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***

1. CLASSIFICATION (TAXONOMY)

- currently known around 145 species in the genus (List of Prokaryotic names with Standing in Nomenclature)
- ***Corynebacterium diphtheriae*** – the ethiologic agent of diphteria
- **respiratory diphteria:** sudden onset with exudative pharyngitis, sore throat, low-grade fever, and malaise; a thick pseudomembrane develops over the pharynx; in critically ill patients, breathing obstruction, cardiac arrhythmia, coma, and death can develop
- **cutaneous diphtheria:** a papule can develop on the skin that progresses to a nonhealing ulcer; systemic signs can develop

MAIN CLINICAL INFECTIONS

1. Respiratory diphtheria (most classic form)

- Affects the nose, throat, and tonsils
- Symptoms:
  - Sore throat, fever, malaise
  - Formation of a thick gray pseudomembrane over the tonsils/pharynx
  - Neck swelling (“bull neck” appearance)
- Major risks:
  - Airway obstruction, systemic toxin absorption

2. Cutaneous diphtheria

- Skin infection, more common in tropical or resource-limited settings
- Symptoms:
  - Chronic, non-healing ulcers
  - Dirty gray membrane covering lesions
- Usually less severe, but can act as a reservoir for transmission

3. Nasal diphtheria

- Milder form, especially in children
- Symptoms:
  - Runny nose (often bloody or serosanguinous)
  - Mild systemic symptoms

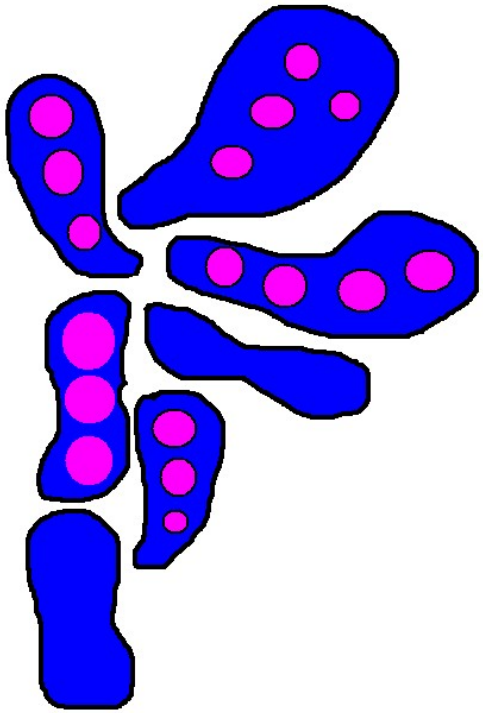
Diphtheria pseudomembrane. CDC.

Respiratory diphteria

A diphtheria skin lesion on the leg

http://en.wikipedia.org/wiki/Diphtheria

2. MICROSCOPY (GRAM-STAIN)



diagram

3. PHYSIOLOGY AND STRUCTURE

- irregularly staining, **pleomorphic rod** (0.3-0.8 × 1.0-8.0 μm)

- **metachromatic granules** have been observed

- after overnight incubation: 1-3mm colonies are observed on blood agar medium (selective, differential media can be used to recover this pathogen from specimens in which other organisms are present

- subdivided into 4 **biotypes** based on their colonial morphology and biochemical properties: ***gravis, intermedius, mitis, belfanti*** (biotypes *intermedius* and *belfanti* are rarely associated with diphtheria)

4. VIRULENCE

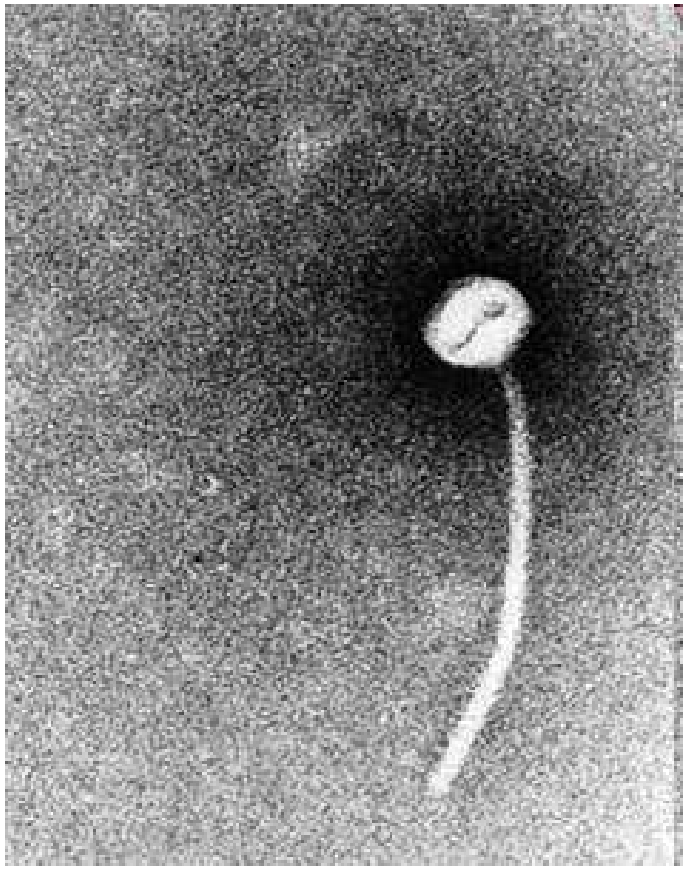
• the toxicity observed in diphtheria is directly attributed to an **exotoxin A-B** (**inhibits protein synthesis** by inactivating elongation factor 2) secreted by the bacteria at the focus of infection. The organism does not need to enter the blood to produce the systemic signs of disease

• the toxin is determined by **tox gene** introduced into *C. diphteriae* strain by a **lysogenic bacteriophage β phage** (not on the bacterial chromosome)

• even though the tox gene is not part of the bacterial chromosome, the regulation of toxin production is under the DtxR (regulatory) chromosom.gene & toxin production depends upon bacterial iron metabolism.

• additional virulence factors are likely (but unknown) because **nontoxigenic strains can cause disease**

The Beta phage

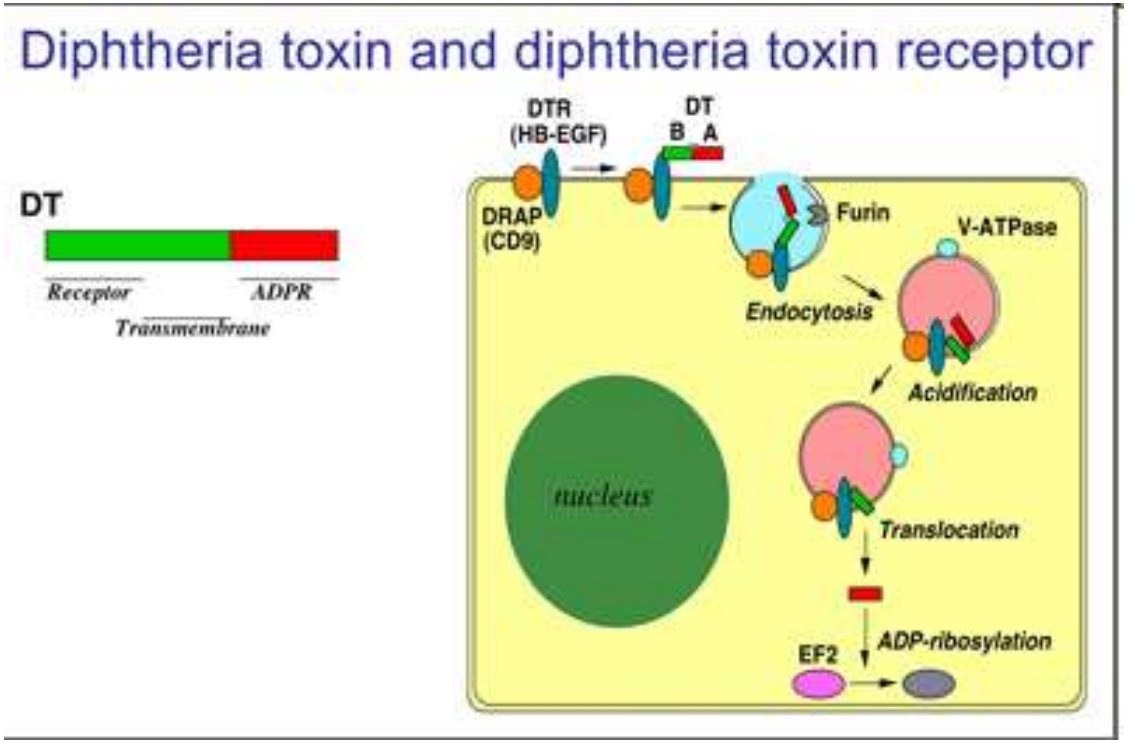


http://www.textbookofbacteriology.net/diphtheria.html

Toxin/colonization/vaccination

Since mass immunization against diphtheria has been practiced, the disease has virtually disappeared, and *C. diphtheriae* is no longer a component of the normal flora of the human throat and pharynx. It may be that the toxin played a key role in the colonization of the throat in nonimmune individuals and, as a consequence of exhaustive immunization, toxigenic strains have become virtually extinct.

http://www.textbookofbacteriology.net/diphtheria.html



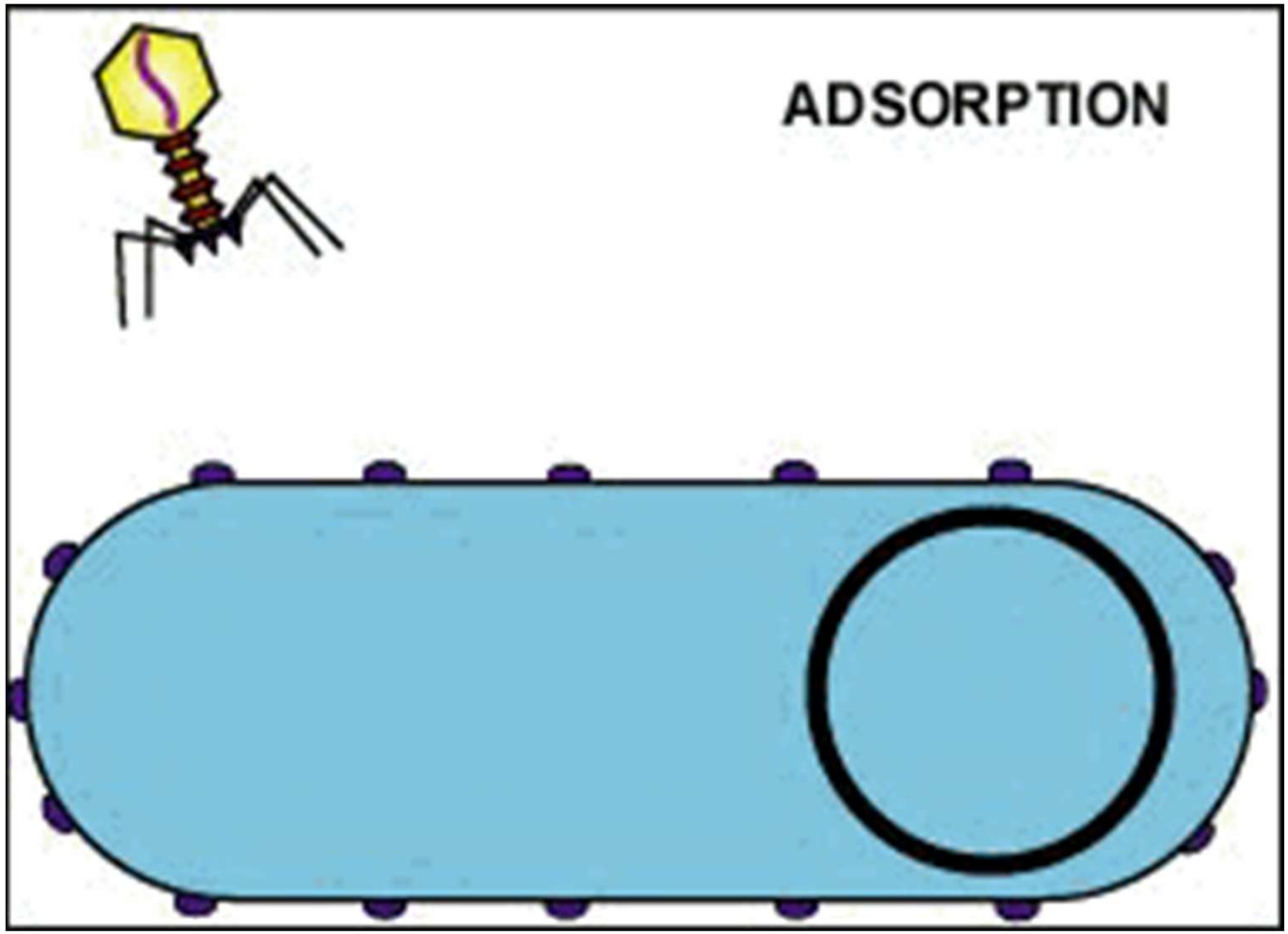
Analysis of the molecules and receptors involved in bacterial infection

Principal Research Scientist
Eisuke Mekada

http://www.biken.osaka-u.ac.jp/COE/eng/project/pro09.html

1. Diphtheria toxin (DT) binds to DT receptor via fragment B. 2. DT receptor forms a complex with membrane proteins such as CD 9.
2. CD 9 facilitates DT binding to DT receptor. DT binds to its receptor and is endocytosed into the endosome.
3. Fragment A then passes through the endosomal membrane into the cytosol and inactivates elongation factor2, (EF2), resulting in the inhibition of protein synthesis in the host cell.

Generally - lytic bacteriophage cycle



http://student.ccbcmd.edu/courses/bio141/lecguide/unit3/viruses/lytlc.html

5. EPIDEMIOLOGY

- **worldwide distribution** maintained in asymptomatic carriers and unvaccinated hosts

- **humans are the only known reservoir**, with carriage in oropharynx or on skin surface

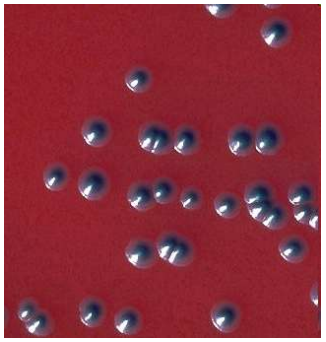
- **Spread** person to person by exposure to respiratory **droplets or skin contact**

- Disease observed in unvaccinated people living in crowded urban areas and in children or adults with waning immunity

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.

7. TREATMENT

- **early administration of diphtheria antitoxin** to specifically neutralize the exotoxin before it is bound by the host cell

- once the cell internalizes the toxin, cell death is inevitable.

- antibiotic therapy with **penicillin** is also used to eliminate *C. diphtheriae* and terminate toxin production.

- after the patient has recovered, immunization with toxoid is required because most patients fail to develop protective antibodies after a natural infection.

8. PREVENTION

- **symptomatic diphtheria can be prevented by actively immunizing people with DT**
- the nontoxic, immunogenic toxoid is prepared by formalin treatment of the toxin. Initially, children are given five injections of this preparation with pertussis and tetanus antigens **(DPT vaccine)** at ages 2, 4, 6, 15 to 18 months, and at 4 to 6 years
- after that time, it is **recommended that booster vaccinations** with diphtheria toxoid combined with tetanus toxoid be given **every 10 years** (recently Czech Republic 20 years). Serum antitoxin antibodies can be measured/tested.

8. CONTROL

- people **coming in close contact** with patients who have **documented diphtheria are** at risk for acquiring the disease.
- **nasopharyngeal specimens** for **culture** should be collected from **all close contacts**, and **antimicrobial prophylaxis** with penicillin or erythromycin started immediately
- **any contact who has not completed** the series of **diphtheria immunizations** or who has not received a booster dose within the previous 5 years **should receive a booster dose of toxoid**
- people exposed to cutaneous diphtheria should be managed in the same manner as those exposed to respiratory diphtheria
- if the respiratory or cutaneous infection is caused by a nontoxigenic strain, it is unnecessary to institute prophylaxis in contacts.

Virulence of other *Corynebacterium* species & diseases & treatment

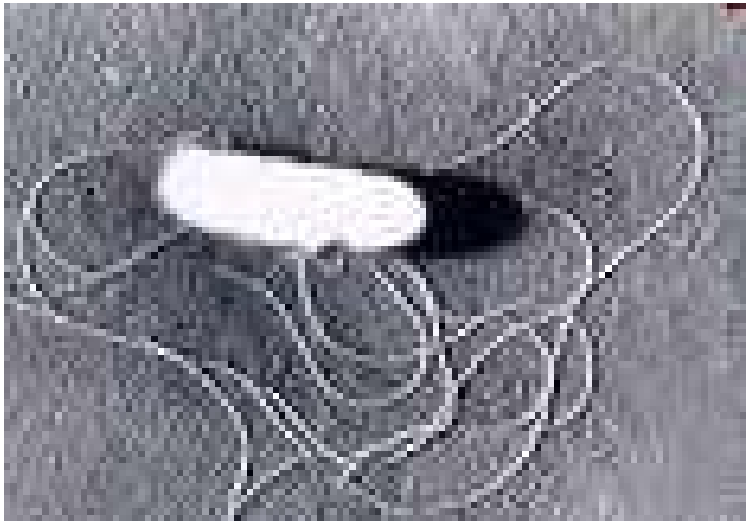
- **diphtheria A-B exotoxin** may be carried by ***C. ulcerans*** and ***C. pseudotuberculosis***
- urinary tract pathogens produce urease (e.g., *C. amycolatum, C. glucuronolyticum, C. riegelii, C. urealyticum*)
- many species able to adhere to foreign bodies (e.g., catheters, shunts, prosthetic devices)
- some species resistant to most antibiotics – usually **agents of HAI** (e.g., *C. amycolatum**, C. jeikeium,** C. urealyticum*)
- **septicemia, endocarditis, foreign body infections, wound infections,** urinary tract infections, respiratory infections, including diphtheria
- treatment with effective antibiotics to eliminate the organism
- removal of foreign body

Listeria

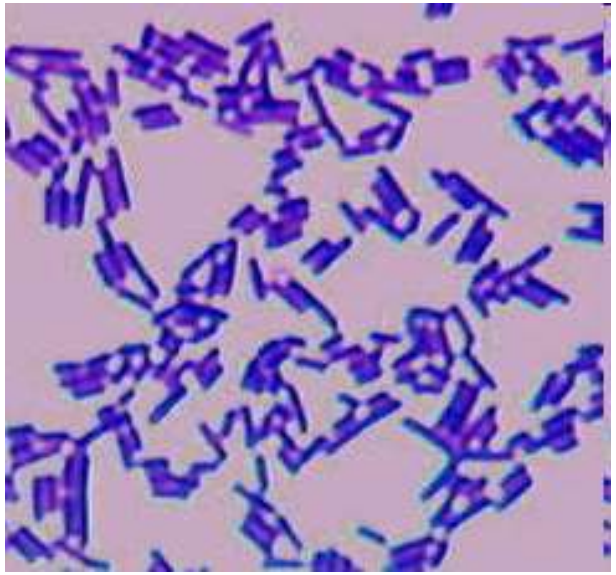
- etymology - Lister, Joseph Baron Lister, 1827-1912; eng. surgeon: introduced antiseptic surgery
- the genus *Listeria* consists of six species
- *L. monocytogenes* and *L. ivanovii* the only recognized pathogens
- *L. monocytogenes* is a short **rod** (to 2.0 μm), nonbranching, **gram-positive, motile** (peritrichous flagella syntetized at 22-25C but not at 37C), facultatively anaerobic rod capable of growth at a broad temperature range (1°C to 45°C) and in a high concentration of salt
- can be mistaken for *Enterococcus*
- **β hemolysis on blood agar** (ussually under white or greyisch colonies)

1. MICROSCOPY

Listeria monocytogenes,source: clinical material from a patient with nosocomial infection, cultivated on Tryptone Soya blood agar



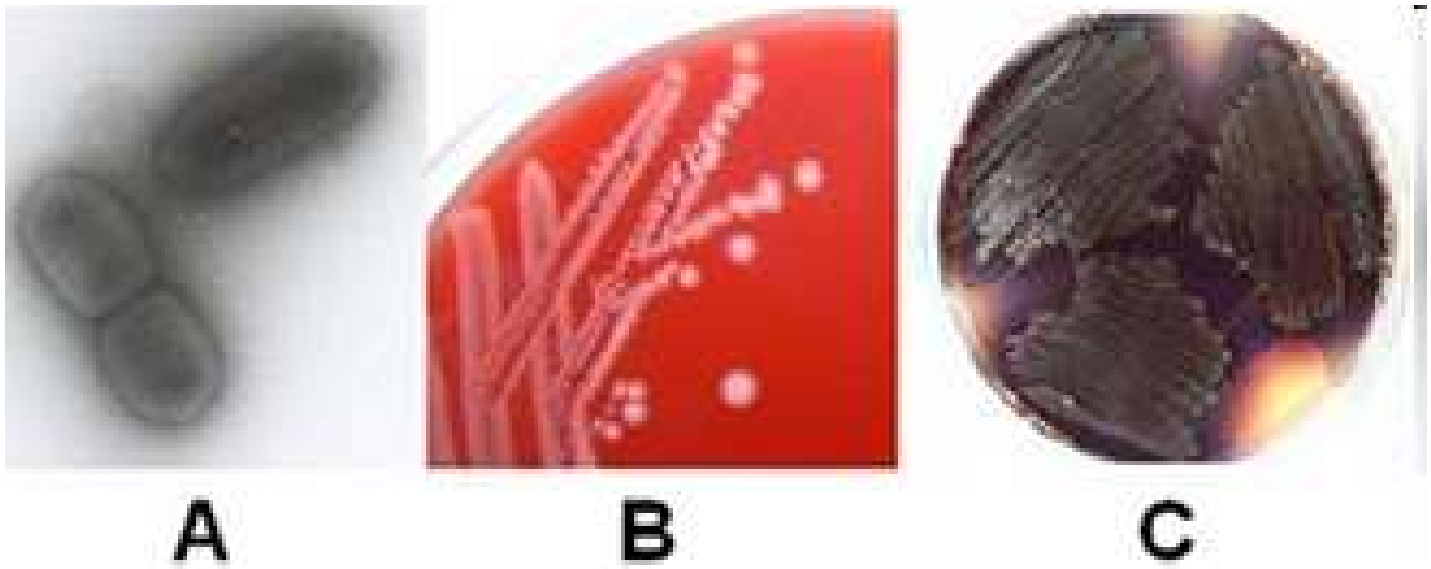
www.textbookofbacteriology.net



Gram-stain

http://www.old.rlbuht.nhs.uk/jps/listeriosis__m.htm

2. ELECTRON MICROSCOPY AND CULTURE



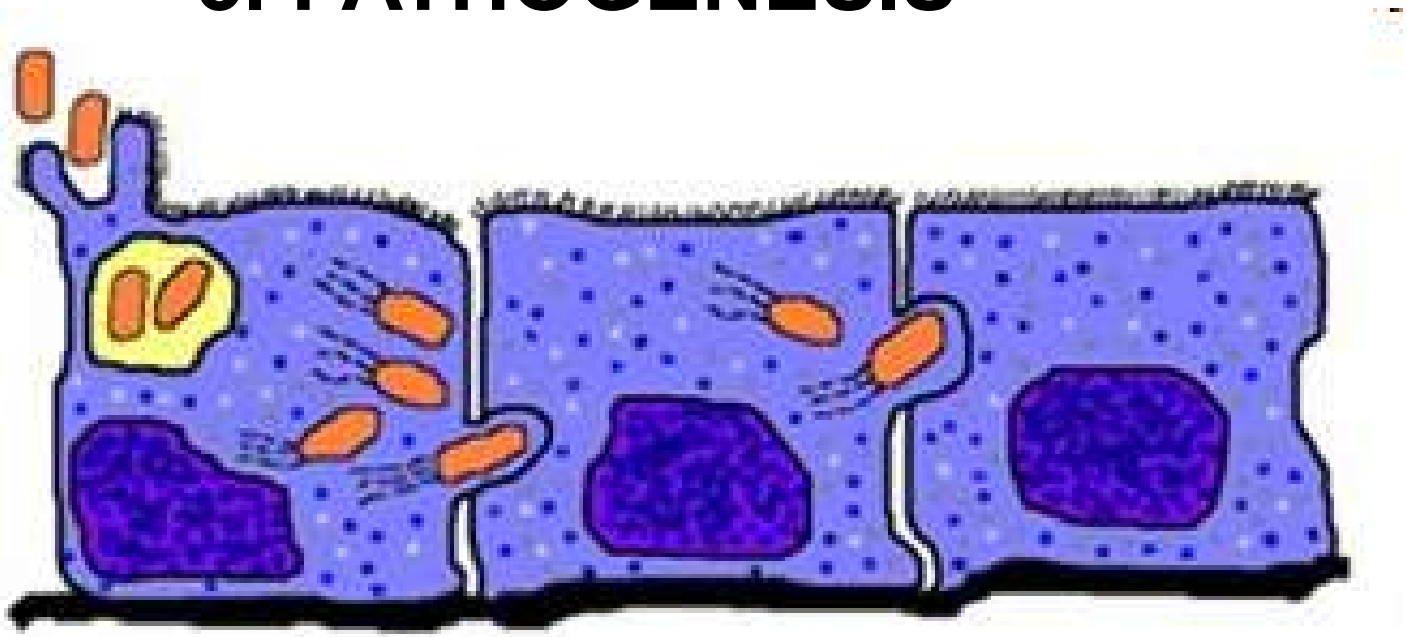
A – electron microscopy, division of a bacterial cell and creation of two dother cells

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

3. PATHOGENESIS



Listeriae infected patient cells (adapted from A.Salyers, Bacterial pathogenesis, 1994)**: Listeriae (brown rods) are engulfed by patient cell (enterocyte) (blue cells). They multiply and move because of a protein actin (dashed lines) and it also shooting them ahead . Source of the actin are the patient cells and listeriae borows it only temporarily.**

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

4. CLINICAL DISEASES IN PATIENTS

• **widely distributed in nature - saprozoonosis**

• human disease is uncommon and is **restricted to several well-defined populations**:

a) **Neonates** (early onset disease - granumlomatous infantiseptica, late-onset d.-after birth, meinigitis, meningoecephalitis), **foetal infection** – miscarriage, stillbirth, preterm labor

b) **the elderly**

c) **pregnant women** (bacteramia, meningitis)

d) **patients with defective cellular immunity**

5. EPIDEMIOLOGY

• isolated in soil, water, and vegetation and from a variety of animals, including humans (low-level gastrointestinal carriage)

• **disease associated with consumption of contaminated food products** (e.g., soft cheese, milk, turkey, raw vegetables [esp. cabbage]) or **transplancental spread from mother to neonate**; sporadic cases and epidemics occur throughout the year but peak in warmer months

• the young, elderly, and pregnant women, as well as patients with defects in cellular immunity, are at increased risk for disease

6. PHYSIOLOGY, STRUCTURE VIRULENCE

* gram-positive shor rods often arranged in pairs resembling enterococci
* **facultative anaerobe**
• **motile at room temperature**, weakly β-hemolytic, and **capable of growth at 4°C** and in high-salt concentrations (useful identification features)
• facultative intracellular pathogen that can avoid antibody-mediated clearance
• virulent strains produce cell **attachment factors (internalins), hemolysins** (**listeriolysin O**, two **phospholipase** Cs), and a protein that mediates actin-directed motility (ActA)
• **growth in contaminated foods in the refrigerator can lead to high concentrations of bacteria !!!**

7. 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*.

7. 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**.

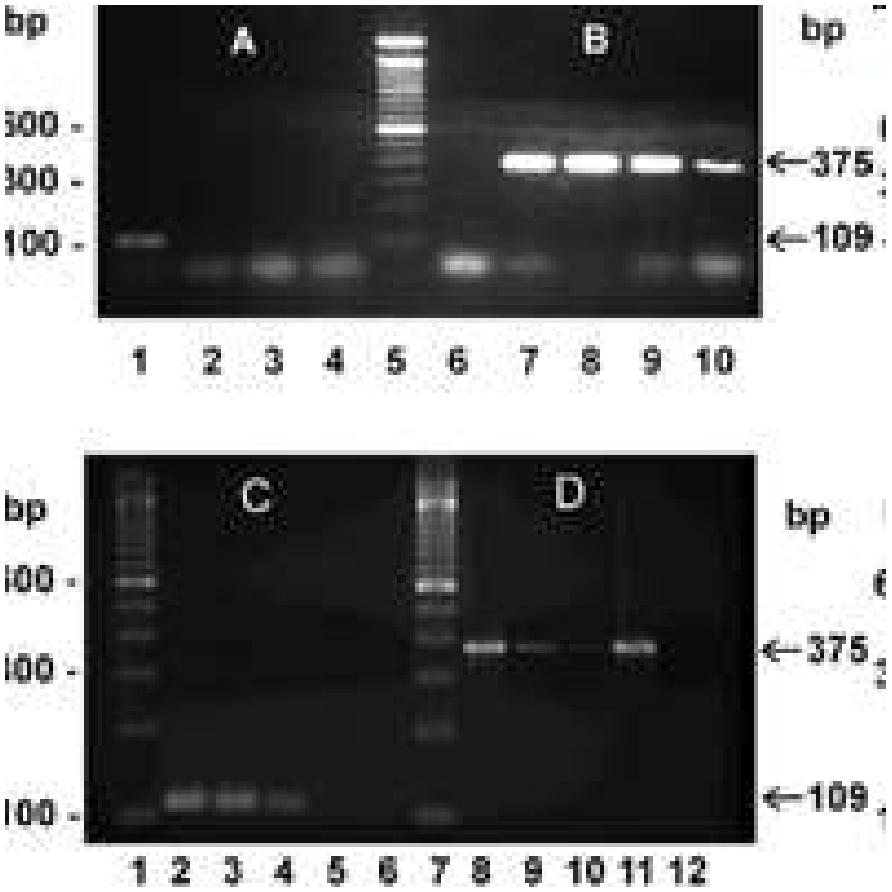
7. LABORATORY DIAGNOSIS

Identification

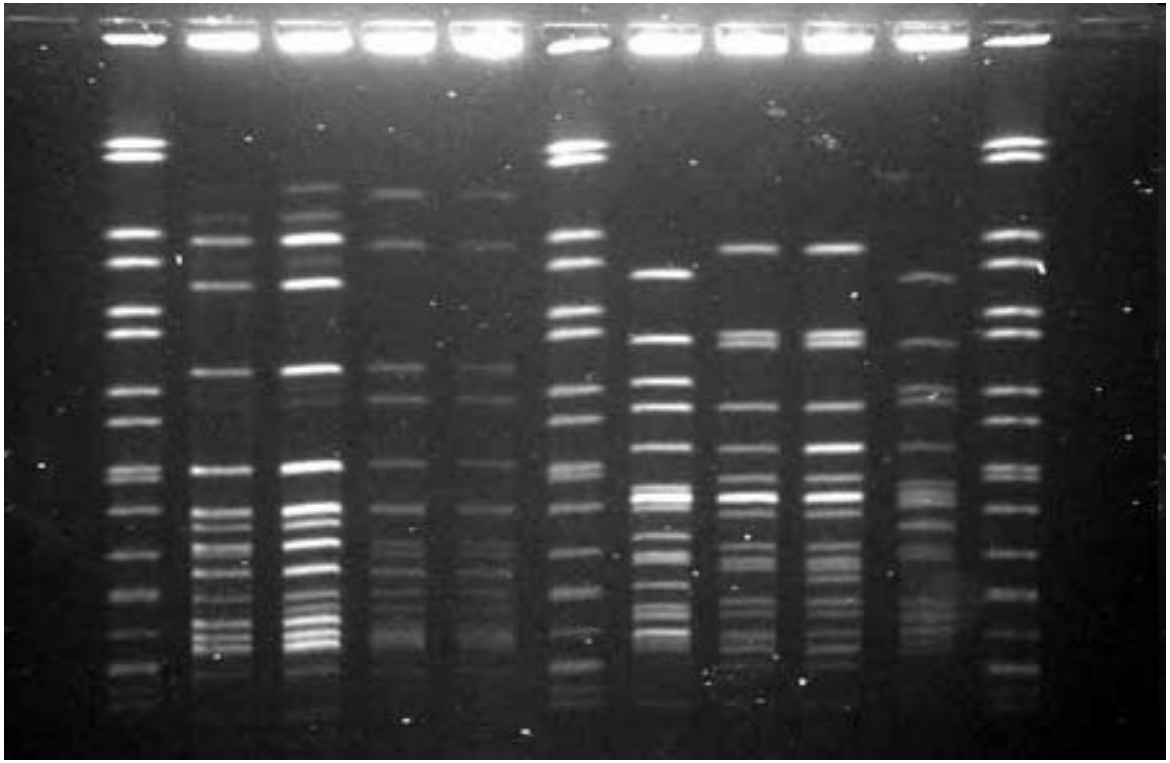
Selected **MALDI TOF** and **serologic tests** are used to identify the pathogen definitively. A total of 13 **serotypes** have been described, with 1/2a, 1/2b, and 4b responsible for most infections in neonates and adults. **Serotyping is generally not useful in epidemiologic investigations**, because **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…)

Detection of specific listerial DNA fragments by PCR



PFGE typing



7. TREATMENT

• currently, **ampicillin**, either **alone or with gentamicin**, is the treatment of choice

• listeriae are **naturally resistant to cephalosporins**.

• Erythromycin can be used in patients allergic to penicillins

• resistance to trimethoprim and the tetracyclines has been observed. Tetracycline resistance was first observed in 1988 and appears to be increasing, in part because of the use of these antibiotics in animal herds.

• Resistance to aminoglycosides has also been reported. Genes for resistance to tetracyclines and aminoglycosides have been found on conjugative plasmids and transposons that originated in enterococci.

8. PREVENTION & CONTROL

• because listeriae are **ubiquitous** and most infections are sporadic, prevention and control are difficult

• **people at high risk of infection should avoid eating raw or partially cooked foods of animal origin, soft cheeses, and unwashed raw vegetables**

• **a vaccine is not available**, and prophylactic antibiotic therapy for high-risk patients has not been evaluated.

Clostridia

(anaerobic spore-forming bacteria)

Anaerobiosis (anaerobic respiration)

* refers to the oxidation of molecules in the absence of oxygen to produce energy, in opposition to aerobic respiration which does use oxygen. Cell respiration is the process of generating ATP through a process of oxidation and where an inorganic molecule is the final electron carrier. Anaerobic respiration processes require another electron acceptor to replace oxygen. Anaerobic respiration is often used interchangeably with fermentation, especially when the glycolytic pathway is used for energy production in a obligate anaerobe cell. They are not synonymous terms. Cellular respiration happens when and organic molecule is the final electron carrier (such as lactic acid, ethanol etc). Anaerobic prokaryotes can generate all of their ATP using an electron transport system and ATP synthase. It is almost as efficient as aerobic respiration but much more efficient than fermentation.

Methods for cultivation of anaerobic bacteria

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**

Tetanus: *Pathogenesis and clinical findings*

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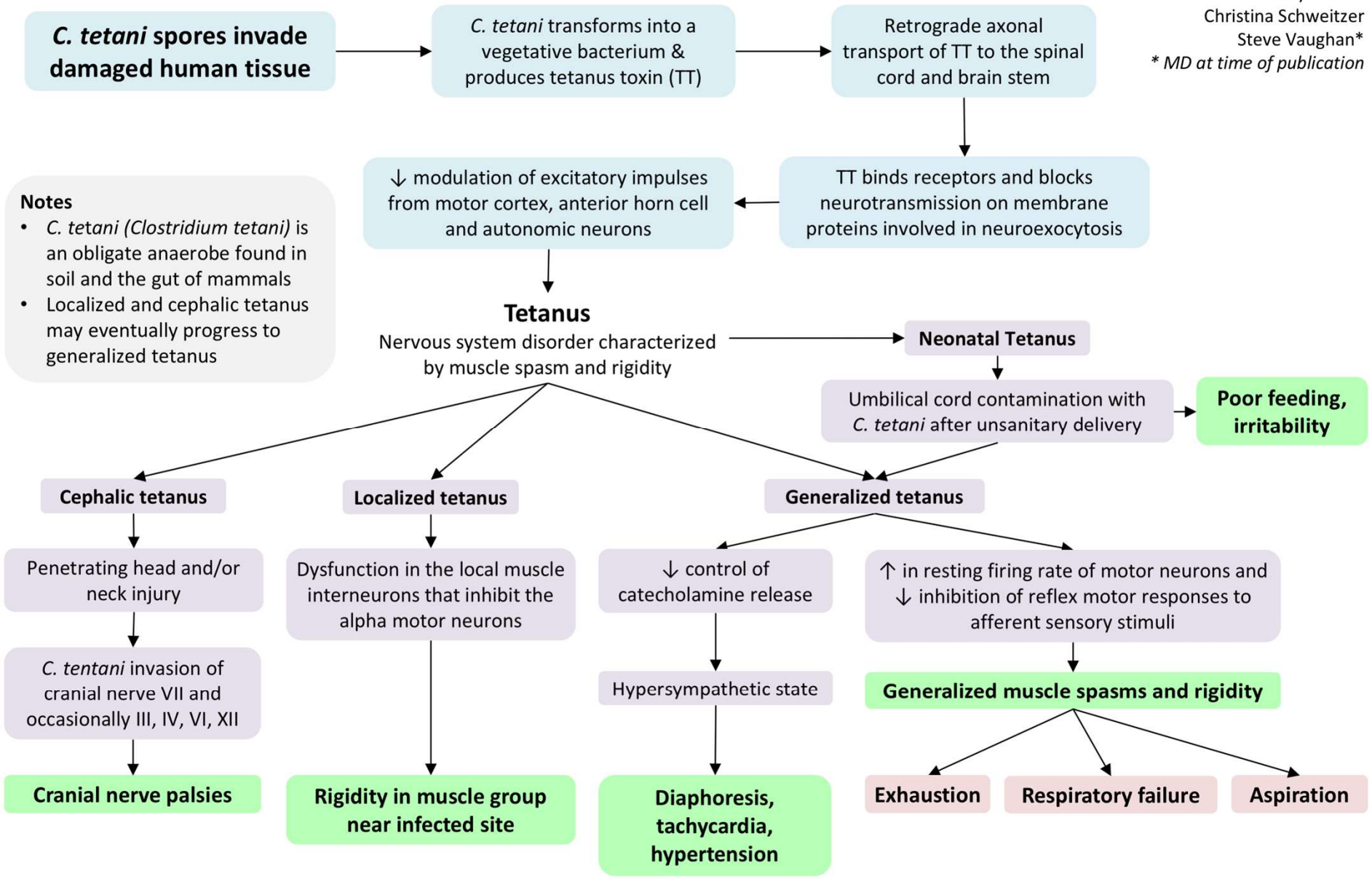

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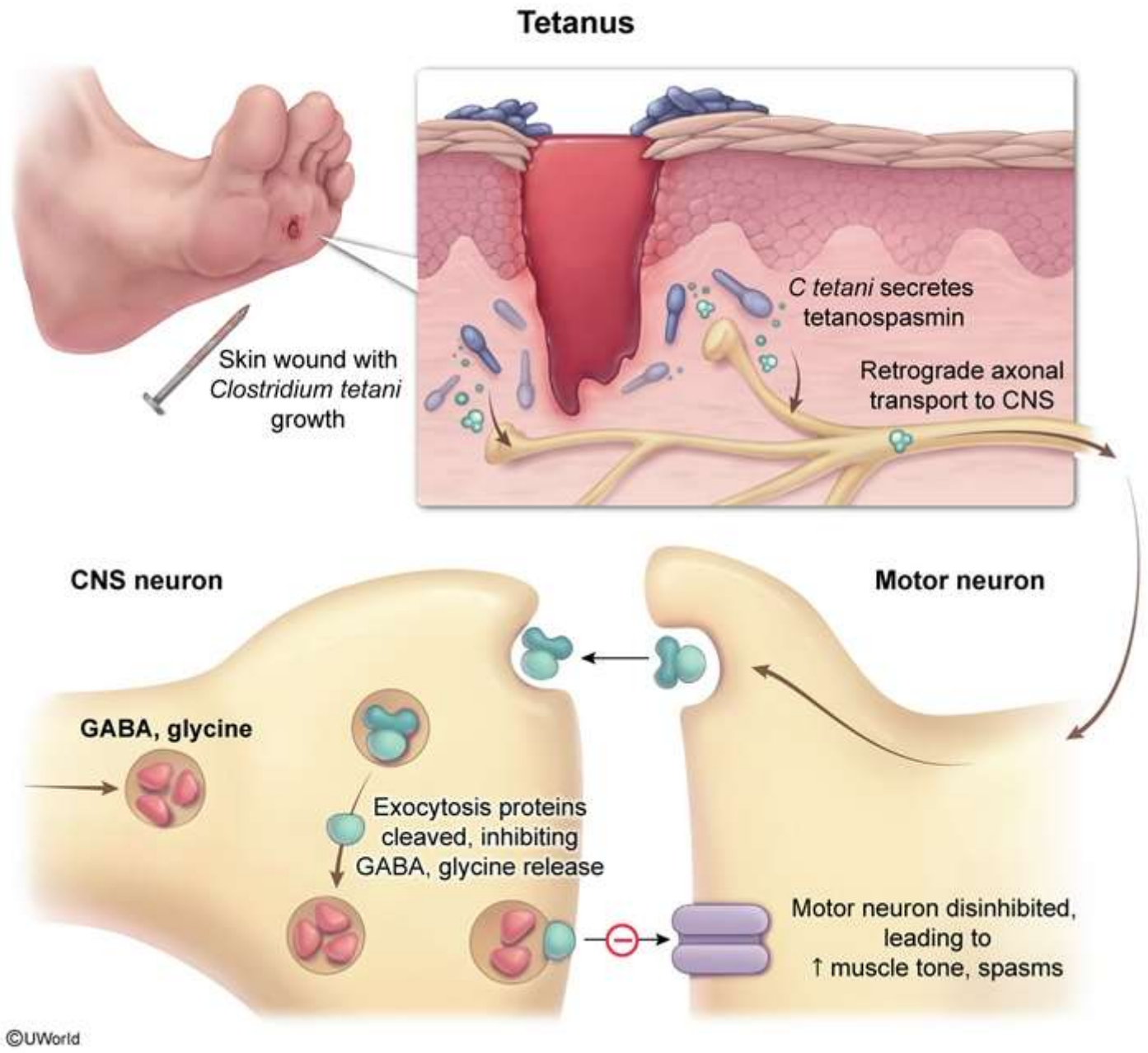
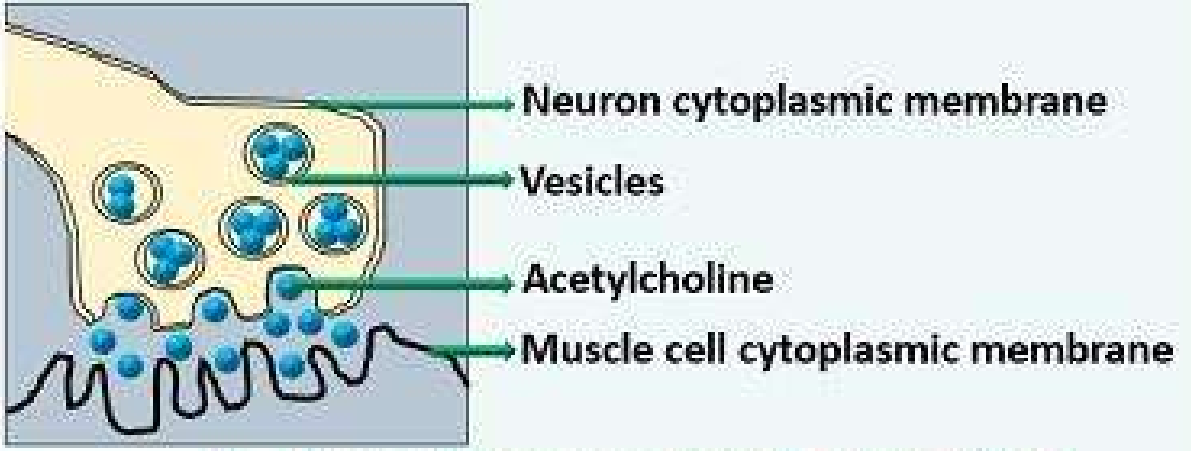


https://medical.uworld.com/library/infectious-diseases/347/tetanus/

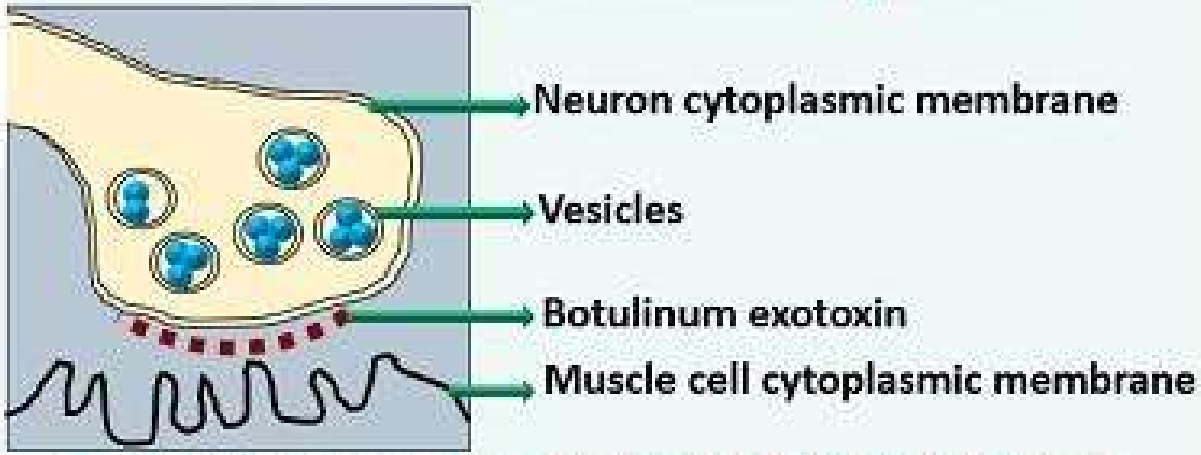
Clostridium botulinum

- **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 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)

Pathogenesis of Clostridium botulinum



1. Normal neuromuscular junction



2. Neuromuscular junction with Botulinum exotoxin

BIOLOGY READER

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)

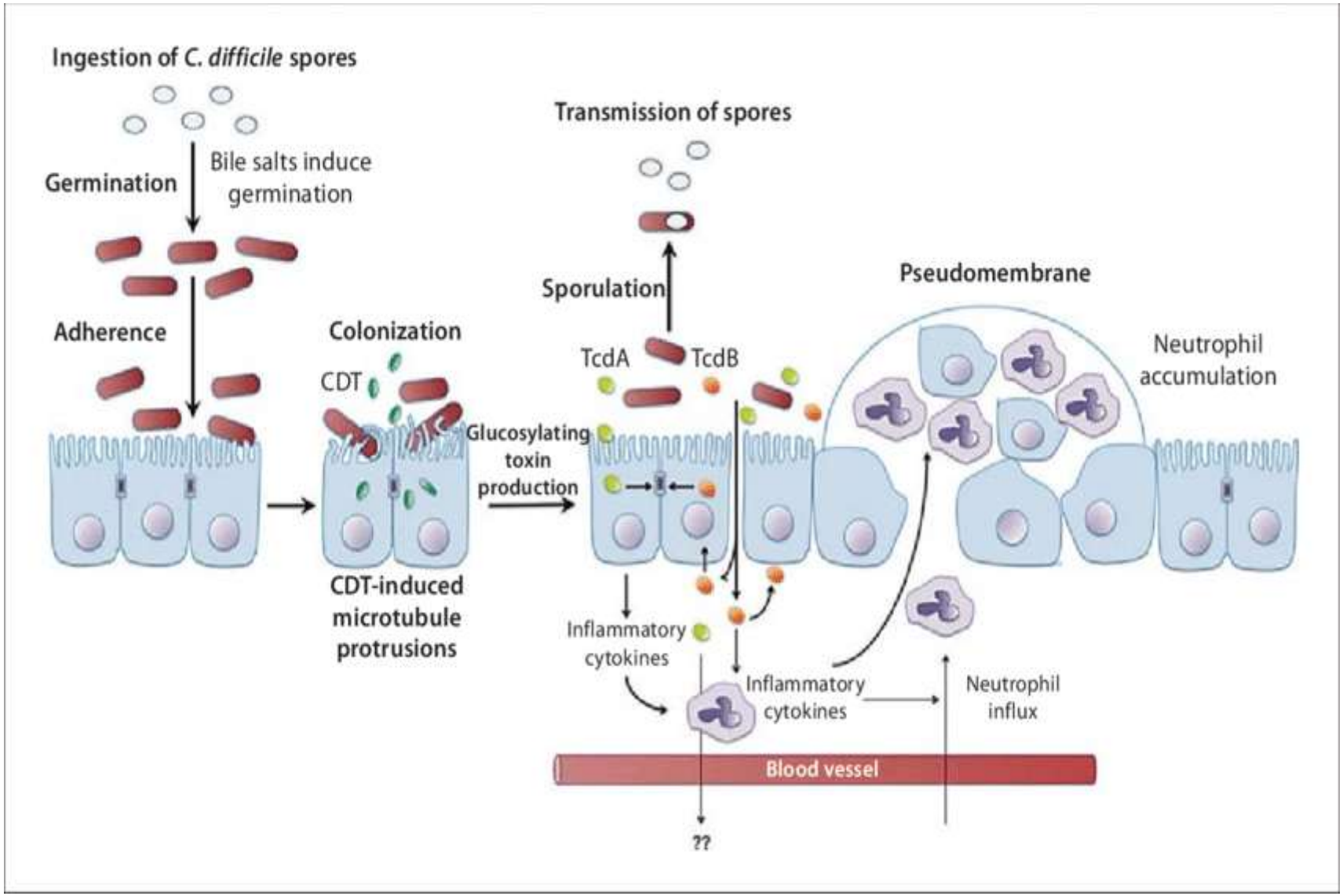
Epidemiology The organism is ubiquitous
* Colonizes the intestines of a small proportion of healthy individuals (<5%)
* Exposure to antibiotics is associated with overgrowth of *C. difficile* and subsequent disease (endogenous infection)
* Spores can be detected in hospital rooms of infected patients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

Pathogenesis of *C. diffcile* infections



https://www.researchgate.net/figure/Pathogenesis-of-Clostridium-difficile-When-C-difficile-spores-are-ingested-they-are_fig1_221740600

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

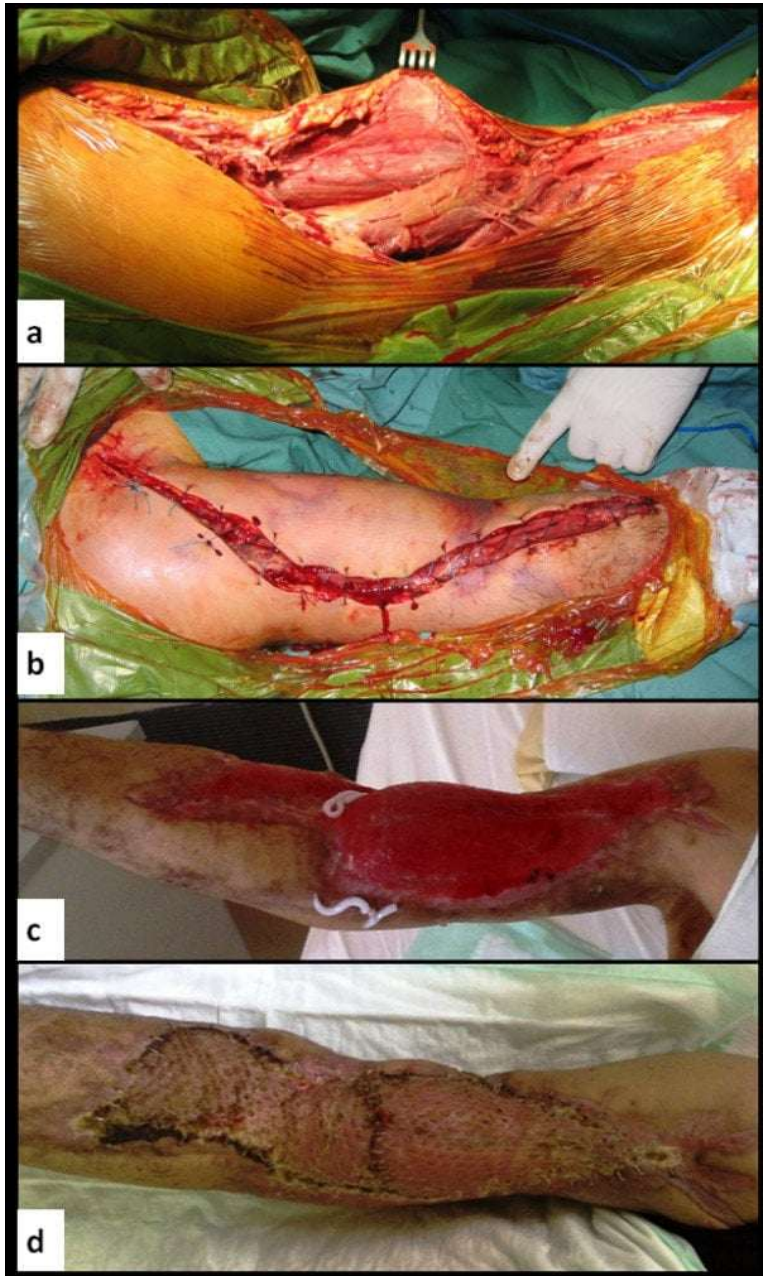
Ubiquitous; present in soil, water, and intestinal tract of humans and animals
Type A is responsible for most human infections
Disease follows exogenous or endogenous exposure

Diagnosis

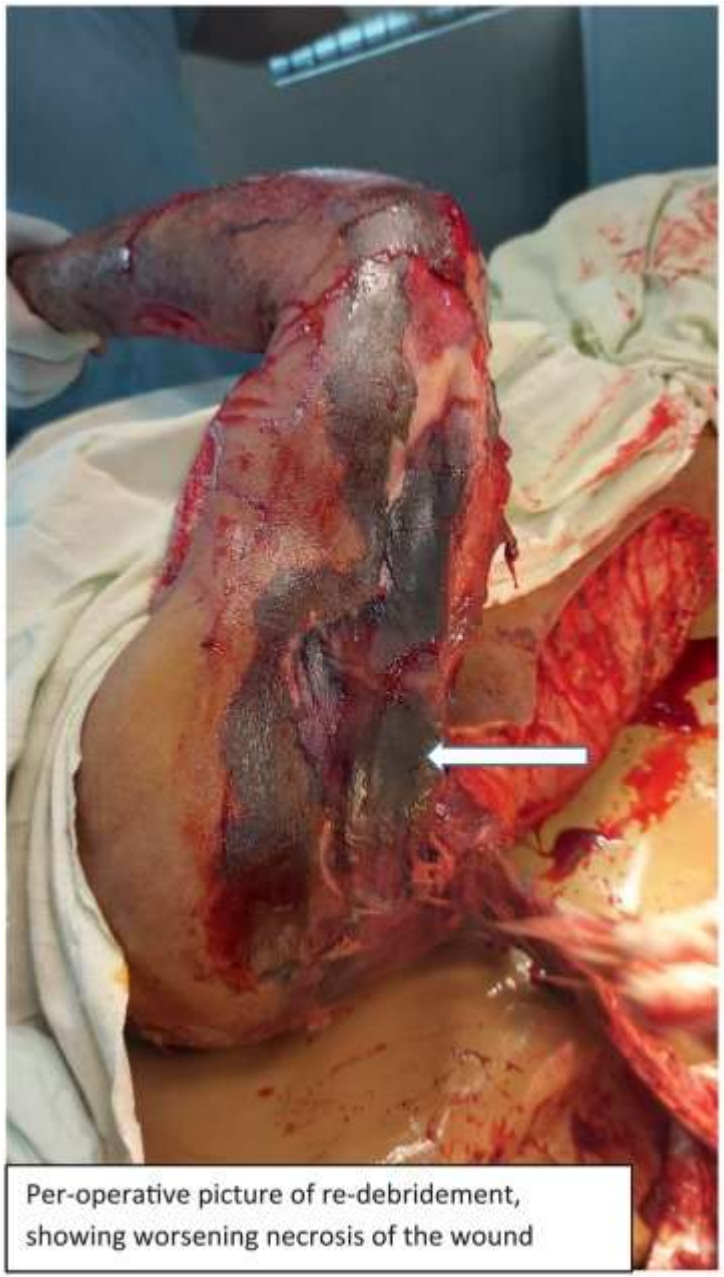
Characteristic forms seen on Gram stain, grows rapidly in culture, PCR from affected tissue

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

Clostridia - Clinical Summaries

Clostridium tetani

Generalized tetanus: Generalized musculature spasms and involvement of the autonomic nervous system in severe disease (e.g., cardiac arrhythmias, fluctuations in blood pressure, profound sweating, dehydration)

Localized tetanus: Musculature spasms restricted to localized area of primary infection

Neonatal tetanus: Neonatal infection primarily involving the umbilical stump; very high mortality

Clostridium botulinum

Foodborne botulism: Initial presentation of blurred vision, dry mouth, constipation, and abdominal pain; progresses to bilateral descending weakness of the peripheral muscles with flaccid paralysis

Infant botulism: Initially nonspecific symptoms (e.g., constipation, weak cry, failure to thrieve) that progress to flaccid paralysis and respiratory arrest

Wound botulism: Clinical presentation same as with foodborne disease, although the incubation period is longer and fewer gastrointestinal symptoms

Inhalation botulism: Inhalation exposure to botulinum toxin would be expected to have a rapid onset of symptoms (flaccid paralysis, pulmonary failure) and high mortality

Clostridia - Clinical Summaries

Clostridium perfringens

Soft-tissue infections

Cellulitis: Localized edema and erythema with gas formation in the soft tissue; generally nonpainful

Suppurative myositis: Accumulation of pus (suppuration) in the muscle planes without muscle necrosis or systemic symptoms

Myonecrosis: Painful, rapid destruction of muscle tissue; systemic spread with high mortality

Gastroenteritis

Food poisoning: Rapid onset of abdominal cramps and watery diarrhea with no fever, nausea, or vomiting; short, self-limited duration

Necrotizing enteritis: Acute, necrotizing destruction of jejunum with abdominal pain, vomiting, bloody diarrhea, and peritonitis

Clostridium difficile

Antibiotic-associated diarrhea: Acute diarrhea generally developing 5 to 10 days after initiation of antibiotic treatment (particularly clindamycin, penicillins, and cephalosporins); may be brief and self-limited or more protracted

Pseudomembranous colitis: Most severe form of *C. difficile* disease with profuse diarrhea, abdominal cramping, and fever; whitish plaques (pseudomembranes) over intact colonic tissue seen on colonoscopy, **toxic megacolon** – fatal complication

DIAGNOSIS OF CLOSTRIDIAL INFECTIONS

(except *C. difficile*)

Clinical signs include:

- **foul-smelling discharge (becuase of the end product of anaerobic metabolism – short-chain fatty-acids)**
- **infection in proximity to a mucosal surface (anaerobes are part of the normal flora)**
- **gas in tissue (production of CO_2 and H_2)**
- **negative aerobic cultures**

The place of molecular genetic methods in the diagnostics of anaerobic bacteria

- diverse group of bacteria
- specimens yielding anaerobic bacteria commonly contain several organisms and often very complex mixtures of aerobic and anaerobic bacteria, considerable time may elapse before the final report.
- Species definition based on phenotypic features is often time-consuming and is not always easy to carry out.
- Molecular genetic methods may help in the everyday clinical microbiological practice (such as 16S rRNA PCR-RFLP profile determination), which can help to distinguish species
- Some anaerobic bacteria are extremely slow growing or not cultivable at all but detectable by molecular methods which also demonstrated the spread of specific resistance genes among the most important anaerobic bacteria.
- Molecular methods (a search for toxin genes and ribotyping) may promote a better understanding of the pathogenic features of some anaerobic infections, such as the nosocomial diarrhoea caused by *C. difficile* and its spread in the hospital environment and the community.