Types of anthrax infection include:

- Skin and soft tissues: cutaneous anthrax is the predominant clinical manifestation. Development of a necrotic skin lesion (malignant pustule) occurs.
- Respiratory: ('wool-sorter's disease'): spores are inhaled (often from wool fibres) causing pulmonary oedema, haemorrhage and commonly, death.
- Gastrointestinal: consumption of contaminated meat results in haemorrhagic diarrhoea. This type of anthrax can also result in death.

Laboratory diagnosis

Normally by direct isolation of the microorganism from infected sites, i.e. sputum or specimens from skin lesions. A safety cabinet should be used to handle such specimens. (practical lecture)

Treatment and prevention

B. anthracis is sensitive to many antibiotics. Common therapeutic agents used are penicillin, erythromycin, ciprofloxacin or doxycycline. Ciprofloxacin or doxycycline may be given for post-exposure prophylaxis. Anthrax vaccinations are available for individuals at high risk, e.g. military personnel, veterinary

practitioners and farm workers. Livestock in endemic areas may also be vaccinated.

B. cereus

B. cereus can grow under anaerobic conditions, is motile and it does not produce lecithinase; however, unlike *B. anthracis*, it produces a zone of haemolysis on blood agar. This microorganism is an important cause of food poisoning, particularly associated with rice dishes and cereals. Pathogenicity is related to the production of enterotoxins.

Food poisoning can present as the emetic form, which has a short incubation period (typically 1–6 hours) and is characterised by nausea and vomiting, or as the diarrhoeal form, for which the incubation period is longer (typically 8–16 hours).

Food-poisoning is self-limiting, therefore antimicrobial therapy is not normally required. *B. cereus* is an infrequent cause of non-gastrointestinal infections, including ocular infections, pneumonia and endocarditis.

Clostridia

Definition

Large Gram-positive bacilli; strictly anaerobic; spore-forming; fermentative. Whilst many *Clostridium* species exist, only some are of medical importance, including *C. perfringens*, *C. difficile*, *C. tetani*, *C. botulinum*, *C. septicum* and *C. tertium*.



Epidemiology

Clostridia are widely distributed in the environment and in the gastrointestinal tract of mammals. They produce highly resistant, transmissible spores, which can survive desiccation, ultraviolet and gamma radiation, extreme temperatures, starvation and disinfection. Spores of clostridia are the vector of infection.

Classification

Based on morphology, biochemical activity, fatty acid production and gene sequencing techniques.

C. perfringens

Morphology and identification

Non-motile, sub-terminal spores. Forms irregular, spreading colonies on blood agar surrounded by a double zone of β -haemolysis (inner zone of complete lysis

due to α -toxin and wider outer zone of partial haemolysis due to β -toxin). Five types (A to E) of *C. perfringens* are recognised based on surface antigens and the types of toxin produced:

- 1 Type A strains: commonly found in human infections; produce only α -toxin
- 2 Type B to E strains: commonly found in animals (lambs, goat, cattle); produce α - and other toxins.

Pathogenicity

Toxin and enzyme production: α -toxin (phospholipase C) is associated with toxæmia seen in gas gangrene; hyaluronidase breaks down cellular cement facilitating spread; collagenase/proteinase– liquefaction of muscle; lipase-lipid breakdown.

Associated infections

- Skin and Soft tissue: gas gangrene, cellulitis;
- Gastrointestinal: necrotising enteritis, food poisoning;
- Gynaecological: septic abortion.

Laboratory diagnosis

Gram stained smears and culture of clinical samples, e.g. blood, pus and tissue may provide evidence of clostridial infection. Recovery of *C. perfringens* on simple or selective agar provides a definitive diagnosis. Identification of *C. perfringens* is determined through biochemical tests (e.g. API 20A, Rapid ID 32A kits). Confirmation of α -toxin and lipase production is established on egg-yolk agar (Nagler plate); toxin-producing strains generate a zone of opalescence around the colonies, which can be inhibited by specific antitoxin to α -toxin.

Treatment and prevention

Gas gangrene: surgical debridement, immediate antibiotic therapy with high dose benzylpenicillin and/or metronidazole and supportive measures.

Co-infecting microorganisms may be present, therefore additional antibiotics may be required.

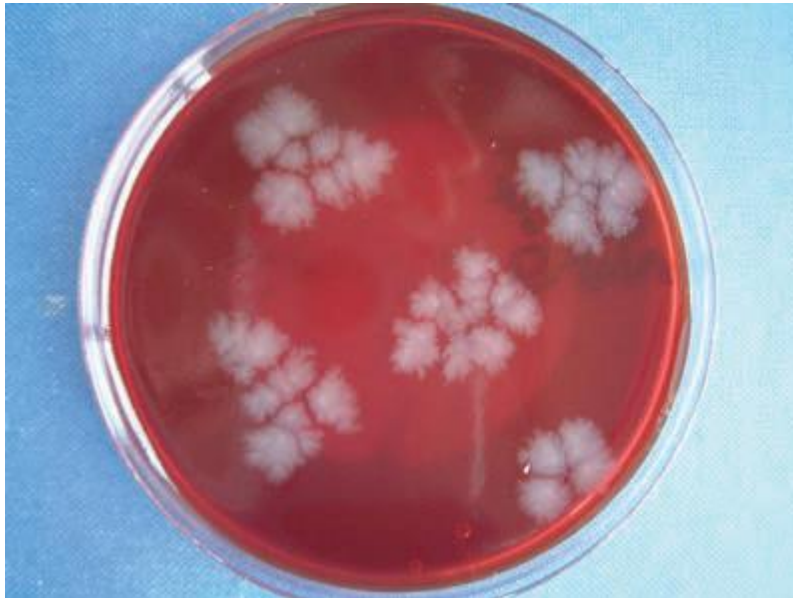
Prophylactic benzylpenicillin may be given for dirty wounds and lower limb amputation.

Food poisoning: self limiting; no antimicrobial therapy warranted.

C. difficile

Morphology and identification

Motile; sub-terminal spores. Forms colonies with irregular edges and a ground glass appearance on blood agar. Colonies have a typical 'horse manure' odour.



Epidemiology

Ubiquitous in the environment and colonises the intestine of 50% of healthy neonates and 4% of healthy adults. A major cause of healthcare-associated infection in the 21st century; patients taking antibiotics, e.g. cephalosporins, clindamycin are at increased risk of developing *C. difficile* antibiotic associated diarrhoea. This is due to suppression of the normal bowel flora and subsequent overgrowth of *C. difficile*. Infection may be endogenous or exogenous (through ingestion of environmental spores).

Pathogenicity

Produces two major toxins: Toxin A (enterotoxin) and Toxin B (cytotoxin). A further binary toxin is present in some strains. Toxin A induces cytokine production with hypersecretion of fluid. Toxin B induces depolymerisation of actin with loss of cytoskeleton. Adhesin factor and hyaluronidase production are also associated virulence factors.

Hypervirulent, hypertoxin producing strains now recognised (e.g. ribotype 027, 078).

Associated infections

- Gastrointestinal: antibiotic associated diarrhoea, pseudomembranous colitis, fulminant colitis.

Laboratory diagnosis

Direct detection of toxin in faeces by various methods: cell toxicity neutralisation assay, commercial assays (e.g. ELISA, latex agglutination) and polymerase chain reaction (PCR). Culture of *C. difficile* on selective agar (e.g. Cycloserine Cefoxatime Fructose Agar); genotyping of isolates by ribotyping where necessary. Assay for glutamate dehydrogenase (GDH) and lactoferrin in faecal samples.

Treatment and prevention

Treatment: Oral Vancomycin or Metronidazole. Prevention of *C. difficile* is multifactorial and includes: clinical awareness, judicious use of antibiotics, infection control strategies, e.g. hand hygiene, environmental decontamination and cleanliness.

C. tetani

Morphology and identification

Motile; terminal spore ('drumstick' appearance); produces a thin spreading film of growth without discrete colonies on blood agar; motile via numerous peritrichous flagella.

Epidemiology

C. tetani is present in mammalian intestines and the environment (particularly manured soil).

Spores are ubiquitous in nature. Incidence of tetanus varies worldwide; more common in developing tropical and subtropical countries; infection is inversely related to living standards, preventative medicine and wound management.

Pathogenicity

Many strains are highly toxigenic, producing oxygen-labile haemolysin (tetanolysin) and a potent neurotoxin (tetanospasmin). Tetanospasmin blocks neurotransmitter release, resulting in the characteristic motor spasms associated with tetanus (e.g. lockjaw, arching of the back).

Associated infections

- Neurological: tetanus.

Laboratory diagnosis

Demonstration of characteristic 'drumstick' bacilli in clinical samples, followed by anaerobic culture on selective or blood agar; serological detection of circulating neurotoxin by enzyme immunoassay.

Treatment and prevention

Treatment includes administration of human tetanus immunoglobulin and benzylpenicillin or metronidazole. Surgical debridement and cleansing of wounds is important in successful treatment. Prevention includes administration of the tetanus toxoid vaccine.

C. botulinum

Morphology and identification

Toxin producing; subterminal spores; motile with peritrichous flagella. Seven main types of *C. botulinum* are recognised (A–G), based on antigenically distinct toxins with identical actions.

Epidemiology

Ubiquitous saprophyte occurring in soil, vegetation, fruit and manure. Infection arises through consumption of contaminated food or wound contamination. Human infection is commonly caused by types A, B and D. Infection in the UK is rare.

Pathogenicity

Production of potent neurotoxin which blocks the release of acetylcholine at neuromuscular junctions, resulting in flaccid paralysis.

Associated infections

- Neurological: botulism, wound botulism, infant botulism.

Laboratory diagnosis

Detection of the microorganism or its toxin in food; toxin may be demonstrated in patient's blood by toxin-antitoxin neutralisation assay.

Treatment and prevention

Treatment includes administration of human tetanus immunoglobulin and benzylpenicillin or metronidazole. Surgical debridement and cleansing of wounds is important. Prevention includes administration of the tetanus toxoid vaccine.

Other clostridial infections

C. septicum is associated with non-traumatic myonecrosis more often in immunocompromised patients. *C. tertium* is associated with traumatic wound infection.

Reference

Elliot, *et al.* Medical Microbiology and Infection "Lecture Notes". 2011 by Blackwell Publishing Ltd

Genus: *Corynebacterium* (Gram Positive Bacilli) *Corynebacteria* (from the Greek words koryne, meaning club, and bacterion, meaning little rod). Gram-positive, aerobic or facultative anaerobic, non-motile, and Catalase-positive rod-shaped bacteria. They have a cell wall with arabinose, galactose, and short-chain mycolic acids. They do not form spores or branch, they have club-shaped, or V-shaped arrangements in normal growth.

Corvnebacterium diphtheriae

C. diphtheriae is the most important species causing diphtheria. Diphtheria is an acute upper respiratory tract illness characterized by sore throat, low-grade fever, and an adherent membrane on the tonsil(s), pharynx, and/or nose.

Cell Wall Components and Antigenic Structure The cell wall contains neuraminidase, arabinose, galactose, mannose, corynemycolic acid, and corynemycolenic acid. The cell walls of the diphtheria bacilli are antigenically heterologous.

Pathogenesis Diphtheria is a classic example of toxin-mediated bacterial disease.

Diphtheria toxin: the exotoxin produced by *C. diphtheriae*, is the key virulence factor of the bacteria and have Biological functions (Neuro and cardiotoxin; inhibits protein synthesis by inactivating elongation factor).

Pathogenesis of diphtheria

1. *C. diphtheriae* usually enters the body through the upper respiratory tract but can also enter through the skin, genital tract, or eye.
2. Infection begins by adherence of the bacteria at the infected site.