Listeriae

Oto Melter

Content

- Taxonomy
- Biological properties
- Pathogenesis
- Listerial cycle
- Epidemiology
- Clinical diseases
- Laboratory diagnosis
- Treatment, prevention and control

Taxonomy

- Genus – 19 species
- Only *L. monocytogenes* is significant human pathogen

Biological properties

- Gram-positive rods, non–spore-forming
- broad temperature range (1-45C)
- tumbinng motility (room temperature)

Pathogenesis

- facultative **intracellular pathogen** (macrophages, enterocytes, epithelial cells, hepatocytes)
- **virulence factors:**

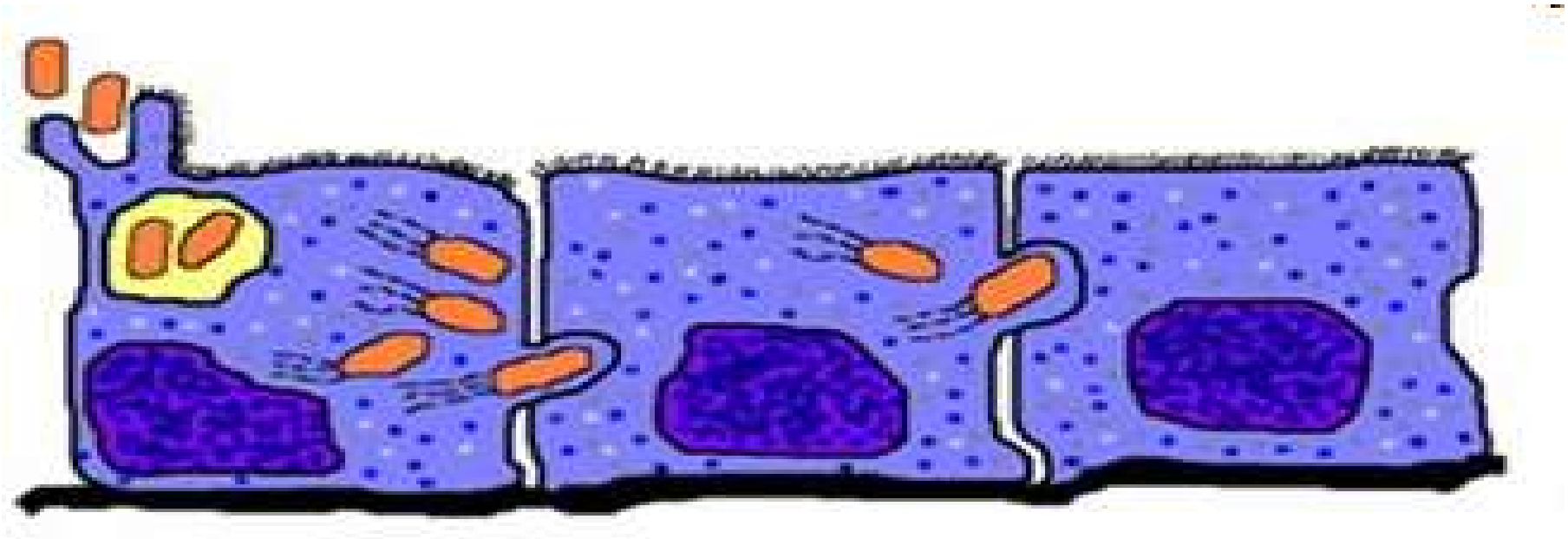
Internalins – interact with receptors on host cells, penetration into host cells

Listeriolysin (exotoxin), phospholipase C (enzymes) – releasing the bacteria into host cell, then bacterial replication

ActA protein – assembly of actin tail – pushing the bacterium into the adjacent cell

Cellular immunity is essential

Listerial cycle



Listeriae (shown as brown rods) are engulfed by a cell (blue). Listeriae multiply and move because actin proteins (dashed lines) which shoots them ahead. Actin is synthetised by the affected host cells (adapted from Wilson, Bacterial pathogenesis, 2011)

Epidemiology of listeriosis

- **Sapro-zoonoses**

(**Greek "sapros" = decaying; "sapron" means in ecology a decaying organic substrate**) are human diseases transmissible from abiotic environment (soil, water, decaying plants, or animal corpses, excreta, and other substrata). The ability of the agent to grow saprophytically and replicate in these substrata (i.e., not only to survive or contaminate them secondarily) are the most important characteristics of a sapronotic microbe.

Zoonoses (Greek "zoon" = animal) are diseases transmissible from living animals to humans (and vice versa)

Clinical diseases

Pregnant women (or patient with cell mediate immune defect) – bacteremia, meningitis

Neonatal disease

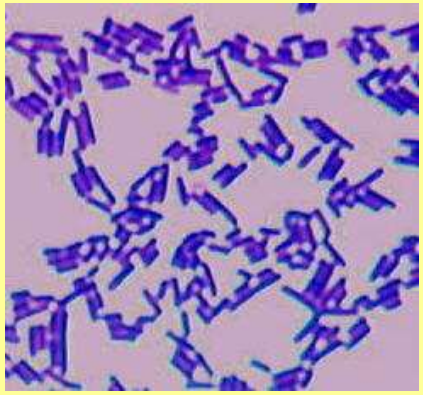
Early-onset disease – granulomatosis infantiseptica - acquired transplacentally in utero, disseminated abscesses and granulomas in multiple organs

Late-onset disease – acquired at or shortly after birth, **meningitis**, **meningoencephalitis with septicemia**

„Healthy“ adults – influenza-like illness with or without gastroenteritis

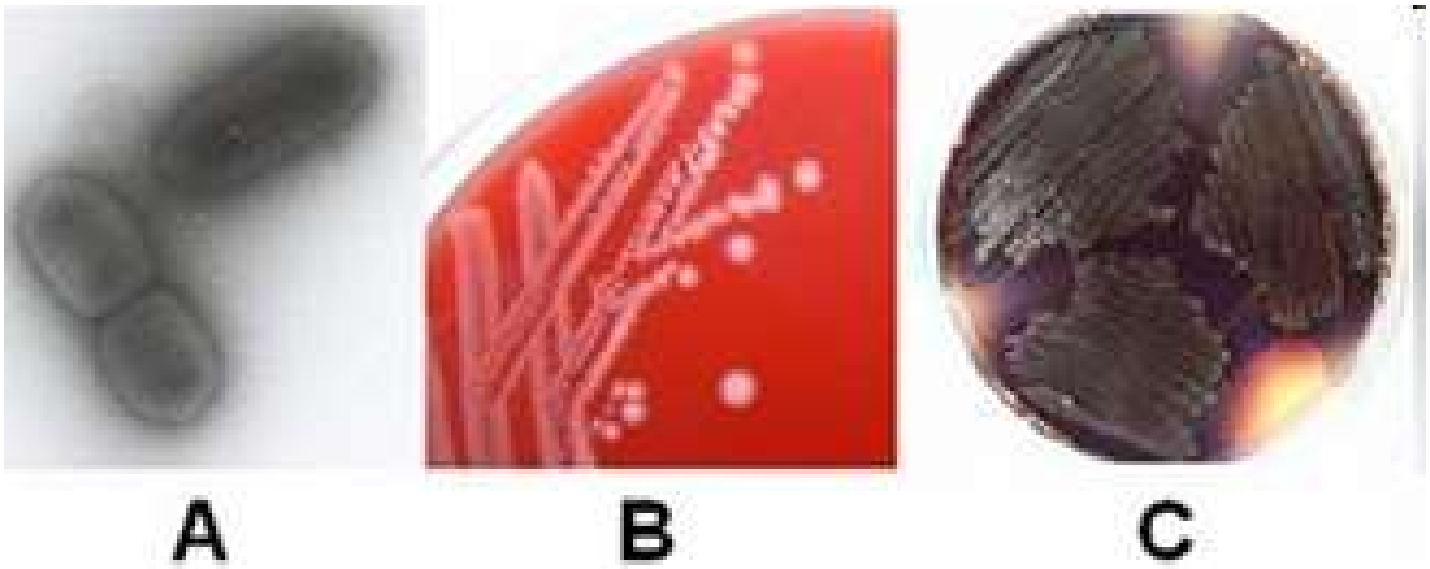
Laboratory diagnosis

- **Material – pus (abscess), CSF, blood, lochia, other**
- **Microscopy**



- **PCR - from infected tissue**
- **Culture – blood agar, hemolysis, CAMP test**
- **Identification – mass spectrometry**

CULTURE



A – electron microscopy, division of a bacterial cell and creation of two dother cells

B – listerial culture on blood agar after overnight cultivation

C – black colonies of *L. monocytogenes* on bile-esculin agar

Melter et al.: http://zdravi.idnes.cz/kdy-se-dari-listeriim-05w-/zdravi.asp?c=A070129_180359_zdravi_ptp

LABORATORY DIAGNOSIS

Microscopy

Gram-stain preparations of cerebrospinal fluid (CSF) typically show no organisms, because the bacteria are generally present in concentrations below the limit of detection (e.g., 10^4 bacteria per ml CSF or less). This is in contrast with most other bacterial pathogens of the central nervous system, which are present in concentrations of 100-fold to 1000-fold higher. If the Gram stain shows organisms, they are intracellular and extracellular gram-positive coccobacilli. Care must be used to distinguish them from other bacteria such as *S. pneumoniae*, *Enterococcus*, and *Corynebacterium*.

LABORATORY DIAGNOSIS

Culture

Listeria grows on most conventional laboratory media, with small, round colonies observed on agar media after incubation for 1 to 2 days. It may be necessary to use selective media and cold enrichment (storage of the specimen in the refrigerator for a prolonged period) to detect listeriae in specimens contaminated with rapidly growing bacteria. β-Hemolysis on sheep blood agar media can serve to distinguish Listeria from morphologically similar bacteria; however, hemolysis is generally weak and may not be observed initially. Hemolysis is enhanced when the organisms are grown next to β-hemolytic Staphylococcus aureus (this enhanced hemolysis is referred to as a positive CAMP [Christie, Atkins, Munch-Petersen] test). The characteristic motility of the organism in a liquid medium or semisolid agar is also helpful for the preliminary identification of listeriae. All gram-positive rods isolated from blood and CSF should be identified (MALDI TOF) to distinguish between Corynebacterium (presumably a contaminant) and Listeria.

LABORATORY DIAGNOSIS

Identification

Selected MALDI TOF and serologic tests are used to identify the pathogen definitively. A total of 13 serotypes have been described, with 1/2a, 1/2b, and 4b responsible for most infections in neonates and adults. Serotyping is generally not useful in epidemiologic investigations, because relatively few serotypes are isolated from humans with disease. Enzyme profiles (i.e., multilocus enzyme electrophoresis [MLEE]) and genomic analysis (e.g., ribotyping, pulsed-field gel electrophoresis [PFGE], random amplified polymorphic DNA [RAPD] typing) are used for epidemiologic investigations.

PCR diagnosis – valuable from primary sterile sites (e.g. CSF, bioptates…)

Treatment, prevention and control

- **Treatment** – penicillin or **ampicilin** (either alone or with gentamicin)
- **Erythromycin** – in case of allergy to penicillin
- **Naturally resistant to cephalosporins** (meningitis can not be treated by cephalosporin like meningitis caused by other agents)
- **High risk people should avoid eating raw or partially cooked foods of animal origin**
- A vaccine is not available

Case 1

A **34-year-old woman** at **11 gestational weeks** was infected by *Listeria monocytogenes* with **clinical symptoms of acute onset of a fever with subsequent headache and neck stiffness**, and was treated with **intravenous ampicillin** at 2 g every 4 h for 3 weeks. **A healthy, unaffected male baby was delivered at term.** Histopathologic examination of the placenta did not reveal any chorioamnionitis, granulomas, microabscesses or vasculitis.

ConclusionThe neonate developed well without any neurologic compromise at a six-week postnatal follow-up visit.

A favorable outcome of maternal listeria infection in the first trimester may be anticipated. Besides, intravenous ampicillin with or without gentamicin should be a reasonable treatment option for maternal listeria infection in the first trimester.

Case 2

A **65-year-old** man with a history of **chronic kidney disease** was admitted with **high-grade fever**, **altered sensorium, and vomiting**. Physical examination revealed him to be febrile (38.8°C) and disoriented with neck stiffness. Investigations included **leukocytosis** with **neutrophilia** and **raised C-reactive protein**. CSF analysis indicated raised protein and low glucose, consistent with bacterial meningitis. **Blood** and **CSF cultures** were sent. The blood culture was positive after 17 hours and was cultured *Listeria monocytogenes.* The isolate was **susceptible** to **ampicilin** and **gentamicin**. The patient received intravenous ampicillin and gentamicin for 14 days. **The patient hada good response to treatment and was discharged stable.**

Case 3

A **72-year-old** male undergoing **chemotherapy** for carcinoma of the cervix presented with fever and vomiting. He had **generalized weakness and an altered mental status**. On examination, he was **febrile** and hypotensive. Laboratory tests showed **leukocytosis** and **elevated CRP**. **Blood cultures** yielded ***Listeria monocytogenes*** within 16 hours.The patient was started on high-dose intravenous **ampicillin** and **gentamicin**. Despite intensive care support, the patient deteriorated and succumbed to multiorgan dysfunction syndrome on day 5 of admission

Corynebacteriae

Taxonomy

- Genus – 116 species
- ***Corynebacterium diphteriae****, C. ulcerans, Arcanobacterium (previously Corynebacterium) haemolyticum* are significant human pathogens and other e.g. *C. jeikeium*, *C. urealyticum* are part of normal human flora and potential pathogens

Biological properties

- **Gram-positive club shaped rods**, non–spore-forming

Virulence factors and pathogenesis

Virulence factors are genetic, biochemical, or structural features that enable an organism to produce disease. **Diphteria toxin** in the corynebacterium is a **bicomponent (A-B) exotoxin**. Fragment B transports the toxic fragment A into the affected cell where it abruptly **stops elongation of proteosynthesis**. Structural genes of the exotoxin are **located on lysogenic β phage so some strains of *C. diphteriae*** that do not own the phage therefore do not produce the diphteria toxin (and the disease)

Virulence factors and pathogenesis

Corenybacterium diphteriae. The infections are spread by droplets or by contact from a patient or a healthy carrier to a susceptible host. **Locally,** sore throat, exudative and pseudomembranous pharyngitis with regional lymphadenitis or cutaneous diphteria (non-healing ulcers) occur. **Systemic disease** procedes as the respiratory tract infection progress with generalized symptoms caused by absorbtion of the diphterial exotoxin (**necrosis and parenchymal degeneration in muscles, heart, kidney, neurons**).

C. ulcerans (pets can be carriers) – can receive also the **β phage and cause an infection simillar to diphteria**

Clinical diagnosis

- The clinical **outcome in diphtheria is improved by the prompt initiation of treatment**.
- **Presumptive diagnosis**, based on several clinical clues: **(1) mildly painful tonsillitis or pharyngitis with associated membrane**, especially if the membrane extends to the uvula and soft palate; **(2) adenopathy and cervical swelling**, especially if associated with **membranous pharyngitis and signs of systemic toxicity**; **(3) hoarseness and stridor**; **(4) palatal paralysis**; **(5) serosanguineous nasal discharge** with associated mucosal membrane; (6) temperature elevation rarely in excess of 102.5° F; and **(7) history of recent travel to a country where diphtheria is endemic.**

Laboratory diagnosis

- **Initial treatment** of a patient with dipheria should be started **on the basis of the clinical diagnosis!**
- **Culture – small hemolytic colonies (**Four subspecies are recognized: ***C. d. mitis*****,** ***C. d. intermedius*****,** ***C. d. gravis*****, and** ***C. d. belfanti***. The four subspecies **differ slightly in their colonial morphology**)

- Diagnostic medium (Clauberg medium) – black colonies

- **Gram-positive club shaped rods**
- **Metachromatic granules** (Albert staining)
- **Detection of tox gene gene (PCR) or** using **immunodifusion test** (**Elek test)**

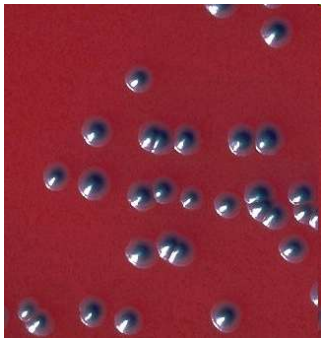
Corynebacteria

- the genus name is derived from Greek term *korynē* - club
- Gram-positive irregularly - club-shaped pleomorphic rods, arranged in „Chinese letters“ or palisades
- aerobic or facultative anaerobic, nonmotile, catalase positive
- are ubiquitous in plants and animals, and they normally colonize the skin, upper respiratory tract, gastrointestinal tract, and urogenital tract in humans
- ussually oportunistic pathogens
- a few are more commonly associated with human disease - most significant *Corynebacterium diphteriae*

6. LABORATORY DIAGNOSIS

blood agar

http://glowingworks.com/med/corynebacteria/index.htm



Cysteine-tellurite agar

Corynebacterium diphtheriae colonies on ?blood agar. CDC

- to confirm the clinical impression if epidemiologically significant (specific treatment must never be delayed if clinical picture is strongly suggestive)
- microscopy/smears (nose, throat): unreliable
- culture: blood agar, diagnostic medium - cysteine-tellurite agar (degaradtion of cystein brown halo, tel.-inhibit growth of respiratory commensals), corynebact. - grey to black color
- identification – MALDI TOF

6. LABORATORY DIAGNOSIS

- **toxigenicity testing**

a) **all isolates should be tested for the production of exotoxin**

b) **historically** by an in vitro immunodiffusion assay **(Elek test),** a tissue culture neutralization assay **using specific antitoxin**, or an in vivo neutralization assay using guinea pigs injected subcutaneously.

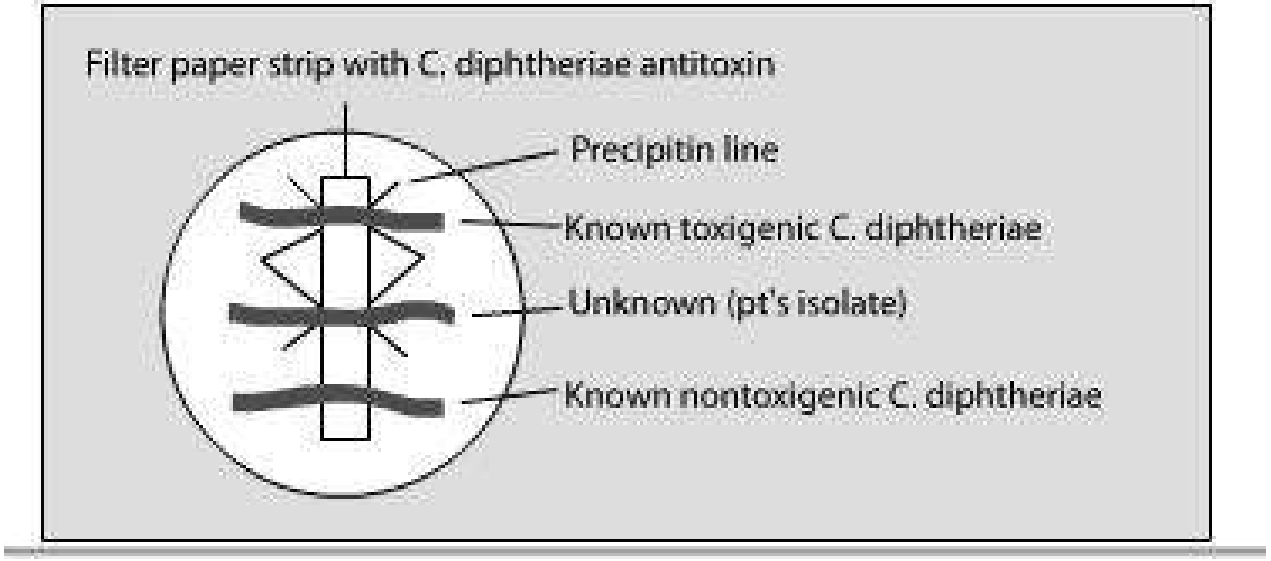
c) nucleic acid amplification test developed by the Centers for Disease Control and Prevention (CDC). This test can detect the ***tox*** **gene in clinical isolates and directly in clinical specimens** (e.g. from the diphtheritic membrane). Although this test is rapid and specific, strains with the *tox* gene not expressed can give a positive signal.

6. LABORATORY DIAGNOSIS

- **Nontoxigenic strains of *C. diphtheriae* should not be ignored,** because these strains have been associated with significant disease, including septicemia, endocarditis, septic arthritis, osteomyelitis, and abscess formation.

Laboratory diagnosis – Elek test

ELEK test:



Treatment, prevention and control

- **Treatment** – **combined therapy** both eliminating the infectious process by **antibiotics** (e.g. penicillin, erythromycin) and **neutralization of the circulating exotoxin by antitoxin** is applied. An important note is that once the toxin is bound to a cell surface receptor it could not be eliminated by the antitoxin.
- **Prevention-** Immunization with **toxoid**, usually administered in **DTP triple vaccine** (tetanus toxoid and pertussis antigen is also included).

Other Corynebacteria

- ***C. jeikeium*** – *lipophilic corynebacteria,* **part of normal human flora**, naturally resistant to penicillins, cephalosporins, aminoglycosides and often to macrolides and fluorochinolones
- **Serious nosocomial infections** – treatment **vankomycin**

Arcanobacterium (Corynebacterium) haemolyticum

- **Acute tonsilitis** in young adults
- **Treatment** – macrolides and linkosamides

A case of fulminant respiratory diphtheria in a 24-year-old Afghan refugee in Austria in May 2022: a case report

24-year-old Afghan refugee accommodated in an Austrian refugee center to a hospital. The patient reported a **massive sore throat and a slightly elevated temperature for four days** (See Fig. 1). Clinical examination showed **enlarged tonsils covered with membranes** with the left tonsil **partly destroyed.** Flexible endoscopy (see fig.) revealed **purulent secretion** and **thick membranes on the whole pharyngeal wall**. The **neck lymph nodes** were **bilaterally swollen**. An **empiric intravenous** (i.v.) antibiotic therapy with **amoxicillin/clavulanate** was started. **A peritonsillar abscess** was ruled out by computed tomography (CT). Because of **increasing constriction of the airway**, **intubation** had to be performed the next day. Over the next hours **oxygenation worsened and resulted in acute respiratory distress syndrome (ARDS).** On day 6 after symptom onset, the **antibiotic therapy was escalated** to **meropenem** and **linezolid** because of markedly rising inflammation markers. **Acute kidney failure** developed. Due to the rapid onset of **multi-organ failure. Diphtheria-antitoxin were administered** i.v. **Bronchoscopy** – inflamation.

Microbiology – 7th day **PCR** from throat confirmed **subunit A and B of *C. diphteriae***

Hypoxemia – extracorporeal membrane oxygenation **(ECMO).**
The young man **died 9 days after the symptom onset of respiratory diphtheria.**

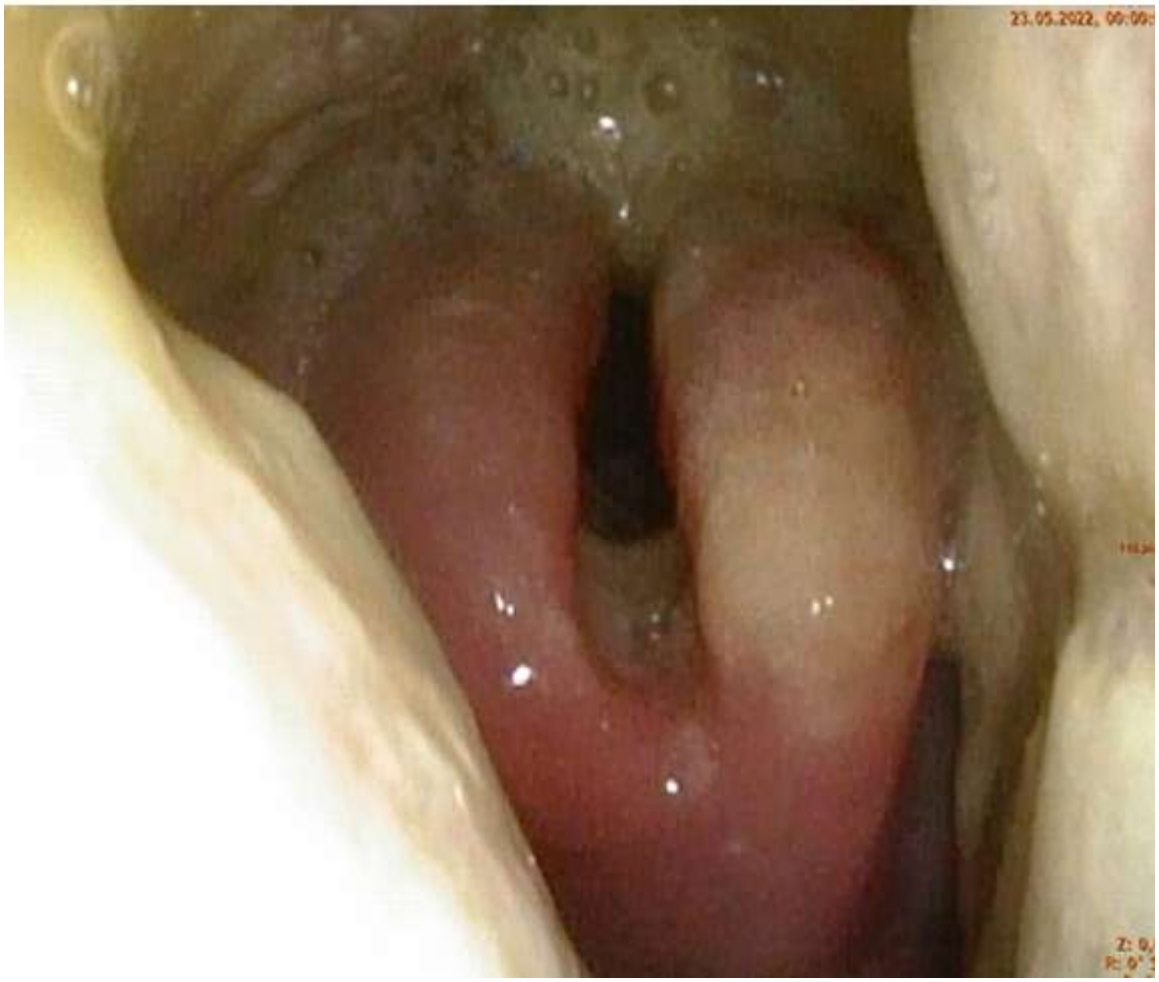


Fig. 1. Flexible endoscopy with **membranes** in the hypopharynx and edematous epiglottis

Clostridia

(anaerobic spore-forming bacteria)

Clinical material

Neurotoxic Clostridia (*Clostridium botulinum, Clostridium tetani*).These produce toxins affecting the nervous system.

Clostridium botulinum (Botulism)

Typical samples:

Serum (blood) → for detecting botulinum toxin
Stool → especially in infant botulism (culture, PCR)
Gastric contents /vomit → early in foodborne cases
Suspected food samples → key for outbreak investigation
Wound samples → in wound botulism
Main reason: detect botulinum toxin, not just the bacteria.

Clostridium tetani (Tetanus)

Typical samples:

Wound tissue or exudate → from the infection site (culture, PCR)
Diagnosis is mainly clinical, because *C. tetani* is hard to isolate and toxin detection is not routine.

Conditions for processing and culture anaerobic samples

Anaerostat works by removing oxygen and replacing it with an anaerobic atmosphere

The principle of culturing anaerobic bacteria in an anaerostat (anaerobic jar/chamber) is to create and maintain an oxygen-free environment so that obligate anaerobes can grow.

***Anaerobic bacteria cannot tolerate oxygen because they lack enzymes like catalase and superoxide dismutase to detoxify reactive oxygen species.**

Clostridium tetani

Physiology and Structure

* Gram-positive rods with prominent terminal spores (drumstick appearance)
* Strict anaerobe (vegetative cells are extremely oxygen sensitive)
* Difficult to isolate from clinical specimens

Virulence

* Spore formation
* Tetanospasmin (heat-labile neurotoxin; blocks release of neurotransmitters
* Tetanolysin (heat-stable hemolysin of unknown significance)

Epidemiology

* Ubiquitous; spores are found in most soils and can colonize gastrointestinal tract of humans and animals
* Exposure to spores is common, but disease is uncommon, except in developing countries where there is poor access to vaccine and medical care
* Risk is greatest for people with inadequate vaccine-induced immunity; disease does not induce immunity
* Diagnosis is **based on clinical presentation**
* **Microscopy, culture, PCR - with poor sensitivity**
* Neither tetanus toxin nor antibodies are typically detected

Treatment, Prevention, and Control. Treatment requires débridement, antibiotic therapy (penicillin G, metronidazole – could be combined by clindamycin), passive immunization with antitoxin globulin, and vaccination with tetanus toxoid

* Prevention - vaccination, consisting of three doses of tetanus toxoid, followed by booster doses every 10 years

Case report

Generalized tetanus is still a **global concern** with a **mortality** rate of **up to 50%,** especially in low and middle-income countries. We reported a 23-year-old **man** from Afghanistan admitted to emergency department, with the chief complaint of **generalized severe spasms** and **lockjaw**. The patient had **skin lesions** and had **never been vaccinated against tetanus**. He intubated and admitted to the intensive care unit (ICU) with **diagnose** of **severe generalized tetanus**. After receiving **tetanus immunoglobulin** and **intravenous metronidazole**, and supportive therapy. Due to the refractory **muscular spasms** intravenous phenobarbital started and little by little recovery was achieved. The patient receiving the first two doses of the **Td vaccine**, and **discharged on Day 42 of hospitalization with no symptom recurrence.**

Clostridium botulinum

- Physiology and Structure Gram-positive, spore-forming rod strict anaerobe, fastidious growth requirements, can produce one of seven distinct botulinum toxins (A to G)
  * Strains associated with human disease produce lipase, digest milk proteins, hydrolyze gelatin, and ferment glucose

  Virulence

  * Spore formation, Botulinum toxin (prevents release of neurotransmitter acetylcholine)

  Epidemiology

  Ubiquitous; *C. botulinum* spores are found in soil worldwide
  * Human diseases associated with toxins A, B, E, and F
  * Infant botulism more common than other forms

  Diagnosis Botulism confirmed by **isolating the organism (or DNA) or detecting the toxin in food products or the patient's feces or serum**

  Treatment, Prevention, and Control Treatment involves administration of metronidazole or penicillin, trivalent botulinum antitoxin, and ventilatory support * Spore germination in foods prevented by maintaining food in an acid pH, by high sugar content (e.g., fruit preserves), or by storing the foods at 4°C or colder * Toxin is heat-labile and therefore can be destroyed by heating of food for 10 minutes at 60° to 100°C
  * Infant botulism is associated with consumption of contaminated foods (particularly honey). Infants younger than 1 year should not be given honey or foods containing it)

Case report of a patient with a **foodborne botulism**.

The **diagnosis** was by **clinical presentation**, evolution and resolution of the clinical features after botulism antitoxin administration and later confirmed by electroneuromyography. Unable isolation of botulinic toxin on biological samples nor in the possible culprit food.

Botulism is a rare neuroparalytic syndrome, caused by a neurotoxin produced by bacteria of the genus *Clostridium*. This syndrome courses initially with symmetrical cranial nerve palsy, may progress to descending flaccid paralysis and ultimately to respiratory arrest. **51-year-old patient** admitted in the Emergency Department with a history of **headache, dizziness, vomiting, bilateral non reactive mydriasis**. The patient **rapidly progressed** to **urinary retention, hypotonia and respiratory arrest**. **Food botulism was hypothesized** and **antitoxin immunoglobulin was administered**. Diagnosis was assumed by **clinical response to antitoxin immunoglobulin and electromyogram alterations.** The patient evolved favorably with **total functional recovery.**

Although a rare entity, it is important to maintain a high suspicion in order to avoid diagnostic delay with increased risk of sequelae and death.

Clostridium difficile

Physiology and Structure Gram-positive, spore-forming rods
* Strict anaerobe (vegetative cells are extremely oxygen sensitive)

Virulence enterotoxin (toxin A) and a cytotoxin (toxin B)

Epidemiology The organism is ubiquitous
* Colonizes the intestines of a small proportion of healthy individuals (<5%)
* Exposure to antibiotics is associated with overgrowth of *C. difficile* and subsequent disease (endogenous infection)
* Spores can be detected in hospital rooms of infected patients (particularly around beds and in the bathrooms); these can be an exogenous source of infection

Diagnosis ***C. difficile* disease is confirmed by detecting the somatic Ag (GDH) , cytotoxin or enterotoxin (A, B) in the patient's liquid feces**, PCR could be also used

Treatment, Prevention, and Control The implicated antibiotic should be discontinued
* Treatment with vancomycin or fidaxomicin (Metronidazol) should be used in severe disease, faecal microbiota transplantation - effective in recurrent inf.

* Relapse is common because antibiotics do not kill spores; a second course of therapy with the same antibiotic is usually successful
* The hospital room should be carefully cleaned after the infected patient is discharged

Clostridium perfringens

Physiology and Structure

Large, rectangular, gram-positive rods, spores rarely seen in specimens or culture
Replicates rapidly, so large, spreading colonies are seen within first day of culture; "double zone" of hemolysis on blood agar (produced by α-and θ-toxins)

Virulence

Produces many toxins and hemolytic enzymes, so white blood cells and platelets are not seen in Gram-stained clinical specimens, lecithinase (phospholipase C)
Subdivided into five types (A to E) on the basis of toxin production

Epidemiology

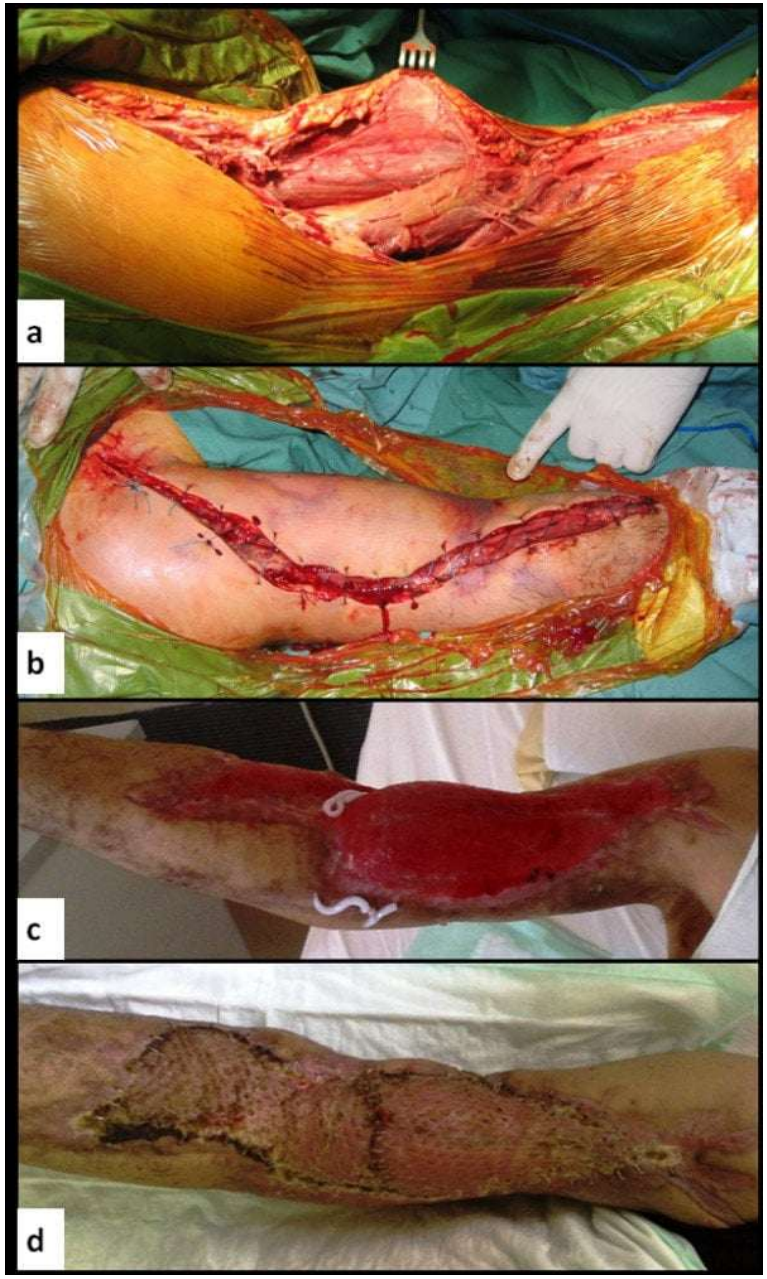
Ubiquitous; present in soil, water, and intestinal tract of humans and animals
Type A is responsible for most human infections
Disease follows exogenous or endogenous exposure

Diagnosis

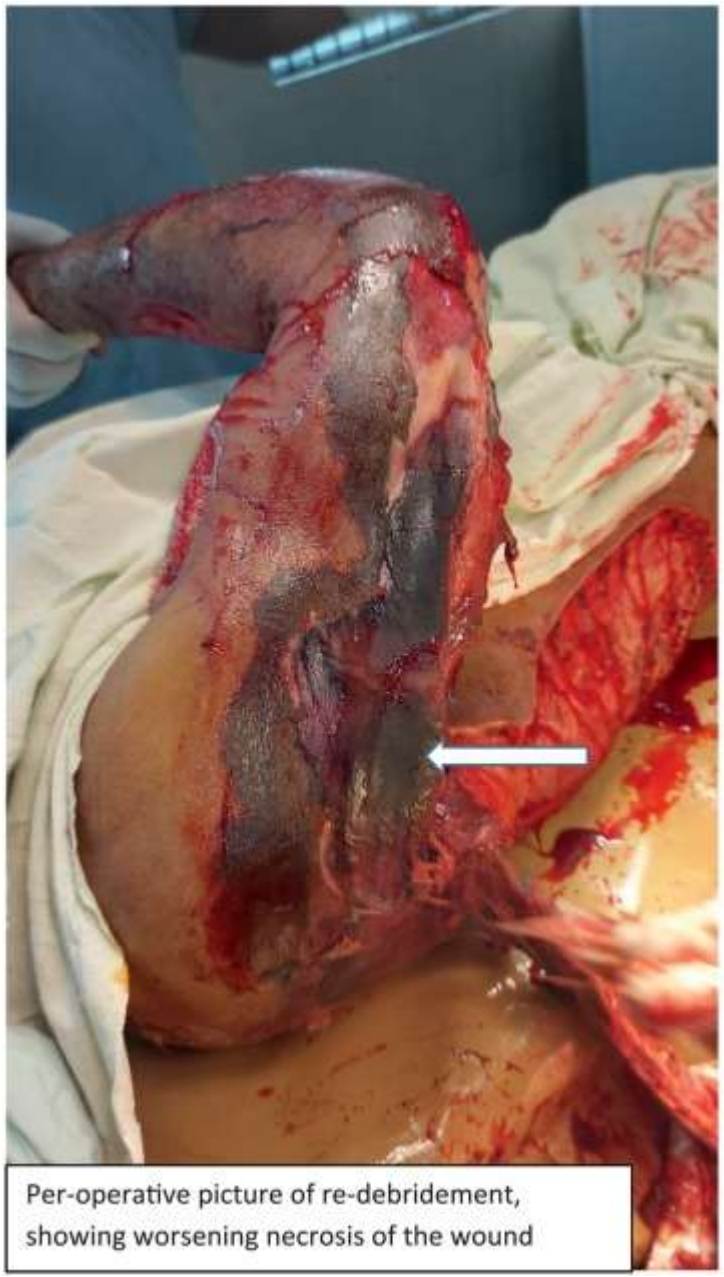
Characteristic forms seen on Gram stain, grows rapidly in culture, PCR from affected tissue

Treatment, Prevention, and Control

Rapid treatment is essential for serious infections
Systemic infections require surgical débridement and high-dose penicillin therapy; antiserum against α-toxin not used today; the value of hyperbaric oxygen treatment is unproven
Treat with débridement and penicillin for localized infections. Symptomatic treatment for food poisoning (not ATB). Proper wound care and judicious use of prophylactic antibiotics will prevent most infections.



https://www.researchgate.net/figure/Surgical-treatment-of-gas-gangrene-with-preservation-of-the-affected-limb-a_fig2_51575850



Per-operative picture of re-debridement, showing worsening necrosis of the wound

https://www.sciencedirect.com/science/article/pii/S2210261225004225

Bacillus anthracis

Clinical Manifestations

Anthrax is caused by *Bacillus anthracis.* Humans acquire the disease directly from contact with infected herbivores or indirectly via their products. The clinical forms include (1) **cutaneous anthrax** (eschar with edema), from handling infected material (this accounts for more than 95 percent of cases); (2) **intestinal anthrax**, from eating infected meat; and (3) **pulmonary anthrax**, from inhaling spore-laden dust.

Bacillus anthracis

Pathogenesis

The virulence factors of *B anthracis* are its capsule and three-component toxin, both encoded on plasmids.

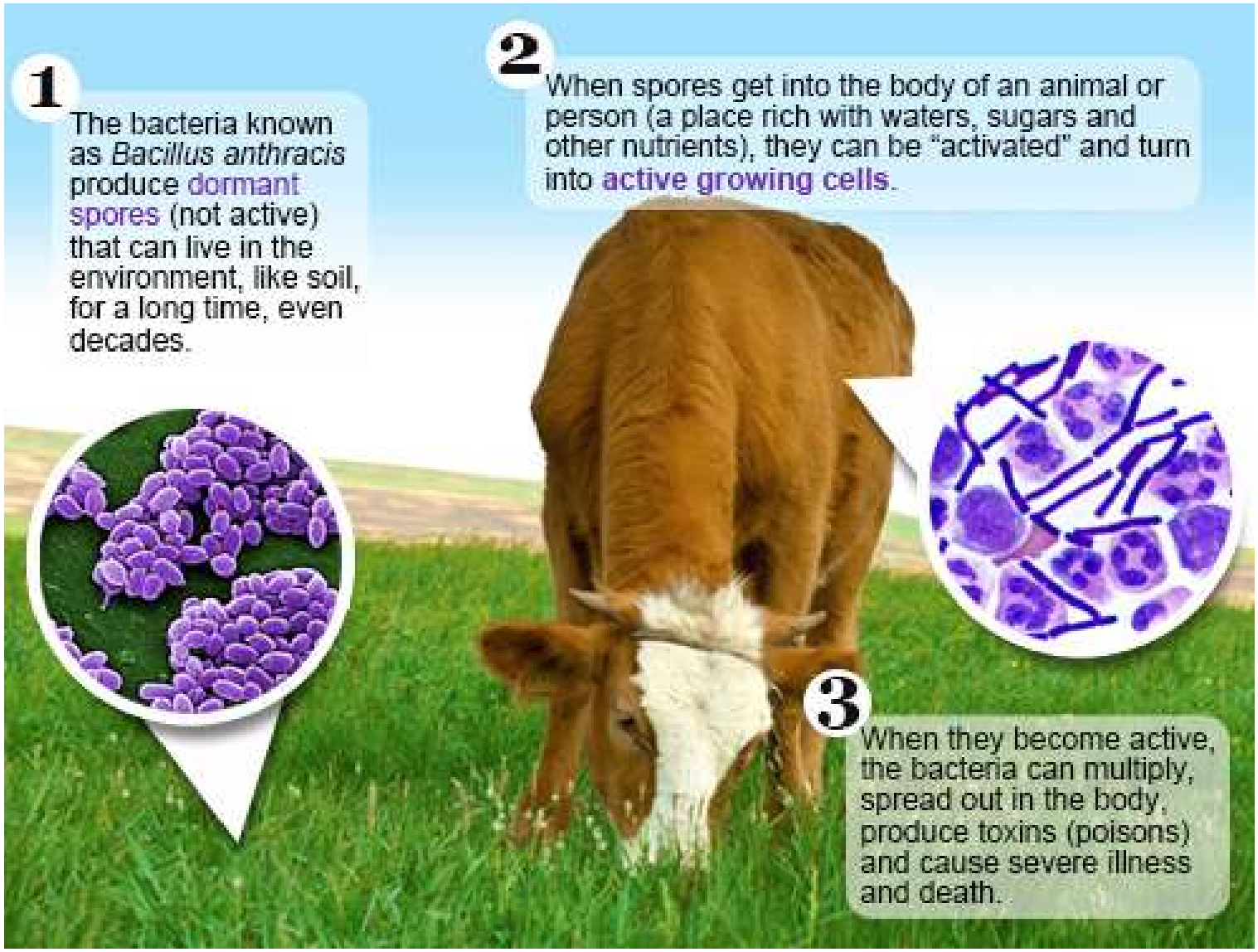
Host Defenses

The reasons for marked differences in susceptibility to anthrax among different animal species are not known. The protective actions of the live-spore animal vaccine or the human chemical vaccines are based on induction of humoral and cell-mediated immunity to the protective antigen component of anthrax toxin.

Epidemiology

Individuals at risk for anthrax include those in contact with infected animals or animal products.

Anthrax epidemiology



References: https://www.cdc.gov/anthrax/basics/index.html

Structure and classification

Bacillus species are **rod-shaped**, **endospore-forming** aerobic or facultatively anaerobic, **Gram-positive** bacteria; in some species cultures may turn Gram-negative with age. The many species of the genus exhibit a wide range of physiologic abilities that allow them to live in every natural environment. Only one endospore is formed per cell. The spores are resistant to heat, cold, radiation, desiccation, and disinfectants. *Bacillus anthracis* needs oxygen to sporulate; this constraint has important consequences for epidemiology and control. In vivo, *B anthracis* produces a polypeptide (polyglutamic acid) capsule that protects it from phagocytosis.

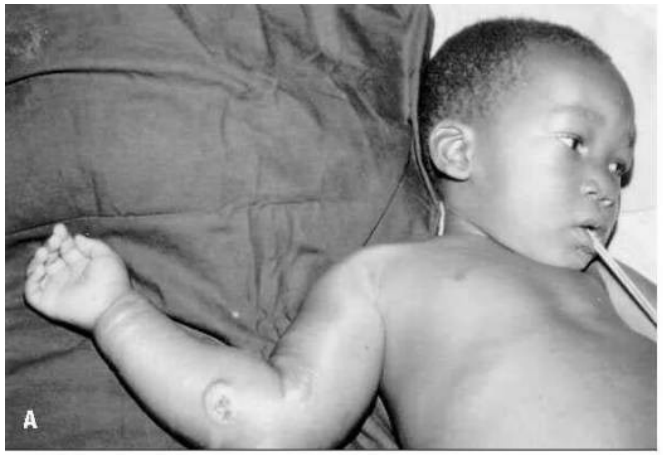
The genera *Bacillus* and *Clostridium* constitute the family Bacillaceae. Species are identified by using morphologic and biochemical criteria.

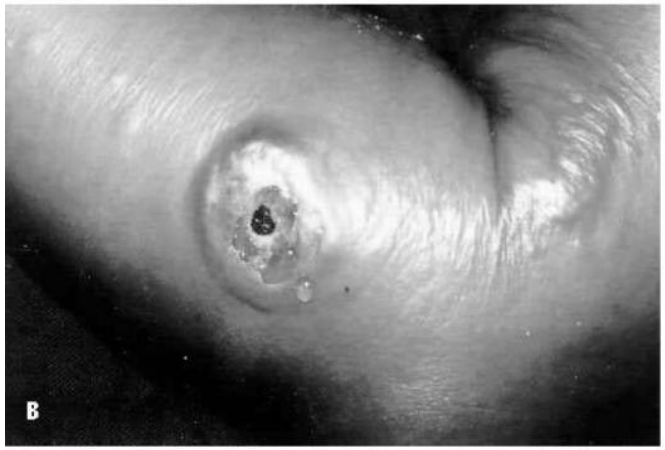
Bacillus anthracis

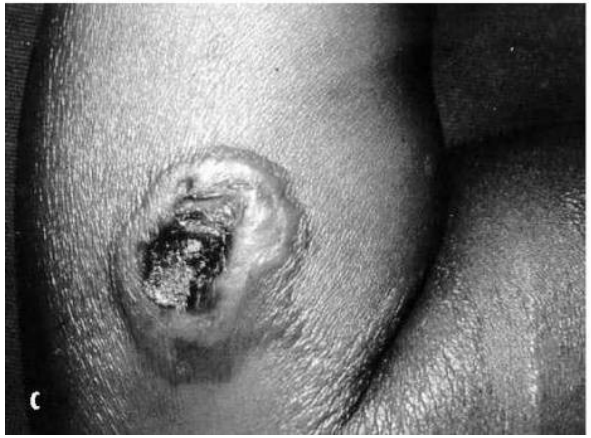
Anthrax

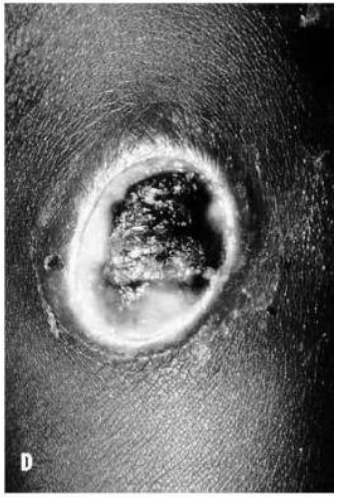
Anthrax is primarily a disease of herbivores. Humans acquire it as a result of contact with infected animals or animal products. In humans the disease takes one of three forms, depending on the route of infection. Cutaneous anthrax, which accounts for more than 95 percent of cases worldwide, results from infection through skin lesions; intestinal anthrax results from ingestion of spores, usually in infected meat; and pulmonary anthrax results from inhalation of spores.

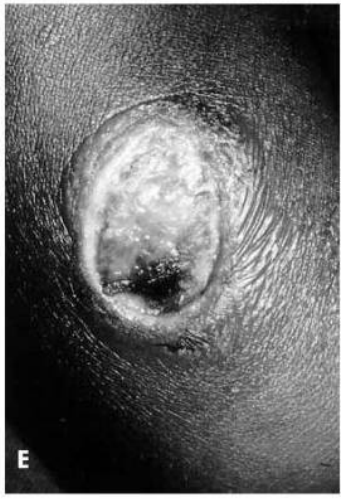
Cutaneous anthrax usually occurs through contamination of a cut or abrasion, although in some countries biting flies may also transmit the disease. After a 2- to 3-day incubation period, a small pimple or papule appears at the inoculation site. A surrounding ring of vesicles develops. Over the next few days, the central papule ulcerates, dries, and blackens to form the characteristic eschar (Fig.). The lesion is painless and is surrounded by marked edema that may extend for some distance. Pus and pain appear only if the lesion becomes infected by a pyogenic organism. Similarly, marked lymphangitis and fever usually point to a secondary infection. In most cases the disease remains limited to the initial lesion and resolves spontaneously. The main dangers are that a lesion on the face or neck may swell to occlude the airway or may give rise to secondary meningitis. If host defenses fail to contain the infection, however, fulminating septicemia develops. Approximately 20 percent of untreated cases of cutaneous anthrax progress to fatal septicemia. However, *B anthracis* is susceptible to penicillin and other common antibiotics, so effective treatment is almost always available.











4 year old boy. (A&B) the lesion when first seen (day 0). Note the arm swollen from the characteristic edema. (C) Day 6. (D) Day 10. (E) Day 15. Although penicillin treatment was begun immediately and the lesion was sterile by about 24 hours, it continued to evolve and resolve as seen. (Turnbull, Medical Microbiology. 4th edition)

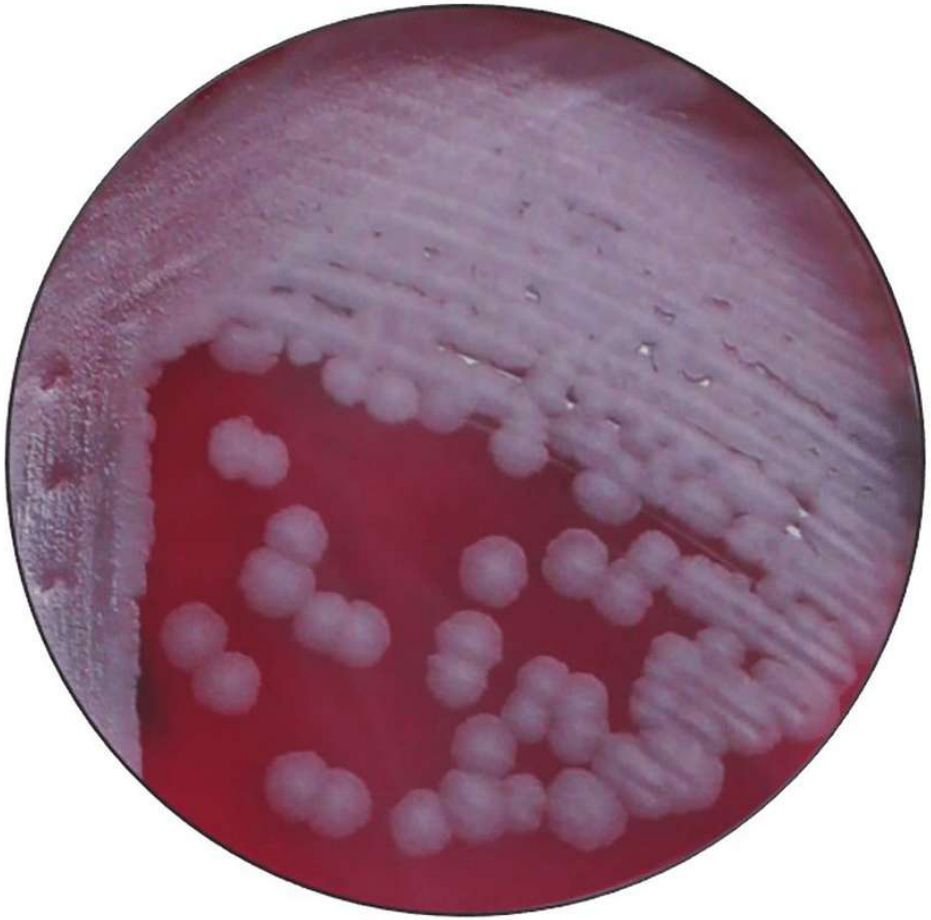
Intestinal anthrax is analogous to cutaneous anthrax but occurs on the intestinal mucosa. As in cutaneous anthrax, the organisms probably invade the mucosa through a preexisting lesion. Generalized disease develops when the organisms spread from the mucosal lesion to the lymphatic system. In pulmonary anthrax, inhaled spores are transported by alveolar macrophages to the mediastinal lymph nodes, where they germinate and multiply to initiate systemic disease. Gastrointestinal and pulmonary anthrax are both more dangerous than the cutaneous form because they are usually identified too late for treatment to be effective.

Herbivorous animals, the primary hosts of *B anthracis*, contract the infection by ingesting spores on forage plants; the spores are derived from soil or dust or are deposited on leaves by flies after feeding on an anthrax-infected carcass. If the spores enter a lesion in the gastrointestinal mucosa, they germinate and are taken into the bloodstream and lymphatics, finally producing systemic anthrax, which is usually fatal.

Symptoms prior to fulminant systemic anthrax may be absent or mild, consisting, for example, of malaise, low fever, and mild gastrointestinal symptoms in the case of gastrointestinal disease. During this phase the organism is multiplying and producing toxin in the regional lymph nodes and spleen. Released toxin causes breakdown of these organs probably of the spleen in particular. This causes the sudden onset of hyperacute illness with dyspnea, cyanosis, high fever, and disorientation, which progress in a few hours to shock, coma, and death. Although symptoms vary somewhat with the host species, this final acute phase is marked by a high-grade bacteremia. In humans, blood cultures are not always positive.

Microbiological diagnosis

The clinical diagnosis of anthrax is **confirmed by directly visualizing or culturing the anthrax bacilli.** Fresh **smears** of vesicular fluid, fluid from under the eschar, blood, lymph node or spleen aspirates, or (in meningitic cases) cerebrospinal fluid are stained with polychrome methylene blue (M'Fadyean's stain) and examined for the characteristic square-ended, blue-black bacilli surrounded by a pink capsule. (It should be remembered that *B anthracis* organisms are not invariably detected in stained blood smears of humans dying of anthrax.) Alternatively, the bacilli may be cultured from these specimens and checked for sensitivity to the anthrax gamma phage, for penicillin sensitivity, and for **capsule formation**. Colonies **grown overnight at 37°C on blood agar** are **gray or white, nonhemolytic**, with a **dry**, ground-glass appearance; they are at least 3 mm in diameter and sometimes have tails. Capsules can be seen in polychrome methylene blue-stained smears of cultures grown on nutrient agar containing 0.7 percent sodium bicarbonate and incubated overnight under CO_2 (e.g., in a candle jar); encapsulated colonies are mucoid. Alternatively, 2 ml of blood (such as commercial defibrinated horse blood) inoculated with a pinhead quantity of material from a suspected colony and incubated at 37°C yields readily demonstrable encapsulated bacilli in 6 hours. Culturing may be unsuccessful if the patient has been treated with antibiotics. **PCR** is valuable from local affection or venous blood.



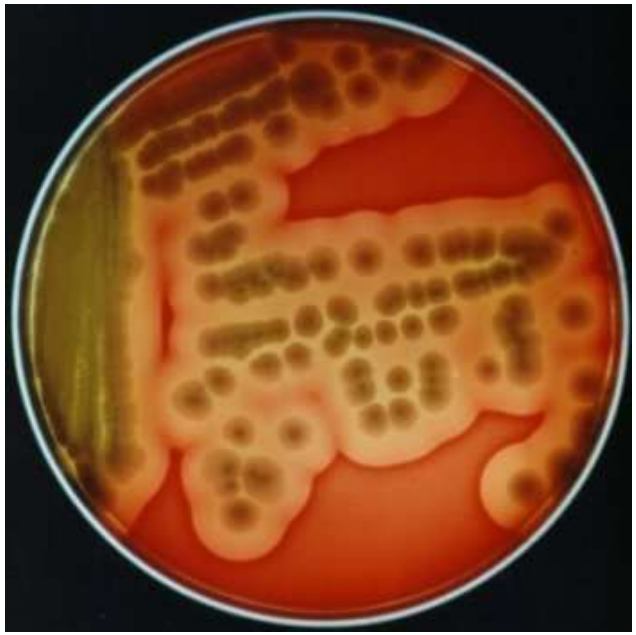
***Bacillus anthracis* 24-hour growth on sheep blood agar from a swab of a cutaneous anthrax lesion**

Other Bacillus spp. and disease

*** Food poisoning (caused mainly by *Bacillus cereus*)**

Types:
- Emetic (vomiting) type
- Diarrheal type

Microbiological diagnosis:
- **Culture of food or stool:** Isolation of *B. cereus*
- **Toxin detection:** Especially for emetic toxin
- **Colony characteristics:** Beta-hemolytic on blood agar
- **Motility test:** Usually motile



B. cereus, blood agar

Rare infections by other *Bacillus* species

Some species (e.g. *Bacillus subtilis*) can cause:
• Opportunistic infections (e.g., bacteremia, wound infections)

Diagnosis:
• **Culture + identification**
• Usually considered contaminants unless repeatedly isolated

B. subtilis,
blood agar