Pharmaceutical microbiology

Chapter 6

Gram-Positive Bacilli:

Aerobic Non–Spore-Forming

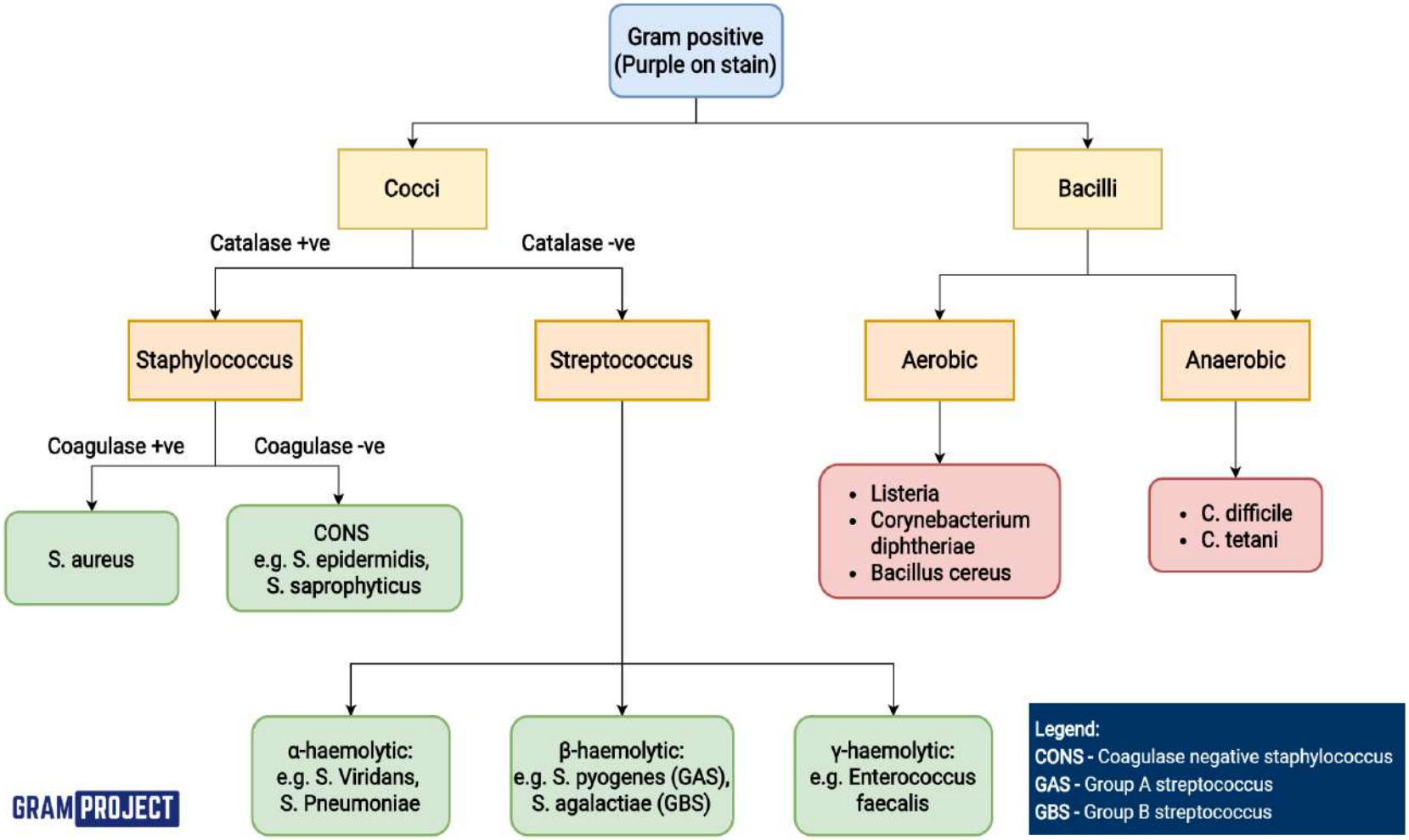
Corynebacterium, Listeria

Dr. Mohammed Hussein Taleb

Lecture 13

08/11/2020

Gram Positive Bacteria



GRAM PROJECT

Among the **aerobic Actinomycetes** are significant pathogens such as ***Corynebacterium diphtheriae***, an organism that produces a **powerful exotoxin that causes diphtheria in humans**,

- ***Listeria monocytogenes*** are **primarily found in animals and occasionally cause severe disease in humans.**
- The **medically important genera of gram-positive bacilli and include some spore-forming and anaerobic genera.**

Corynebacterium Diphtheriae

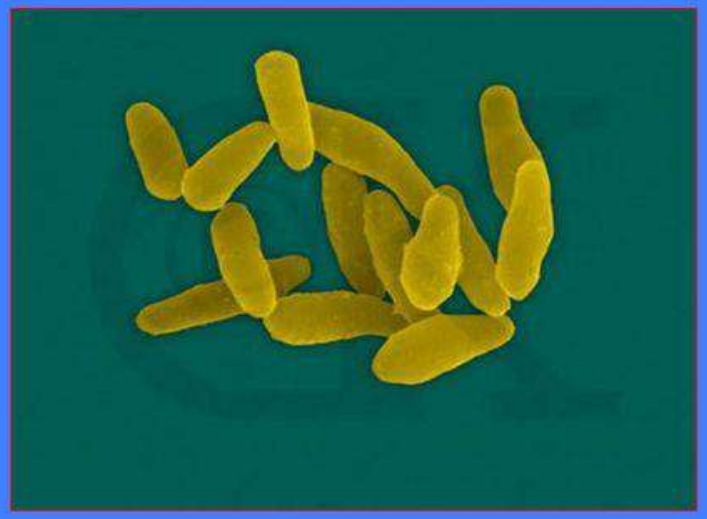
- ## Morphology and Identification

- **Corynebacteria** are **0.5–1 µm** in diameter and several micrometers long. Characteristically, they possess irregular swellings at one end that give them the **"club-shaped" appearance** .

- *C. diphtheriae* **and other corynebacteria grow aerobically on most ordinary laboratory media.** **On Loeffler serum medium**, Corynebacteria grow much more readily than other respiratory organisms

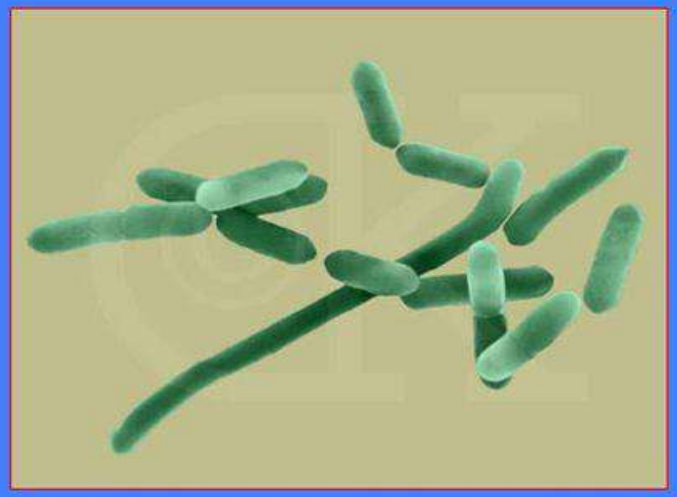
Corynebacterium & Listeria

Two aerobic Gram-positive bacilli



C. diphtheriae

L. monocytogenes



Pathogenesis

- The **principal human pathogen of the genus Corynebacterium is *C.diphtheriae*, the causative agent of respiratory or cutaneous diphtheria.**
- In nature, C diphtheriae occurs in the respiratory tract, in wounds, or on the skin of infected persons or normal carriers. **It is spread by droplets or by contact to susceptible individuals; the bacilli then grow on mucous membranes or in skin abrasions, and those that are toxigenic start producing toxin.**
- All **toxigenic *C. diphtheriae* are capable of elaborating the same disease-producing exotoxin.**

Diphtheria toxin

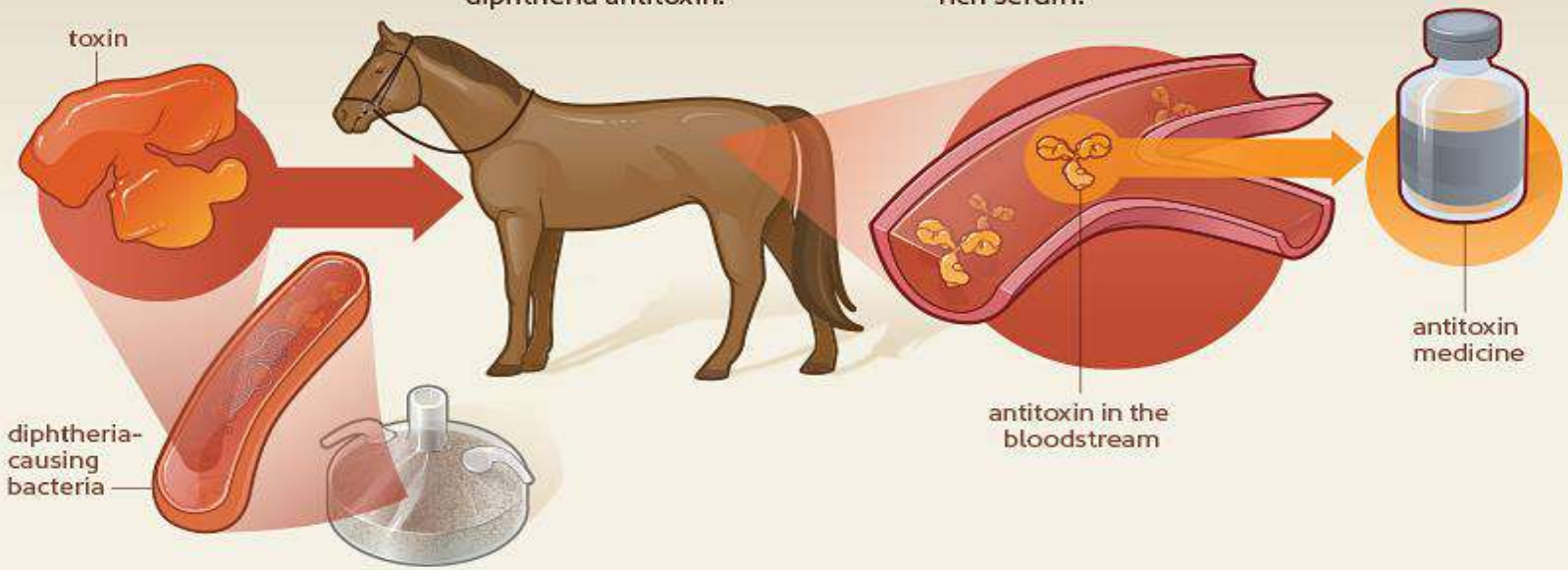
- **Diphtheria toxin** a heat-labile polypeptide (molecular weight [MW], 62,000) **that can be lethal in a dose of 0.1 μg/kg**.
- It is assumed that the **abrupt arrest of protein synthesis** **is responsible for the necrotizing and neurotoxic effects of diphtheria toxin.**
- **An exotoxin with a similar mode of action can be produced by strains of *Pseudomonas aeruginosa.***

How did they make diphtheria antitoxin?

HOW DID THEY MAKE DIPHTHERIA ANTITOXIN?

SCIENTISTS LEARNED TO HARNESS THE IMMUNE SYSTEMS of some animals to produce antitoxin serums to use as medicines. Diphtheria antitoxin was one of these medicines. Doctors used diphtheria antitoxin to treat and prevent diphtheria, an often deadly childhood disease.

1. Scientists grow diphtheria-causing bacteria in the laboratory and harvest its toxin.
2. Next, researchers inject horses with the diphtheria toxin. As an immune response, the animals' blood produces diphtheria antitoxin.
3. Scientists collect blood from the horses and separate out the antitoxin rich serum.
4. Then, researchers purify the antitoxin serum for use as a medicine for people.



Pathology

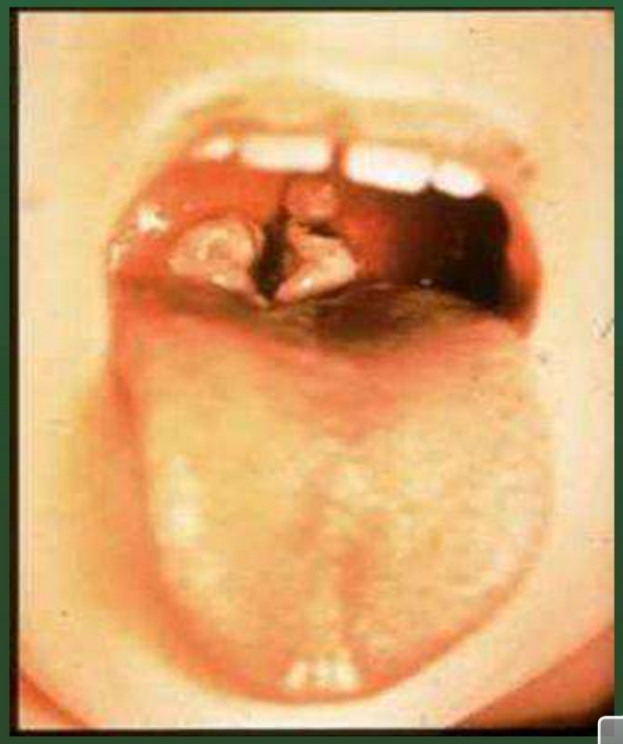
- Diphtheria toxin is absorbed into the mucous membranes and causes destruction of epithelium and a superficial inflammatory response.
- The necrotic epithelium becomes embedded in exuding fibrin and red and white cells, so that a grayish "pseudomembrane" is formed—commonly over the tonsils, pharynx, or larynx.

- Any attempt to remove the pseudomembrane exposes and tears the capillaries and thus results in bleeding.
- The regional lymph nodes in the neck enlarge, and there may be marked edema of the entire neck, with distortion of the airway, often referred to as "bull neck" clinically. The diphtheria bacilli within the membrane continue to produce toxin actively.

- The small amount of toxin that is absorbed during skin infection promotes development of antitoxin antibodies.
- The "virulence" of diphtheria bacilli is attributable to their
- capacity for establishing infection,
- growing rapidly, and
- then quickly elaborating toxin that is effectively absorbed.

Diseases and Pathogenesis of Disease Caused by *Corynebacterium diphtheriae*

- Normally found in the throats of healthy carriers.
- Organism can cause:
  - Diphtheria which starts as a local infection of the mucous membrane causing a membranous pharyngitis
  - Cutaneous diphtheria which necrotic lesions with occasional formation of a local pseudomembrane occur.
    - More common in tropical and subtropical areas.
- Toxigenic strains cause severe disease.
  - Secretes a potent heat-labile polypeptide exotoxin that cause diphtheria.
    - Exotoxin causes diphtheria, not the organism.
- In order for toxin production to occur:
  - Bacterium must be lysogenized by a bacteriophage that carries the tox gene.
  - Aerobic conditions, alkaline pH of 7.8-8.0, and a low iron level.



Treatment

- The **treatment of diphtheria rests largely on** rapid suppression of toxin-producing bacteria by antimicrobial drugs and the early administration of specific antitoxin against the toxin formed by the organisms at their site of entry and multiplication.
- **Diphtheria antitoxin is produced in various animals (horses, sheep, goats, and rabbits) by the repeated injection of purified and concentrated toxoid.**
- **Treatment with antitoxin is mandatory when** there is strong clinical suspicion of diphtheria. **Antimicrobial drugs (penicillin, erythromycin) inhibit the growth of diphtheria bacilli.**
- Although **these drugs have virtually no effect on the disease process, they arrest toxin production.**
- **Active immunization in childhood with diphtheria toxoid yields antitoxin levels that are generally adequate until adulthood.**

Listeria Monocytogenes

Listeria Monocytogenes

- There are several species in the genus *Listeria*. Of these, *L monocytogenes* is important as a cause of a wide spectrum of disease in animals and humans.
- Therefore, it is able to overcome food preservation and safety barriers, making it an important foodborne pathogen.

Morphology and Identification , Culture and Growth Characteristics

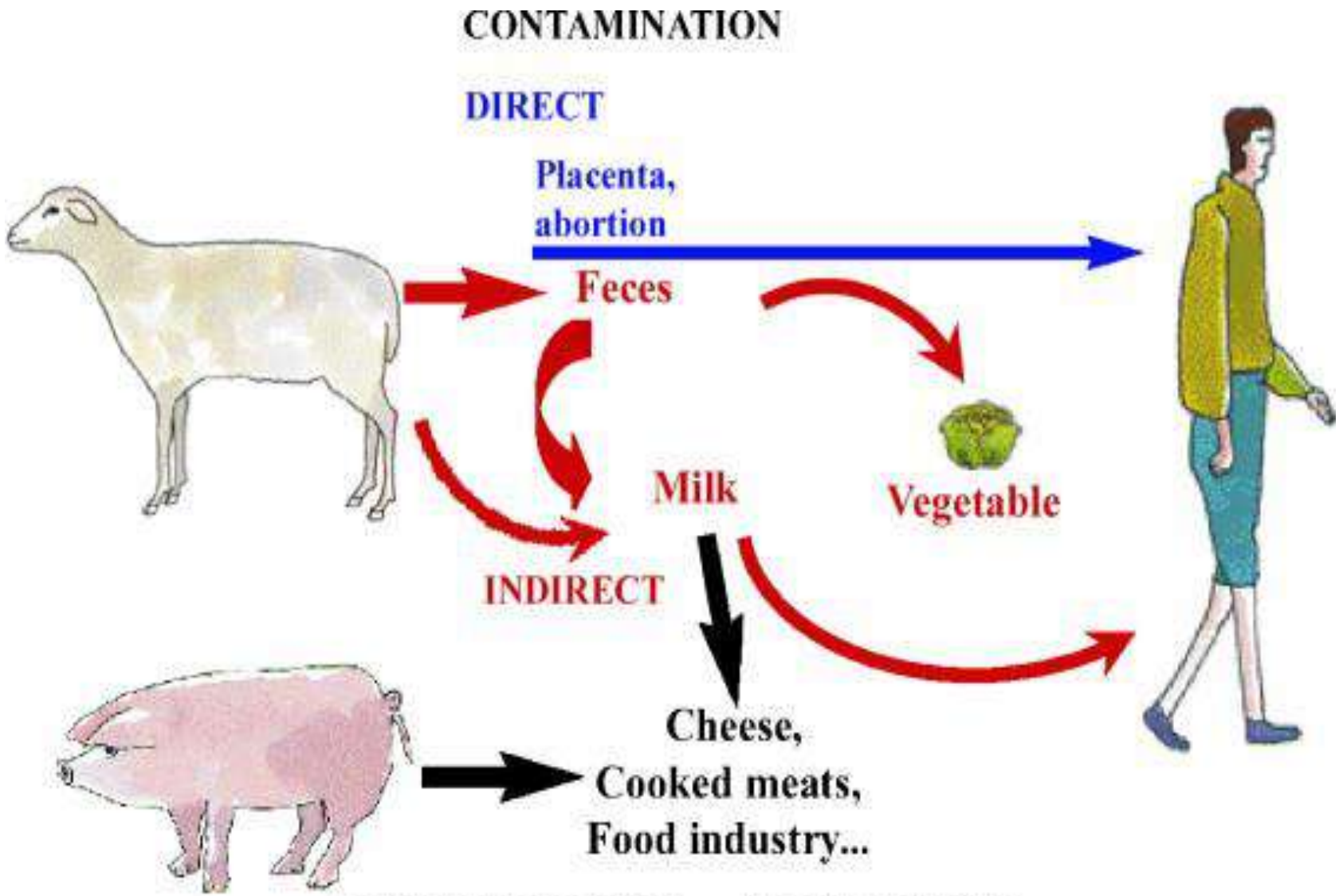
L. monocytogenes is a short, gram-positive, non–spore-forming rod .

The organism is a facultative anaerobe and is catalase positive,

L monocytogenes is capable of growing and surviving over a wide range of environmental conditions. It can survive at refrigerator temperatures (4°C), under conditions of low pH and high salt conditions.

Pathogenesis and Immunity

- *L. monocytogenes* enters the body through the gastrointestinal tract after ingestion of contaminated foods such as cheese or vegetables. The organism has several adhesin proteins (Ami, Fbp A, and flagellin proteins) that facilitate bacterial binding to the host cells and that contribute to virulence.
- It has cell wall surface proteins called internalins A and B that interact with E-cadherin, a receptor on epithelial cells, promoting phagocytosis into the epithelial cells. After phagocytosis, the bacterium is enclosed in a phagolysosome, where the low pH activates the bacterium to produce listeriolysin O.



LISTERIOSIS = ZOONOSIS

LISTERIOSIS

What is it?

Listeriosis is **food poisoning** caused by eating foods contaminated with the **Listeria monocytogenes bacteria.**

Who's at risk?

- Pregnant women
- Children
- Elderly
- People with weakened immune systems

Symptoms

Fever
Muscle aches
Nausea or diarrhoea
Headache
Confusion
Convulsions

Source of outbreak

Enterprise Foods factory in Polokwane

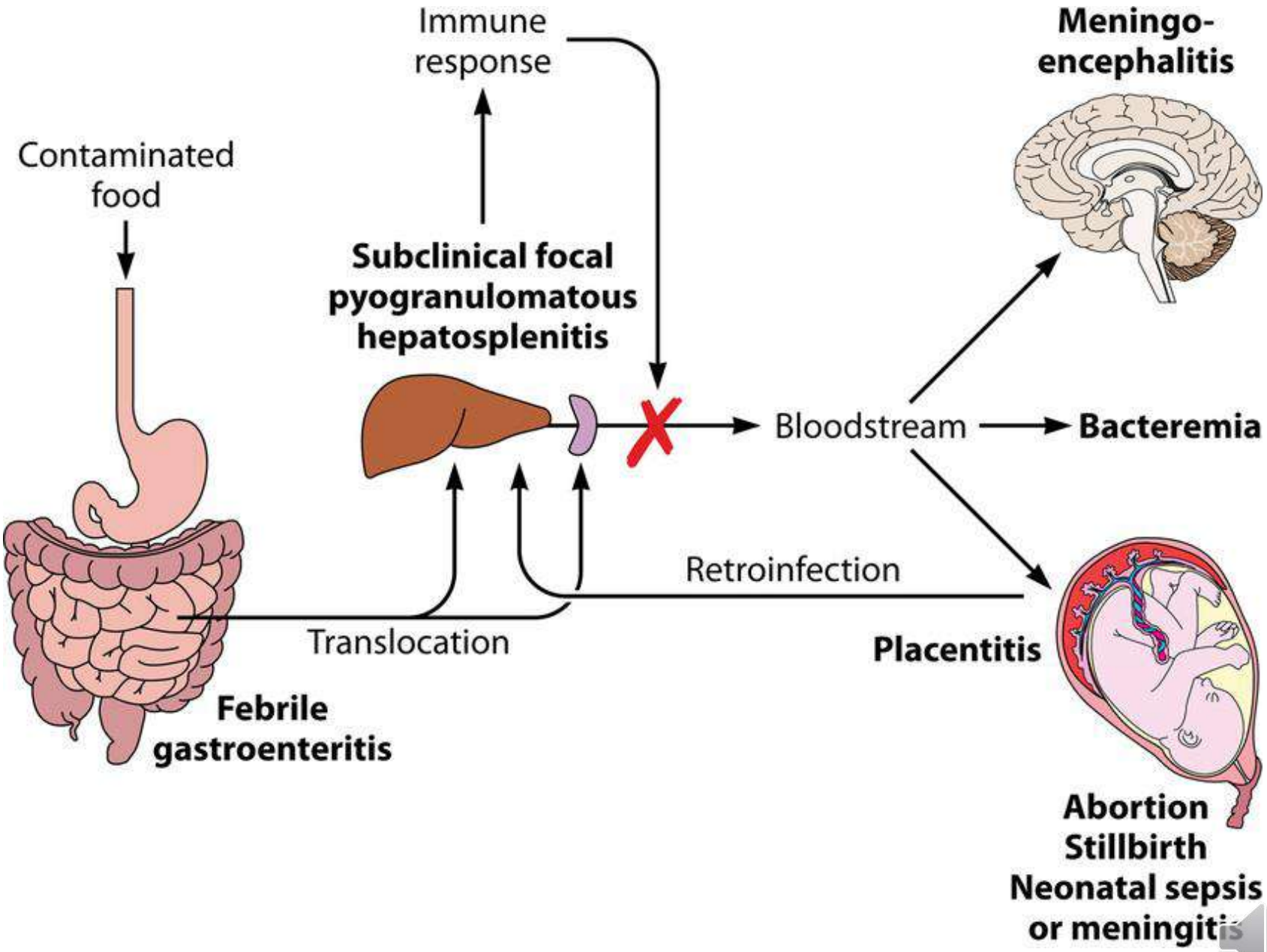
Foods to avoid

Ready-to-eat cold meat products including

Polony

Viennas and other sausages

Cold meats


Immune response
Meningo-encephalitis
Contaminated food
Subclinical focal pyogranulomatous hepatosplenitis
Bloodstream
Bacteremia
Retroinfection
Translocation
Placentitis
Febrile gastroenteritis
Abortion
Stillbirth
Neonatal sepsis or meningitis

Treatment

- Many antimicrobial drugs inhibit *Listeria* species in vitro. Clinical cures have been obtained with ampicillin, erythromycin, or intravenous trimethoprim–sulfamethoxazole.
- Cephalosporins and fluoroquinol ones are not active against *L monocytogenes.*
- Ampicillin plus gentamicin is often recommended for therapy, but gentamicin does not enter host cells and may not help treat the Listeria infection.
- Trimethoprim–sulfamethoxazole is the drug of choice for central nervous system infections in patients who are allergic to penicillin.

Pharmaceutical microbiology

Chapter 7

Gram-Positive Bacilli:

Aerobic –Spore-Forming

Bacillus Species

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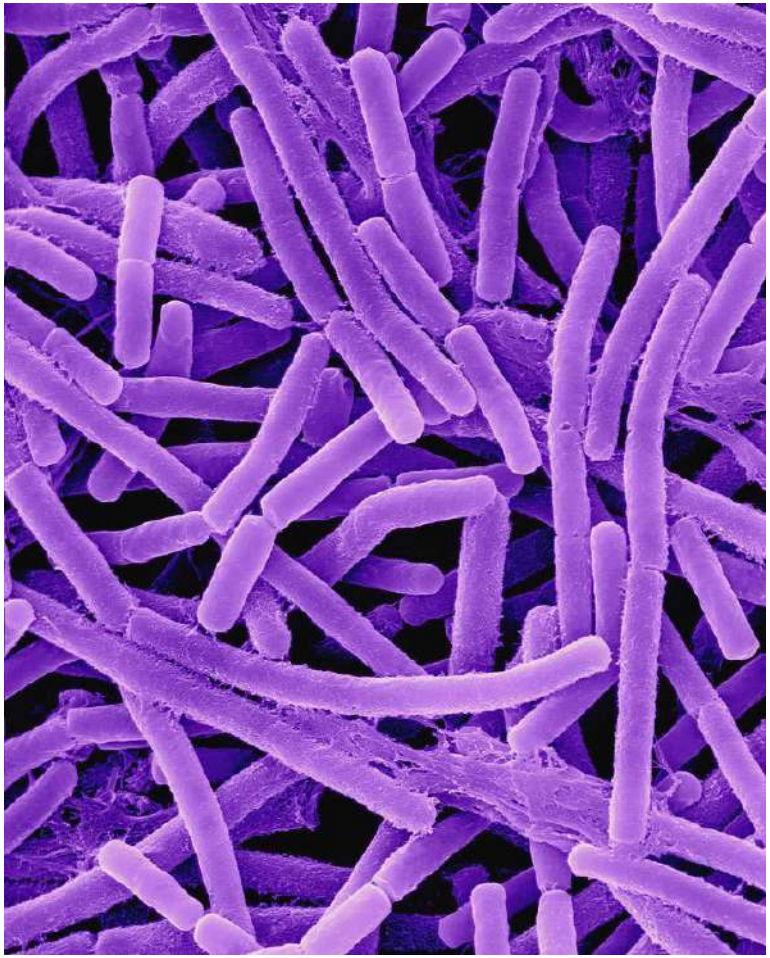
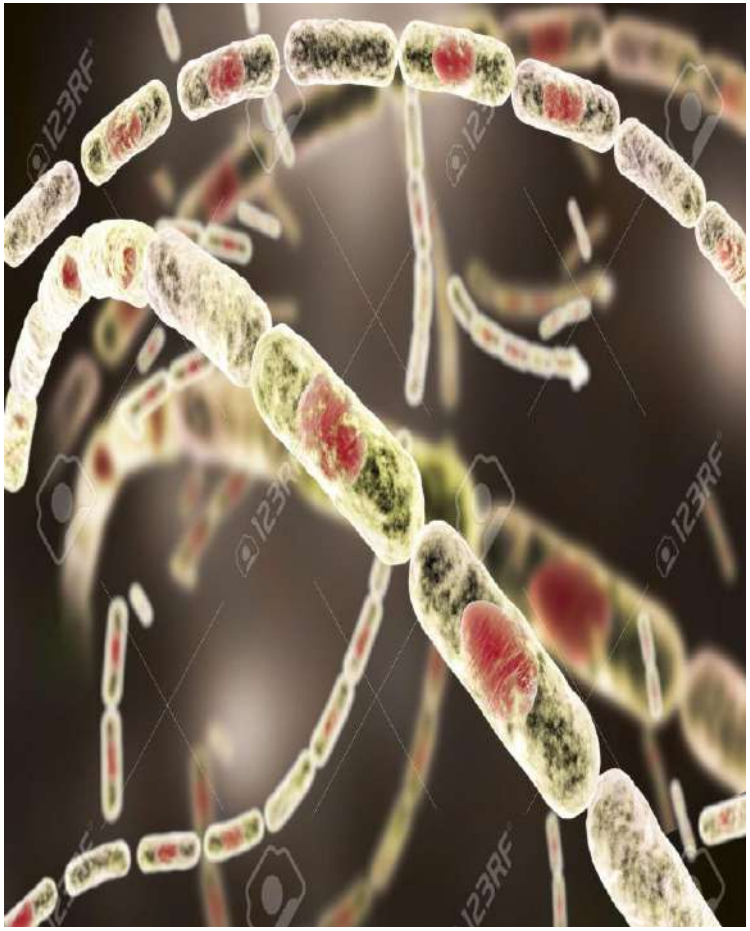
Lecture 14

12/11/2020

- The gram-positive spore-forming bacilli are the *Bacillus* and *Clostridium* species.
- These bacilli because they form spores, they can survive in the environment for many years.
- Whereas the *Bacillus* species are aerobes, the *Clostridium* species are anaerobes.
- Anthrax remains an important disease of animals and occasionally of humans.
- Because of its potent toxins, *B. anthracis* is a major potential agent of bioterrorism and biologic warfare.

Bacillus anthracis

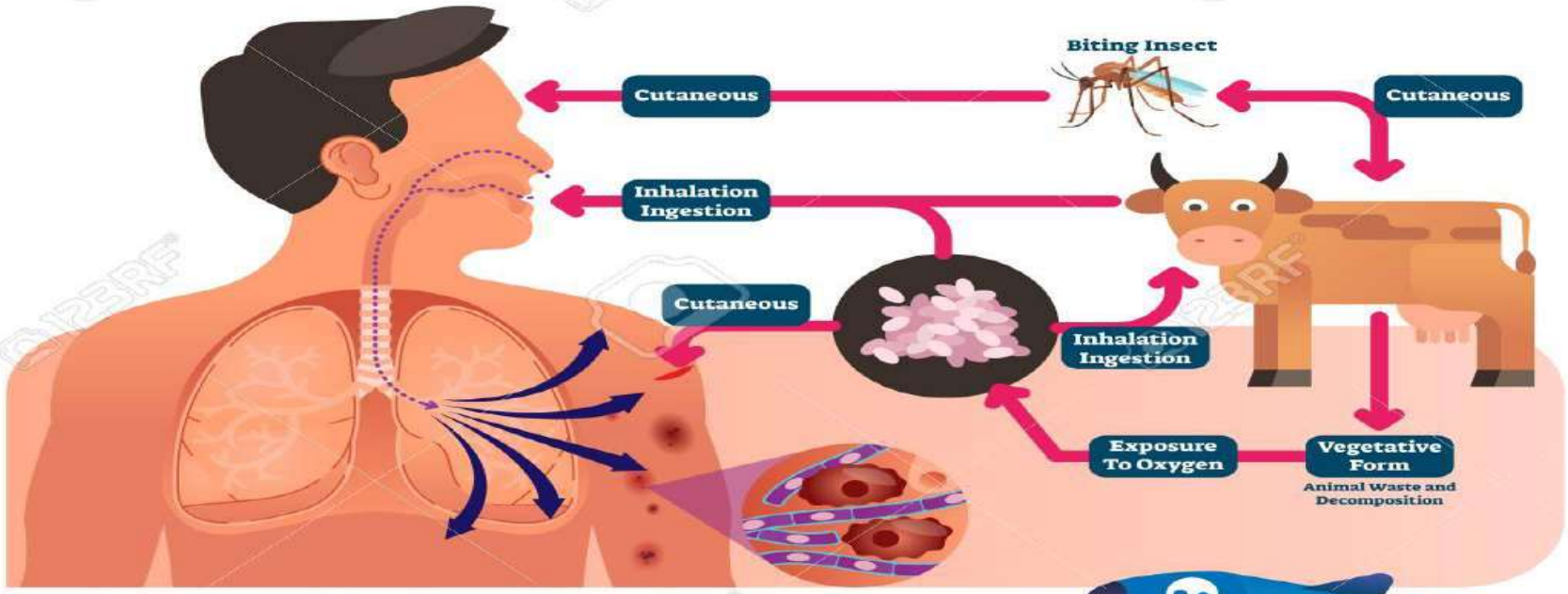




Bacillus anthracis

- Gram Positive
- Rod shaped
- Produces lethal poison
  - Poisons leave the cell and affect the area around it
- Lives in the soil
  - Alkaline rich soil is a good place to grow
- Contain spores
- It is the only bacterium known to synthesize a protein capsule
- Are highly resilient bacteria that can survive harsh temperatures, chemicals, or low nutrient environments

ANTHRAX



Anthrax Spores Have Bee
Used as a Biological Warfare Weapon

Bacillus Species

- *B. cereus* can grow in foods and cause food poisoning by producing either an enterotoxin (diarrhea) or an emetic toxin (vomiting). Both *B. cereus* and *B thuringiensis* occasionally produce disease in immunocompromised humans (eg, meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastroenteritis).
- ***B. anthracis,*** **which causes anthrax, is the principal pathogen of the genus.**

Morphology and identification

A. Typical Organisms

The typical cells, measuring 1 × 3–4 μm, have square ends and are arranged in long chains; spores are located in the center of the nonmotile bacilli.

B. growth Characteristics

The saprophytic bacilli use simple sources of nitrogen and carbon for energy and growth. **The spores are resistant to environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods, and persist for years in dry earth. Animal products contaminated with anthrax spores (eg, hides, bristles, hair, wool, bone) can be sterilized by autoclaving.**

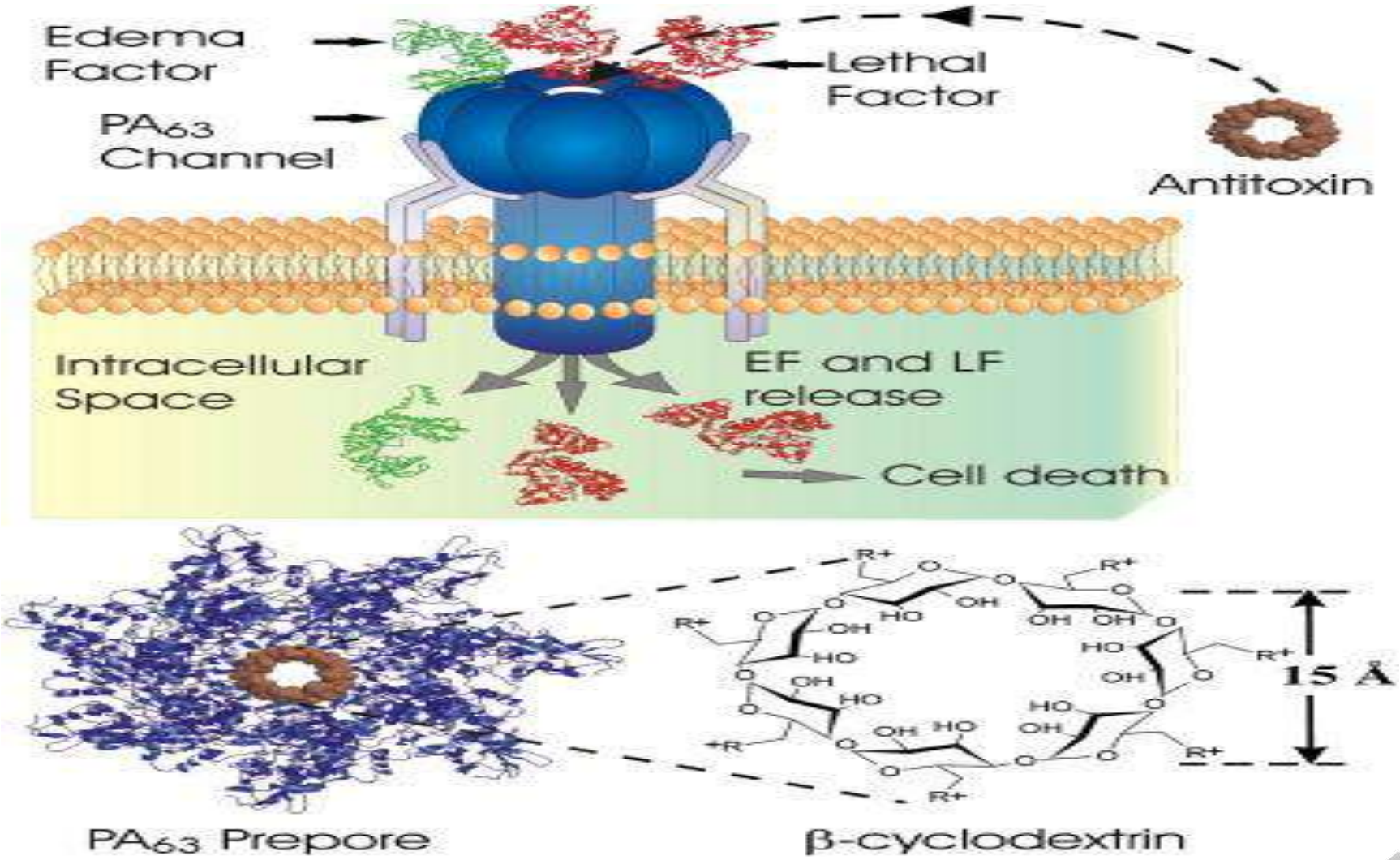
Bacillus Anthracis

•Pathogenesis

- Anthrax is primarily a disease of herbivores—goats, sheep, cattle, horses, and so on; other animals (eg, rats) are relatively resistant to the infection. Humans become infected incidentally by contact with infected animals or their products.
- In animals, the portal of entry is the mouth and the gastrointestinal tract. Spores from contaminated soil find easy access when ingested with spiny or irritating vegetation.

- In humans, the infection is usually acquired by the entry of spores through injured skin (cutaneous anthrax) or rarely the mucous membranes (gastrointestinal anthrax) or by inhalation of spores into the lung (inhalation anthrax).
- The spores germinate in the tissue at the site of entry, and growth of the vegetative organisms results in formation of a gelatinous edema and congestion.

Anthrax exotoxin mechanism



- Anthrax toxins are made up of three proteins, protective antigen (PA), edema factor (EF), and lethal factor (LF).

- PA binds to specific cell receptors, and after proteolytic activation, it forms a membrane channel that mediates entry of EF and LF into the cell.

- EF is an adenylate cyclase (AC); with PA (EF +PA) , it forms a toxin known as **edema toxin.**
- LF + PA form lethal toxin, which is a major virulence factor and cause of death

Clinical Findings

In humans, approximately 95% of cases are cutaneous anthrax

- 5% are inhalation.
- Gastrointestinal anthrax is very rare; when people have eaten meat from infected animals.
- Cutaneous anthrax generally occurs on exposed surfaces of the arms or hands followed in frequency by the face and neck.
- In as many as 20% of patients, cutaneous anthrax can lead to sepsis, the consequences of systemic infection—including meningitis—and death.

The fatality rate in inhalation anthrax is high in the setting of known exposure; it is higher when the diagnosis is not initially suspected.

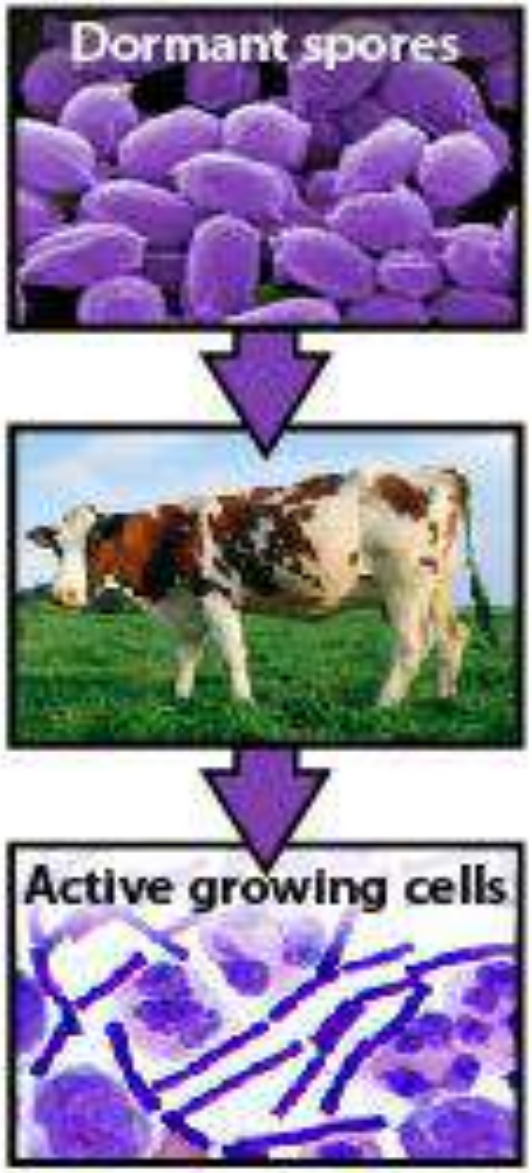
Animals acquire anthrax through ingestion of spores and spread of the organisms from the intestinal tract. This is rare in humans, and gastrointestinal anthrax is extremely uncommon. Abdominal pain, vomiting, and bloody diarrhea are clinical signs.

Cutaneous anthrax

1 The bacteria known as *Bacillus anthracis* produce **spores that are dormant** (not active) and can live in the environment, like soil, for a long time, even decades.

2 When spores **get into the body** of an animal or person (a place rich with water, sugars, and other nutrients), they can be "activated" and turn into **active growing cells**.

3 When they become active, the bacteria can multiply, spread out in the body, produce toxins (poisons) and cause severe illness and death.



Treatment

- **Ciprofloxacin is recommended for treatment; penicillin G, along with gentamicin or streptomycin, has previously been used to treat anthrax.**
- In the **setting of potential exposure to B. anthracis as an agent of biologic warfare**, **prophylaxis with ciprofloxacin or doxycycline** should be **continued for 4 weeks while 3doses of vaccine are being given or for 8 weeks if no vaccine is administered.**

- Some other gram-positive bacilli, such as B cereus, are resistant to penicillin by virtue of β-lactamase production.
- Doxycycline, erythromycin, and ciprofloxacin may be effective alternatives to penicillin.

Epidemiology, Prevention, and Control

- **Soil is contaminated with anthrax spores from the carcasses of dead animals. These spores remain viable for decades. Perhaps spores can germinate in soil at a pH of 6.5 at proper temperature.**
- Grazing animals infected through injured mucous membranes serve to perpetuate the chain of infection. Contact with infected animals or with their hides, hair, and bristles is the source of infection in humans. Control measures include
- (1) disposal of animal carcasses by burning or by deep burial in lime pits,
- (2) decontamination (usually by autoclaving) o animal products,
- (3) protective clothing and gloves for handling potentially infected materials
- (4) active immunization of domestic animals with live attenuated vaccines.
- Persons with high occupational risk should be immunized.

Pharmaceutical microbiology

Chapter 8

Gram-Positive Bacilli:

Non Aerobic –Spore-Forming

Clostridium Species

Dr. Mohammed Hussein Taleb

Lecture 15

12/11/2020

Clostridium

- Clostridia cause several important toxin mediated diseases, including *Clostridium tetani,* tetanus; *Clostridium botulinum,* botulism; *Clostridium perfringens,* gas gangrene; and *Clostridium difficile,* pseudomembranous colitis.
- Other clostridia are also found in mixed anaerobic infections in humans

Clostridium Species

- The clostridia are large anaerobic, gram-positive, motile rods. Among the pathogens are the organisms causing botulism, tetanus, gas gangrene, and pseudomembranous colitis.

- ## Morphology and Identification

- A. Typical Organisms

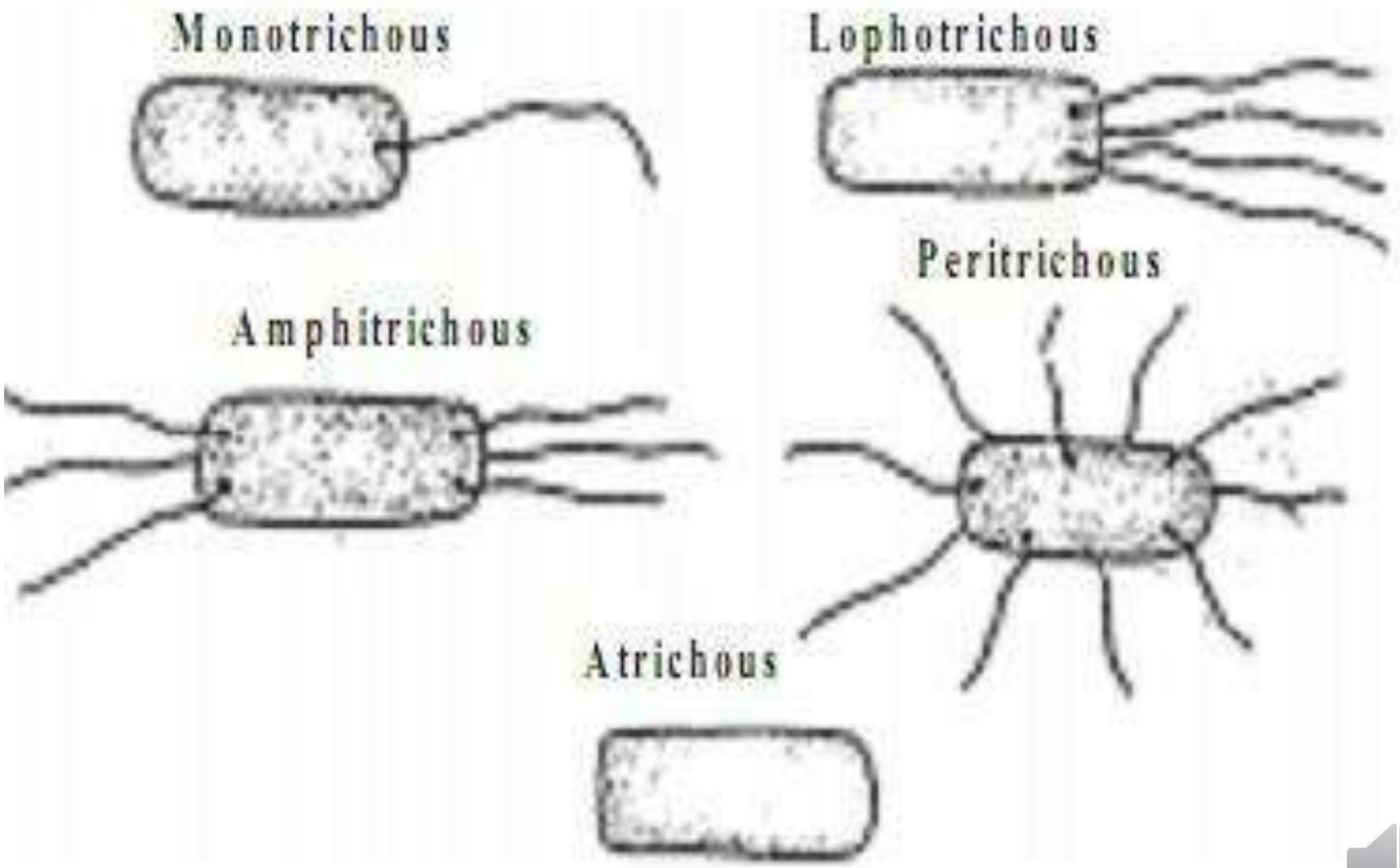
- Most species of clostridia are motile and possess peritrichous flagella.

- A Gram stain of a *Clostridium* species with terminal spores

- B. Culture

- Clostridia are anaerobes and grow under anaerobic conditions; a few species are aerotolerant and also grow in ambient air.

Types of flagellum



- **D. Growth Characteristics**
- Clostridia can ferment a variety of sugars; many can digest proteins. These metabolic characteristics are used to divide the Clostridia into groups, saccharolytic or proteolytic.
- **E. Antigenic Characteristics**
- Clostridia share some antigens but also possess specific **soluble antigens that permit grouping by precipitin tests or other assays.**

(1) Clostridium Botulinum

- **C. botulinum,** which causes **botulism,** is worldwide in distribution; it is **found in soil and in intestinal tracts of birds , animal , and fish (Gills) . Also Cannd food**
- **Types of C. botulinum** are distinguished **by the antigenic type of toxin they produce.** Spores of the organism are **subterminal spores,** highly resistant to heat, withstanding 100°C for several hours.
- **Toxin**
- During the growth of **C. botulinum** and during **autolysis of the bacteria, toxin is liberated into the environment.**

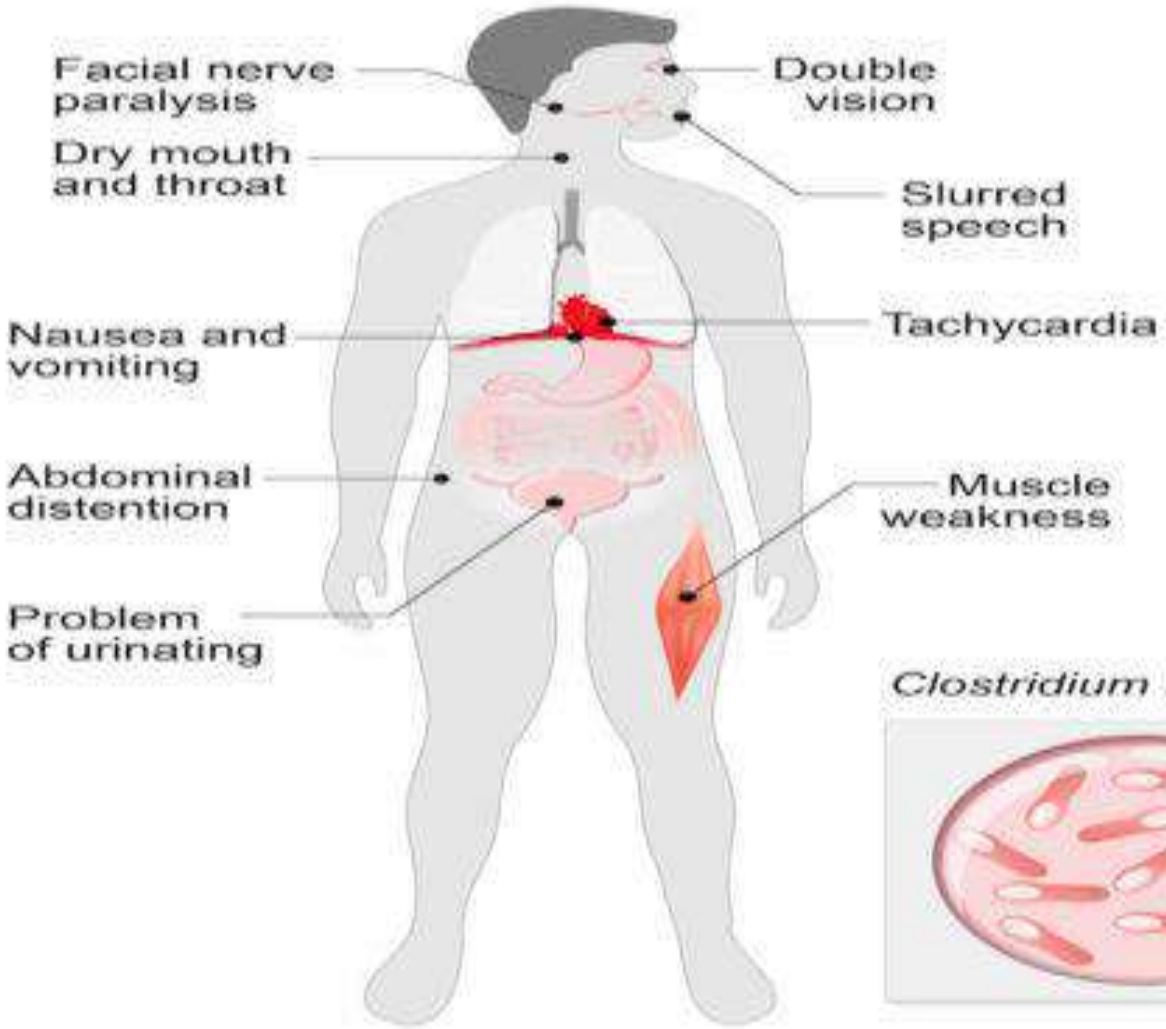
Canned Food and Honey

Pathogenesis

- Most cases of **botulism represent an intoxication resulting from the ingestion of food in which *C. botulinum* has grown and produced toxin.**
- The most **common offenders are spiced, smoked, vacuum packed, or canned alkaline foods that are eaten without cooking.**
- In such **foods, spores of *C. botulinum* germinate; that is, under anaerobic conditions, vegetative forms grow and produce toxin.**
- **In infant botulism, honey is the most frequent vehicle of infection.**

BOTULISM

SIGNS AND SYMPTOMS



Clostridium botulinum

Botulism is caused by the toxins that are produced by clostridium botulinum bacteria and is rare but potentially fatal condition

Bacteria

Common or General Symptoms of Botulism

- Visual disturbance or blurred vision
- Drooping eyelids
- Dry mouth and speaking problems
- Vomiting
- Swallowing problems
- Nausea
- Abdominal cramps

Diagnostic Laboratory Tests

- Toxin can often be demonstrated in serum, gastric secretions, or stool from the patient, and toxin may be found in leftover food.

Treatment

- Potent antitoxins to three types of botulinum toxins have been prepared in horses. Because the type responsible for an individual case is usually not known, trivalent (A, B, E) antitoxin must be promptly administered intravenously with customary precautions.
- Adequate respiration must be maintained by mechanical ventilation if necessary. These measures have reduced the mortality rate from 65% to below 25%.
- Although most infants with botulism recover with supportive care alone, antitoxin therapy is recommended.

Epidemiology, Prevention, and Control

- Because spores of *C. botulinum* are widely distributed in soil, they often contaminate vegetables, fruits, and other materials.
- When such foods are canned or otherwise preserved, they either must be sufficiently heated to ensure destruction of spores or must be boiled for 20 minutes before consumption.
- Strict regulation of commercial canning has largely overcome the danger of widespread outbreaks, but commercially prepared foods have caused deaths.

- A chief risk factor for botulism lies in home-canned foods, particularly string beans, corn, peppers, olives, peas, and smoked fish or vacuum-packed fresh fish in plastic bags.
- Toxic foods may be spoiled and rancid, and cans may "swell," or the appearance may be innocuous.
- The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption.
- Botulinum toxin is considered to be a major agent for bioterrorism and biologic warfare .

Pharmaceutical microbiology

Chapter 8

Gram-Positive Bacilli:

Non Aerobic –Spore-Forming

Clostridium Species

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Lecture 16

26/11/2020

(2) Clostridium Tetani

- ***C. tetani,*** which causes **tetanus**, is worldwide in **distribution in the soil and in the feces of horses and other animals. Several types of *C. tetani* can be distinguished by specific flagellar antigens.**
- All share a common **O (somatic) antigen** which may be masked, and all produce the **same antigenic type of neurotoxin, tetanospasmin.**

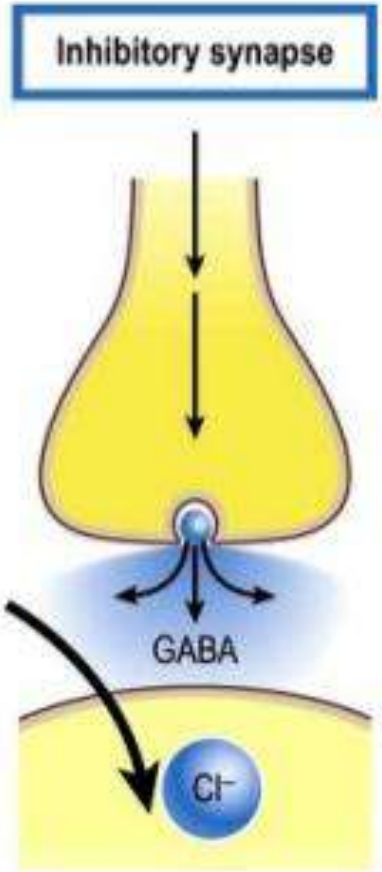
Toxin

- The vegetative cells of ***C. tetani*** produce the **toxin tetanospasmin** The toxin initially binds to receptors on the presynaptic membranes of motor neurons. It then migrates by the retrograde axonal transport system to the cell bodies of these neurons to the spinal cord and brainstem.

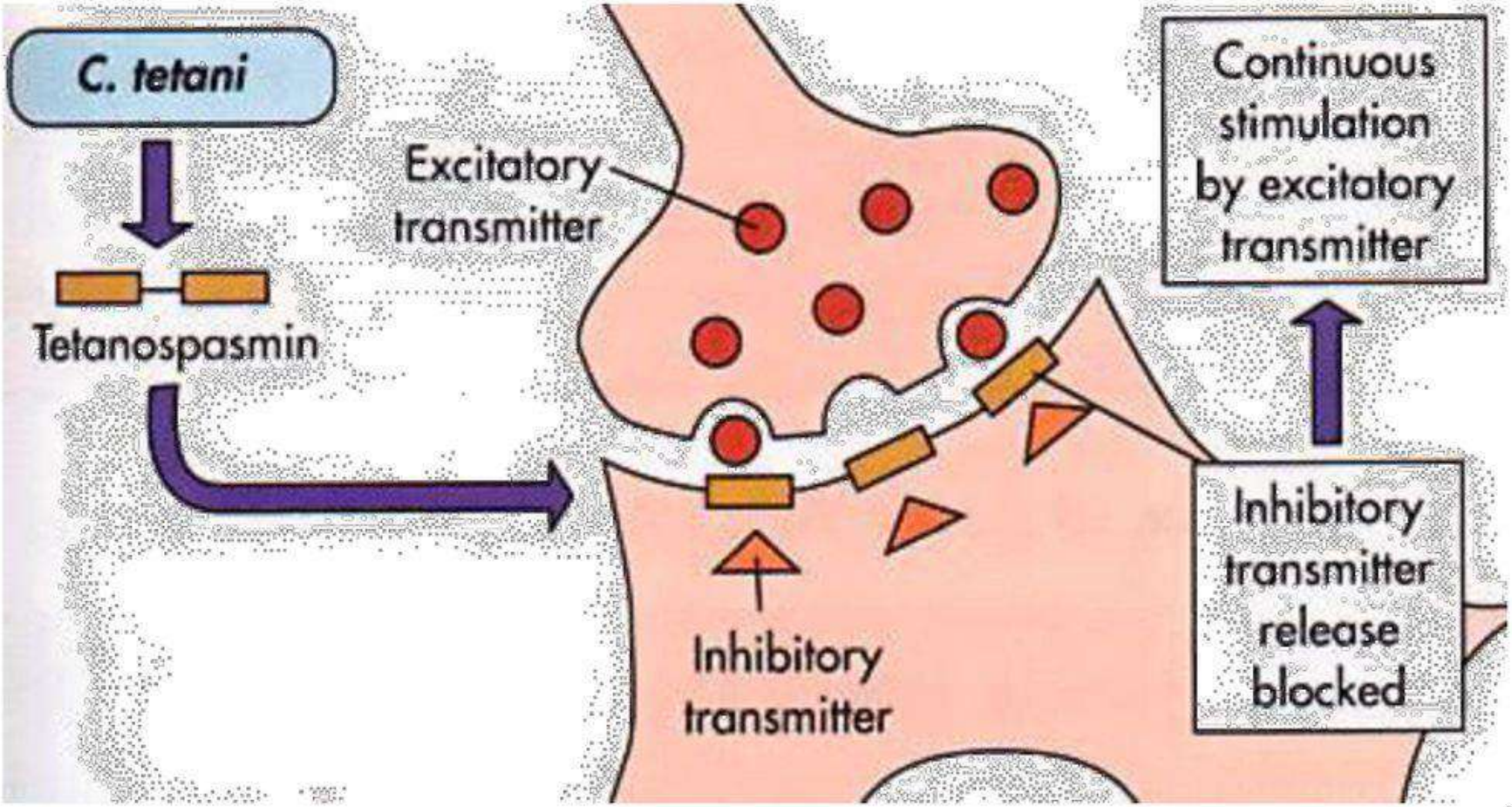
MECHANISUM OF ACTION

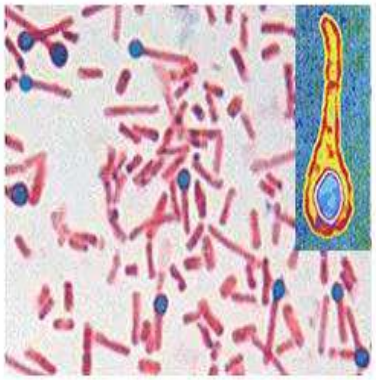
Inhibition of inhibitory neurotransmittor

- Glycine is the neurotransmitter for primary inhibitory interneurons such as the Renshaw cell
  - Tetanospasmin prevents Ca^{2+}-dependent release of glycine
- GABA is the inhibitory transmitter for descending pathways.
  - Blocking GABA inhibits the inhibitory action on motor neurons

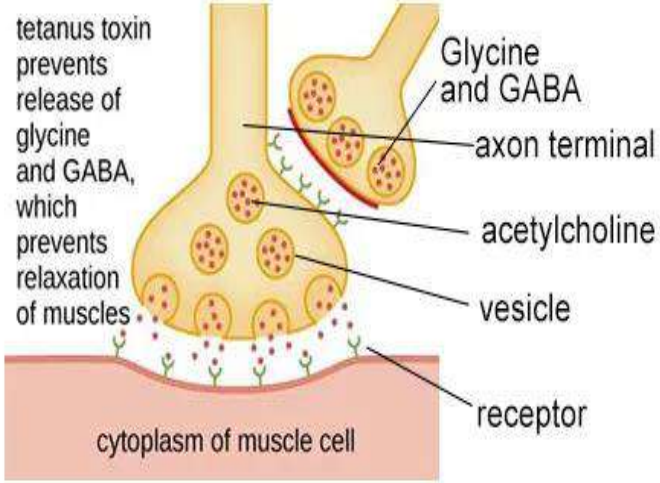


Mechanism of Action of Tetanus Toxin





'Drum Stick' or
'Tennis Racket' appearance



Mechanism of Tetanus Toxin

Clostridium tetani

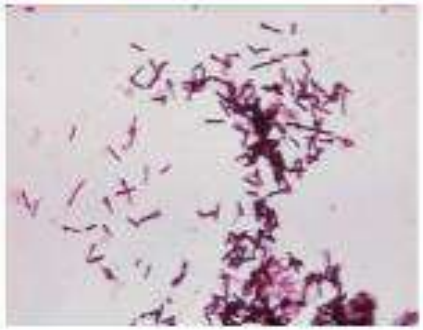
Opisthonus

microbeonline

Risus Sardonicus

What is Tetanus?

- An infectious disease caused by contamination of wounds from the bacteria *Clostridium tetani* or the spores they produce that live in the soil, and animal feces.
- *Greek words -"tetanos and teinein"*, meaning rigid and stretched, which describe the condition of the muscles affected by the toxin, tetanospasmin, produced by *Clostridium tetani.*
- The estimated minimum human lethal dose is 2.5 nanograms per kilogram of body weight, or 175 nanograms in a 70 kg (154 lb) human.
- Also known as lockjaw, it is characterised by muscular spasms.
- In the most common type the spasms begin in the jaw and then progresses to the rest of the body.
- These spasms usually last a few minutes each time and occur frequently for three to four weeks.



TETANIC TRIAD

RISUS SARDONICUS

"SARDINIAN GRIN"

↳ GRIN caused by FACIAL MUSCLE SPASM

TRISMUS

"LOCKJAW"

↳ MILD → SEVERE SPASMS of LOWER JAW

OPISTHOTONOS

↳ SEVERE SIMULTANEOUS SPASM of ALL MUSCLES

TETANUS
(Lockjaw)

* Intact Sensorium
* Headache
* Difficult Swallowing
* Sore Throat
* Irritability
* Tonic Spasms
* Prevention - Childhood Immunizations

* Spasms of Facial Muscles
  - Fixed Smile
  - Elevated Eyebrows
* Jaw Stiffness
* Fever
* Restlessness
* Chills
* Exaggerated Reflexes
* Profuse Sweating

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- The **toxin diffuses to terminals of inhibitory cells, including both glycinergic interneurons and γ-aminobutyric acid (GABA)– secreting neurons from the brainstem.**
- The **toxin degrades synaptobrevin, a protein required for docking of neurotransmitter vesicles on the presynaptic membrane.**
- Release of the **inhibitory glycine and GABA is blocked, and the motor neurons are not inhibited. Hyperreflexia, muscle spasms, and spastic paralysis result. Extremely small amounts of toxin can be lethal for humans.**

Pathogenesis

- *C. tetani* is not an invasive organism. The infection remains strictly localized in the area of devitalized tissue (wound, burn, injury, umbilical stump, surgical suture) into which the spores have been introduced. The volume of infected tissue is small, and the disease is almost entirely a toxemia.
- The patient is fully conscious, and pain may be intense.
- Death usually results from interference with the mechanics of respiration.
- The mortality rate in generalized tetanus is very high.

Diagnosis

- **The diagnosis rests on the clinical picture and a history of injury,** The primary differential diagnosis of tetanus is strychnine poisoning.
- **Anaerobic culture of tissues from contaminated wounds may yield *C. tetani*, .**

Prevention and Treatment

- The results of treatment of tetanus are not satisfactory. Therefore, prevention is all important. Prevention of tetanus depends on
- (1) active immunization with toxoids,
- (2) proper care of wounds contaminated with soil
- (3) prophylactic use of antitoxin, and
- (4) administration of penicillin.

- The intramuscular administration of 250–500 units of human antitoxin (tetanus immune globulin) gives adequate systemic protection (0.01 unit or more per milliliter of serum) for 2–4 weeks.
- It neutralizes the toxin that has not been fixed to nervous tissue. Active immunization with tetanus toxoid should accompany antitoxin prophylaxis.
- Patients who develop symptoms of tetanus should receive muscle relaxants, sedation, and assisted ventilation.

- Sometimes they are given very large doses of antitoxin (3000–10,000 units of tetanus immune globulin) intravenously in an effort to neutralize toxin that has not yet been bound to nervous tissue.
- However, the efficacy of antitoxin for treatment is doubtful except in neonatal tetanus, in which it may be lifesaving.

- Surgical debridement is vitally important because it removes the necrotic tissue that is essential for proliferation of the organisms.

- Penicillin strongly inhibits the growth of C. tetani and stops further toxin production. Antibiotics may also control associated pyogenic infection.
- When a previously immunized individual sustains a potentially dangerous wound, an additional dose of toxoid should be injected to restimulate antitoxin production.

TABLE 3

Fundamentals of Tetanus Treatment

Control of spasms and autonomic dysfunction with sedation, muscle relaxants, and paralysis
Wound care with debridement and IV antibiotics
Neutralization of unbound tetanospasmin with tetanus immunoglobulin
Supportive care, including IV fluid hydration, nutrition, and prevention of complications

Control

- Tetanus is a **totally preventable disease.** **Universal active immunization with tetanus toxoid** should be **mandatory.**
- **Tetanus toxoid is produced by detoxifying the toxin with formalin and then concentrating it. Aluminum salt–adsorbed toxoids are used.**
- **Initial immunization should be carried out in all children during the first year of life. A "booster" injection of toxoid is given upon entry into school.**

(3) Clostridium perfringens

Clostridia That Produce Invasive Infections , Many different toxin-producing clostridia (*C perfringens* and related clostridia) can produce invasive infection (including **myonecrosis** and **gas gangrene**) if introduced into damaged tissue.

About 30 species of clostridia may produce such an effect, but the most common in invasive disease is *C. perfringens* (90%). An enterotoxin of *C perfringens* is a common cause of food poisoning.

Toxins

The invasive clostridia produce a large variety of toxins and enzymes that result in a spreading infection. Gas gangrene bacilli. *Clostridium perfringens* typically does not form spores when grown on laboratory media.

Figure 11-3 Gas gangrene bacilli. *Clostridium perfringens* typically does not form spores when grown on laboratory media.

- The α toxin of C perfringens type A is a lecithinase, and its lethal action is proportionate to the rate at which it splits lecithin (an important constituent of cell membranes) to phosphorylcholine and diglyceride.

- The theta toxin has similar hemolytic and necrotizing effects but is not a lecithinase. DNase and hyaluronidase, a collagenase that digests collagen of subcutaneous tissue and muscle, are also produced.

Pathogenesis

- In invasive clostridial infections, spores reach tissue either by contamination of traumatized areas (soil, feces) or from the intestinal tract. The spores germinate at low oxidation reduction potential; vegetative cells multiply, ferment carbohydrates present in tissue, and produce gas.

- The distention of tissue and interference with blood supply, together with the secretion of necrotizing toxin and hyaluronidase, favor the spread of infection. Tissue necrosis extends, providing an opportunity for increased bacterial growth; hemolytic anemia; and, ultimately, severe toxemia and death.

Clinical Findings

- From a contaminated wound (eg, a compound fracture, postpartum uterus), the infection spreads in 1–3 days to produce crepitation in the subcutaneous tissue and muscle, foul-smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death.

- Treatment is with early surgery (amputation) and antibiotic administration. Until the advent of specific therapy, early amputation was the only treatment. At times, the infection results only in anaerobic fasciitis or cellulitis.

- ***C perfringens*** **food poisoning usually follows the ingestion of large numbers of clostridia that have grown in warmed meat dishes. The toxin forms when the organisms sporulate in the gut, with the onset of diarrhea—usually without vomiting or fever—in 7–30 hours. The illness lasts only 1–2 days**.

Treatment

- The most important aspect of treatment is prompt and extensive surgical debridement of the involved area and excision of all devitalized tissue, in which the organisms are prone to grow.
- **Administration of antimicrobial drugs, particularly penicillin, is begun at the same time. Hyperbaric oxygen may be of help in the medical management of clostridial tissue infections.**
- **Antitoxins are available against the toxins of *C perfringens***

(4)Clostridium Difficile

- Pseudomembranous Colitis
- Pseudomembranous colitis is diagnosed by detection of one or both *C difficile* toxins in stool and by endoscopic observation of pseudomembranes or microabscesses in patients who have diarrhea and have been given antibiotics.

- The diarrhea may be watery or bloody, and the patient frequently has associated abdominal cramps, leukocytosis, and fever.
- Although many antibiotics have been associated with pseudomembranous colitis, the most common are ampicillin and clindamycin and more recently, the fluoroquinolones.

- Administration of antibiotics results in proliferation of drug-resistant *C difficile* that produces two toxins.

- Toxin A, a potent enterotoxin that also has some cytotoxic activity, binds to the brush border membranes of the gut at receptor sites. Toxin B is a potent cytotoxin.

- Both toxins are usually found in the stools of patients with pseudomembranous colitis.

Antibiotic-Associated Diarrhea

- The administration of antibiotics frequently leads to a mild to moderate form of diarrhea, termed antibiotic-associated diarrhea. This disease is generally less severe than the classic form of pseudomembranous colitis.
- As many as 25% of cases of antibiotic-associated diarrhea are caused by C difficile infection.

- The disease is treated by **discontinuing administration** of the offending antibiotic and orally giving either **metronidazole or vancomycin**.

The end of clostridium

Pharmaceutical microbiology

Chapter 9

Gram negative

Bacilli

A- Enterobacteriaceae

Dr. Mohammed Hussein Taleb

Lecture 17

Enterobacteriaceae

- The **most important bacterial family in human medicine** is the **Enterobacteriaceae**.
- **This family includes genera and species that cause well-defined diseases with typical clinical symptoms (typhoid fever, dysentery, plague) as well as many opportunists that cause main nosocomial infections (urinary tract infections, pneumonias, wound infections, sepsis).**
- **Enterobacteriaceae are Gram-negative, usually motile, facultatively anaerobic rod bacteria.**
- **The high levels of metabolic activity observed in them are made use of in identification procedures.**

- The species are subdivided into epidemiologically significant serovars based on O, H, and K antigens. The most important pathogenicity factors of Enterobacteriaceae are colonizing factors, invasins, endotoxin, and various exotoxins.
- Enterobacteriaceae are the most significant contributors to intestinal infections, which are among the most frequent diseases of all among the developing world populac.

The taxonomy

- The taxonomic system applied to Enterobacteriaceae is based **on varying patterns of metabolic processes .**
- **Lactose-positive Enterobacteriaceae are grouped together as coliform Enterobacteriaceae.**
- **Salmonellae and most of the shigellae are lactose-negative.**

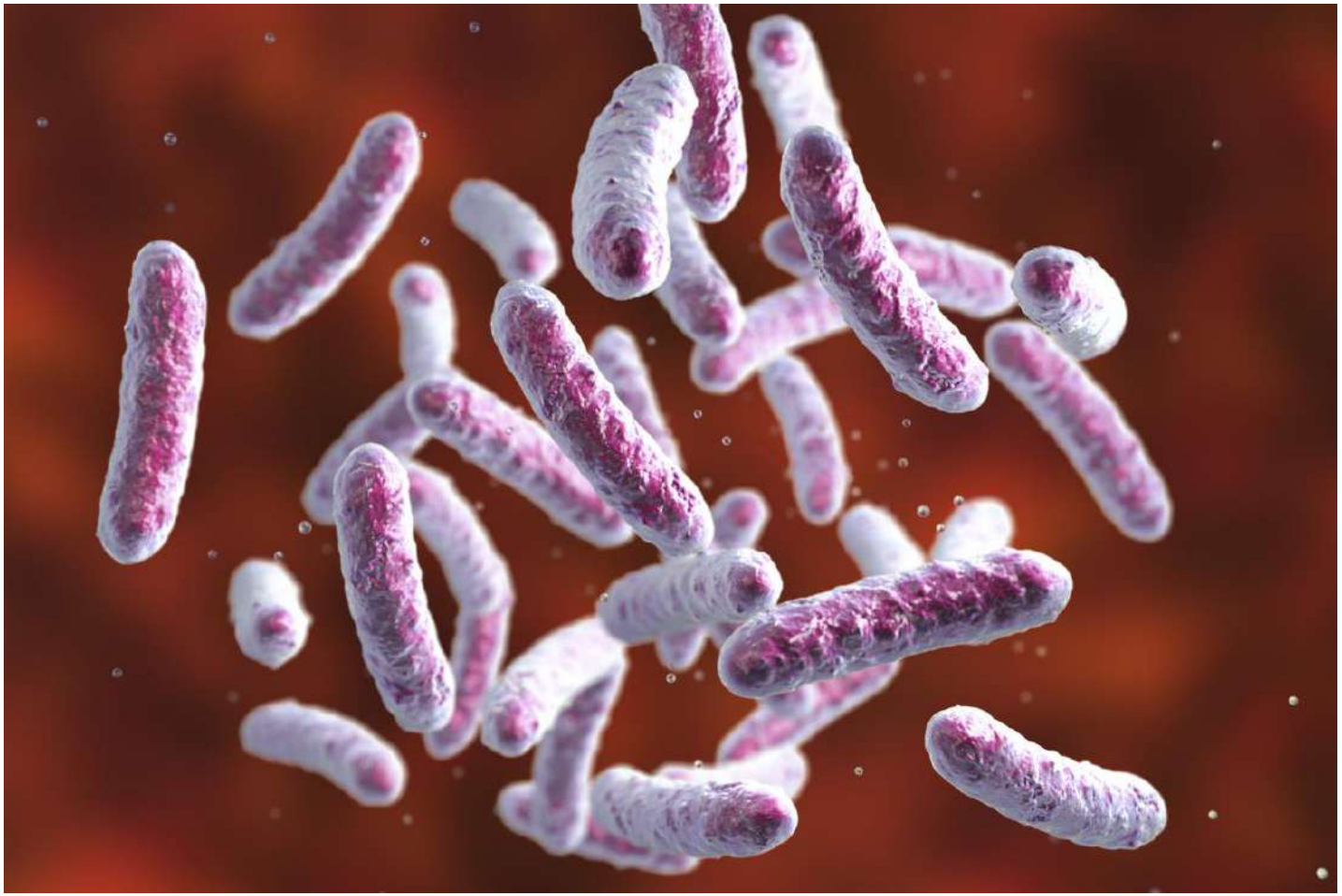
Antigen structure.

- The most important antigens of **Enterobacteriaceae** are:
- **O antigens.** **Specific polysaccharide** chains in the **lipopolysaccharide complex of the outer membrane .**
- **H antigens.** **Flagellar antigens consisting of protein.**
- **K antigens.** **Linear polymers of the outer membrane built up of a repeated series of carbohydrate units** (sometimes proteins as well). They can cover the **cell densely and render them O inagglutinable.**
- **F antigens.** **Antigens of protein attachment fimbriae.**

Pathogenicity determinants

- A number of factors are known to play a role in the pathogenicity of various Enterobacteriaceae infections. The most important are:
- Adhesion factors. **Attachment fimbriae, attachment pili, colonizing factor antigens (CFAs).**
- Invasive factors. **Proteins localized in the outer membrane (invasins) that facilitate the invasion of target cells.**
- Exotoxins.— Enterotoxins **disturb the normal functioning of enterocytes. Stimulation of adenylate or guanylate cyclase; increased production of cAMP**
- **This results in the loss of large amounts of electrolytes and water.**
- — Cytotoxins exert a direct toxic effect on cells (enterocytes, endothelial cells).
- Endotoxin. **Toxic effect of lipoid A as a component of LPS**
- Phagocyte resistance. **Makes survival in phagocytes possible.**

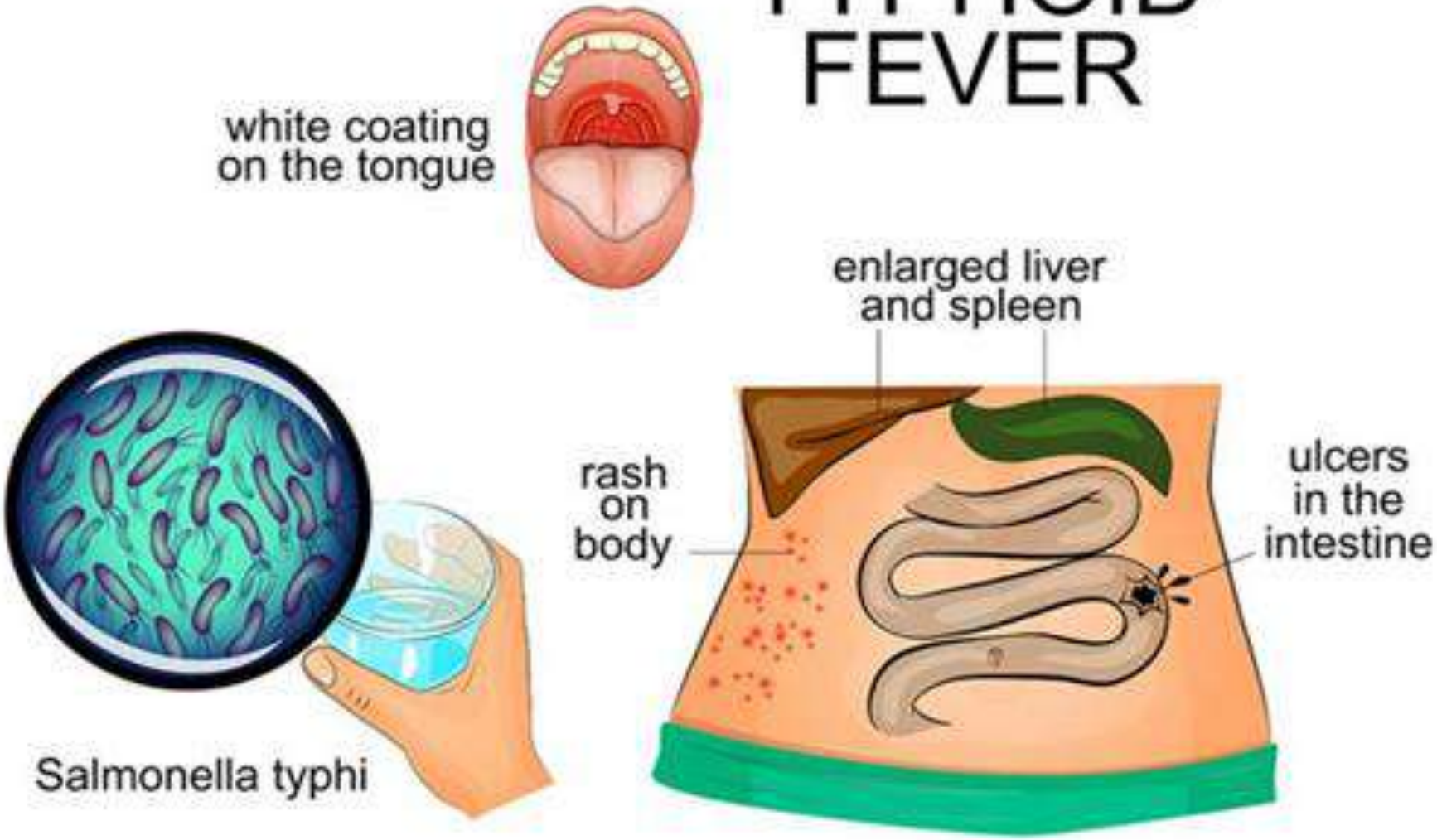
1- Salmonella



Salmonella (Gastroenteritis, Typhoid Fever, Paratyphoid Fever)

- Typhoid salmonelloses are caused by the serovars typhi and paratyphi A, B, and C. The salmonellae are taken up orally and the invasion pathway is through the intestinal tract, from where they enter lymphatic tissue, first spreading lymphogenously, then hematogenously.
- A generalized septic clinical picture results. **Human carriers are the only source of infection.** Transmission is either direct by **smear infection** or **indirect via food and drinking water.**

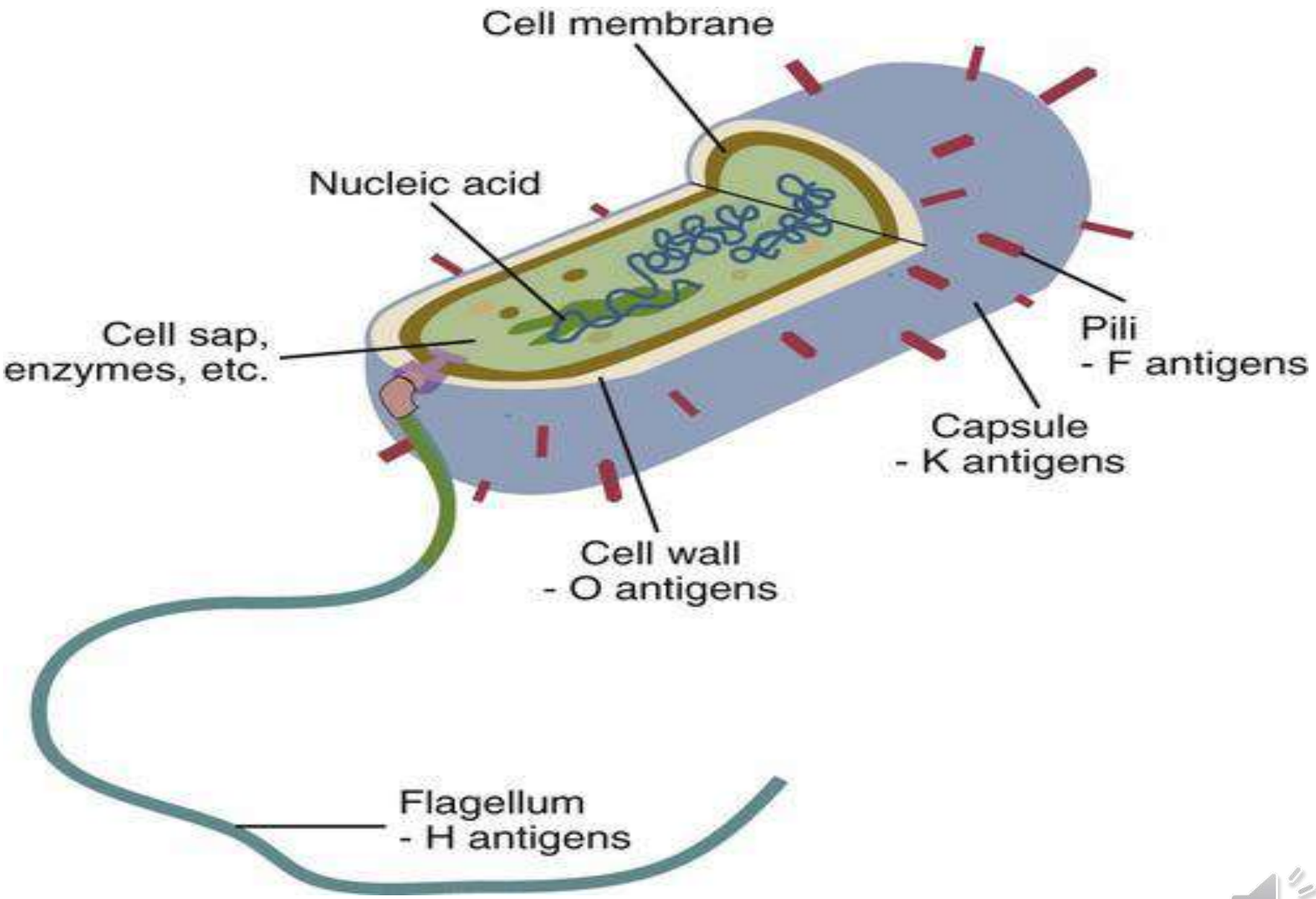
TYPHOID FEVER



- Anti-infective agents are required for therapy (**ampicillin, cotrimoxazole, 4-quinolones**). **An active vaccine is available to protect against typhoid fever.**
- **Enteric salmonelloses develop when pathogens are taken up with food. The primary infection source is usually livestock. These relatively frequent infections remain restricted to the gastrointestinal tract.**

Pathogenesis and clinical pictures.

- Salmonellae are classified as either typhoid or enteric regarding the **relevant clinical pictures and epidemiologies.**
- It is not known why **typhoid salmonellae only cause systemic disease in humans, whereas enteric salmonella infections occur in animals as well and are usually restricted to the intestinal tract.**


Cell membrane
Nucleic acid
Cell sap,
enzymes, etc.
Pili
- F antigens
Capsule
- K antigens
Cell wall
- O antigens
Flagellum
- H antigens

- Typhoid salmonelloses.
- Attachment of typhoid salmonellae to cells of the jejunum (M cells). **Invasion** by means of endocytosis, transfer, and exocytosis.
- Enteric salmonelloses.
- Attachment to enterocytes of the ileum and colon.

Invasion of mucosa induced by inasin proteins on the surface of the salmonella cells.

Diagnosis

- The method of choice is **detection of the pathogens in cultures. Selective indicator mediums are used to isolate salmonellae in stool.** Identification is done using metabolic patterns .Serovar classification is determined with specific antisera in the slide agglutination test.
- Culturing requires at least two days. **Typhoid salmonelloses can be diagnosed indirectly by measuring the titer of agglutinating antibodies to O and H antigens (according to Gruber-Widal).**

Widal - Test
Toxoplasma
ASO (Anti-Strep
Widal's test
Weil-Felix
VDRL
C4
CH50
LE Cell
TIS VIRUS
MONITORING

Therapy.

- **Typhoid salmonelloses** must be **treated with anti-infective agents, whereas symptomatic treatment will sufficient for enteric infections.** Symptomatic treatment **encompasses slowing down intestinal activity** (e.g., with **loperamide**) and replacing fluid and electrolyte losses orally as required.
- Anti-infective agents are required for therapy **(ampicillin, cotrimoxazole, 4-quinolones). An active vaccine is available to protect against typhoid fever.**

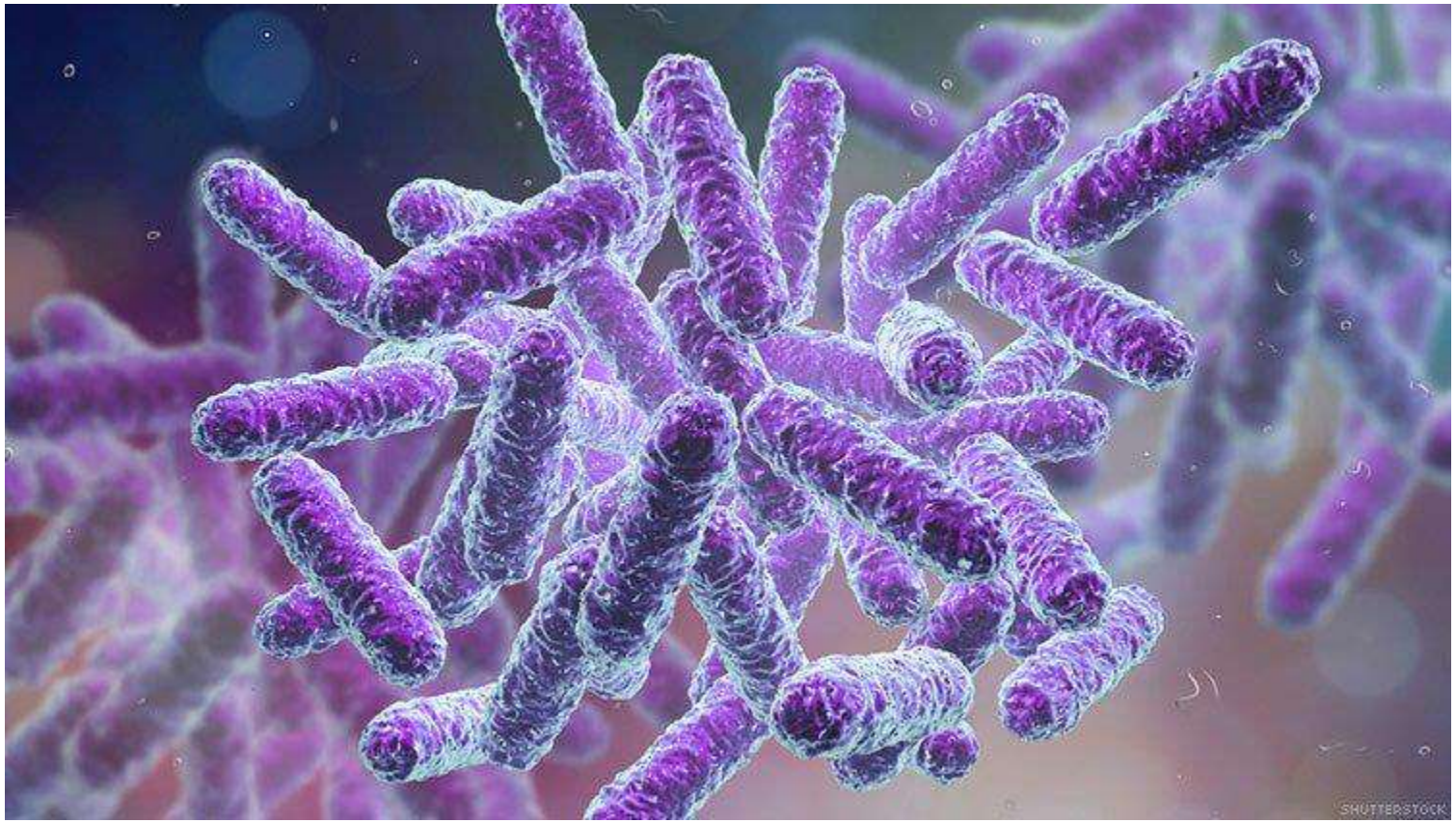
Epidemiology

- **Humans are the only primary source of infection in** the cases of typhoid salmonellosis
- By contrast, **enteric salmonelloses occur in this population both endemically and epidemically.** Livestock represents the most important source of infection. **The pathogens are transmitted to humans in food.**

Prevention.

- The main method of effective prevention is to avoid exposure: this means clean drinking water, prevention of food contamination, avoidance of uncooked foods in countries where salmonellae occur frequently.
- Typhoid fever vaccinations for travelers to endemic areas can best be done with the oral attenuated vaccine

2- Shigella



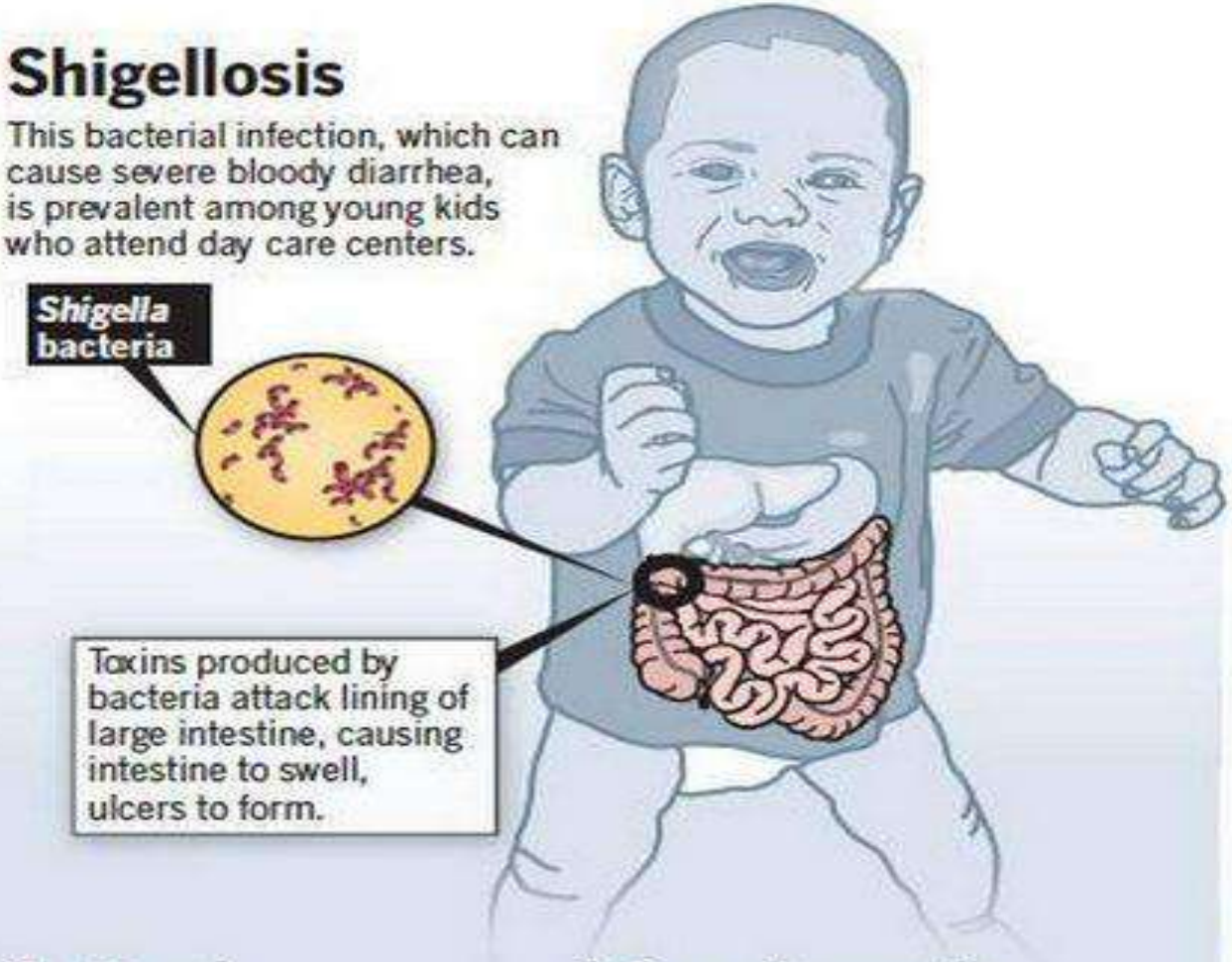
Shigella (Bacterial Dysentery)

Shigella is the causative pathogen in **bacterial dysentery**. **Shigellae are characterized by invasive properties**. They can **penetrate the colonic mucosa to cause local necrotic infections**. Humans are the **sole source of infection since shigellae are pathologically active in humans only**. The pathogens are **transmitted directly, more frequently indirectly, via food and drinking water.**

Shigellae are nonmotile and therefore have no flagellar (H) antigens.

Shigellosis

This bacterial infection, which can cause severe bloody diarrhea, is prevalent among young kids who attend day care centers.



Treatment

- Antibiotics; fluids to prevent dehydration.
- No antidiarrheal drugs; they could worsen infection.
- Severe cases may require hospitalization.

Spread, prevention

- Spread by contact with soiled hands of infected person, or by ingesting food and water contaminated by feces.
- Controlled by proper hygiene, drinking only treated water, washing raw foods well.

Source: U.S. Centers for Disease Control and Prevention, U.S. Library of Medicine

MCCLATCHY-TRIBUNE

Pathogenesis.

- Shigellae are only pathogenic in humans. **The invasion** is **facilitated by outer membrane polypeptides, the invasins,** which are coded by inv genes localized on plasmids.

- **Shigella dysenteriae produces shigatoxin, the prototype for the family of shigalike toxins (or verocytotoxins), which also occur in several other Enterobacteriaceae.**

- **The toxin inhibits protein synthesis in eukaryotic cells .**

- **Shigatoxin contributes to the colonic epithelial damage, the small intestine diarrhea with watery stools at the onset of shigellosis**

Clinical picture.

- Following an **incubation period of two to five days**, the disease manifests with **profuse watery diarrhea (= small intestine diarrhea).** Later, stools may contain **mucus, pus, and blood**. **Intestinal cramps, painful stool elimination (tenesmus), and fever are observed in the further course of the infection. Complications include massive intestinal bleeding and perforation peritonitis. These severe effects are caused mainly by S. dysenteriae, whereas S. sonnei infections usually involve only diarrhea.**

Therapy.

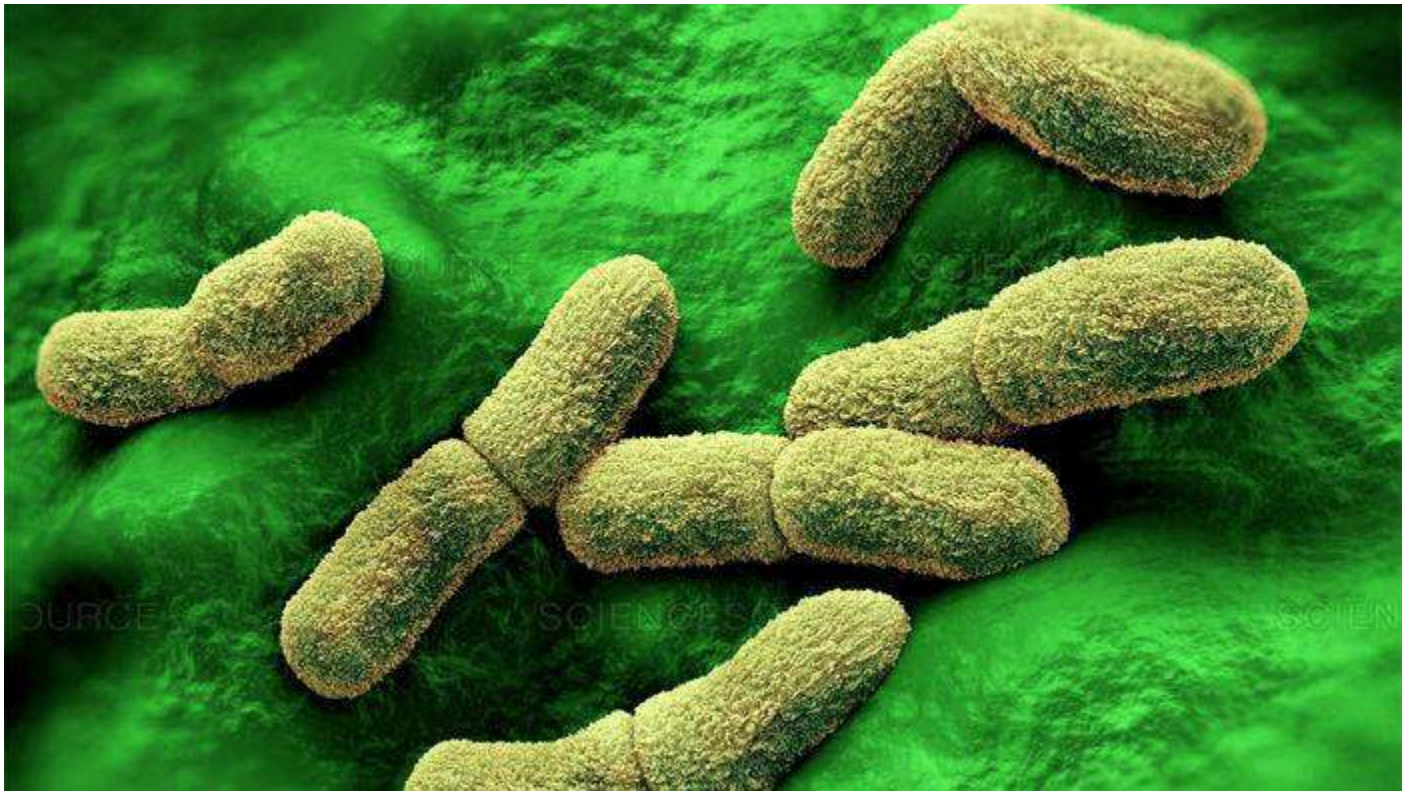
- Anti-infective agents are the first line of treatment (aminopenicillins, 4-quinolones, cephalosporins). Losses of water and electrolytes may have to be replaced.

- **Epidemiology and prevention.**

- The source of infection is always humans, in most cases infected persons whose stools contain pathogens for up to six weeks after the disease has abated.

- Transmission is by direct contact (smear infection) or indirect uptake via food, surface water, or flies.

3- Yersinia

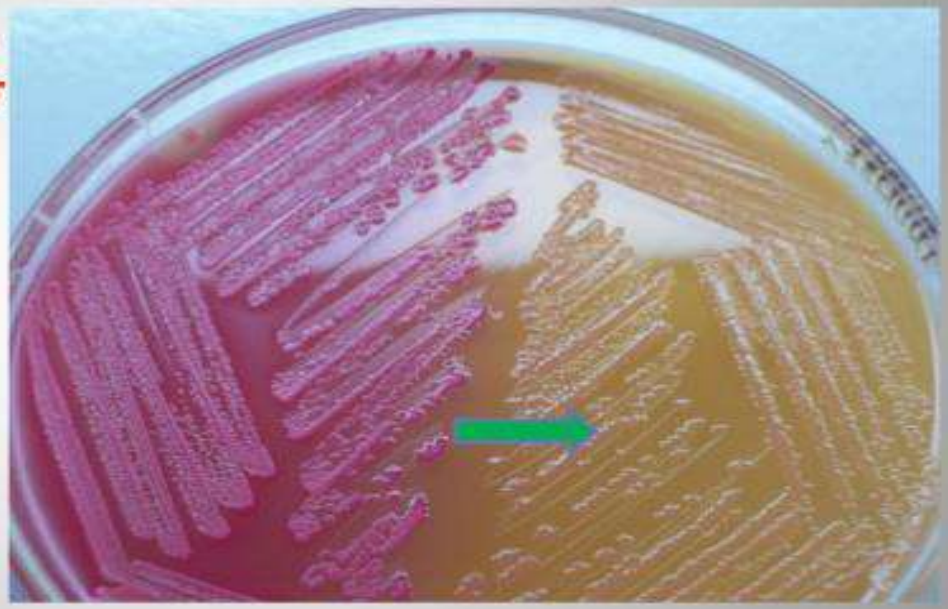


Non lactose fermenter enterobacteriacea

Enterobacteriaceae\ Non-lactose fermenters

Pale-colour colonies on MacConkey agar

- *Proteus*
- *Shigella*
- *Salmonella*
- *Yersinia*



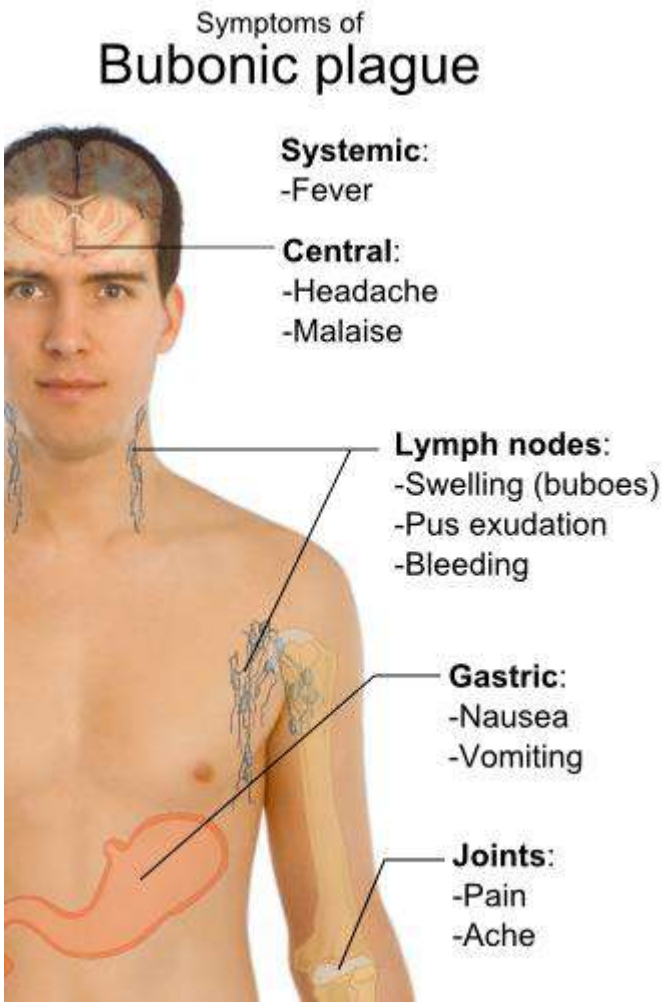
Yersinia (Plague, Enteritis)

- Y. pestis is a nonflagellated, short, encapsulated, Gram-negative rod bacteria .
- The plague is primarily a disease of rodents (rats). It spreads among them by direct contact or via the rat flea.
- The pathogen breaches the skin through dermal injuries. From such a location, the bacteria reach regional lymph nodes in which they proliferate.

- 2-5 days after infection, hemorrhagically altered, blue, and swollen lymph nodes (buboes) are observed. Over 90% of pestis infections show the "bubonic plague" course.
- Y. pestis is the causative pathogen of plague (black death, bubonic plague). Plague is a classic rodent zoonosis.
- The pathogens penetrate into the skin through microtraumata, from where they reach regional lymph nodes in which they proliferate, resulting in the characteristic buboes.

- In the next stage, **the pathogens may enter the bloodstream or the infection may generalize to affect other organs.**
- **Contact with such patients can result in primary pulmonary plague infections due to direct, aerogenic transmission. Left untreated, this form of plague is lethal in nearl 100% of cases.**
- **Therapy requires use of antibiotics.**

bubonic plague



Epidemiology and prevention

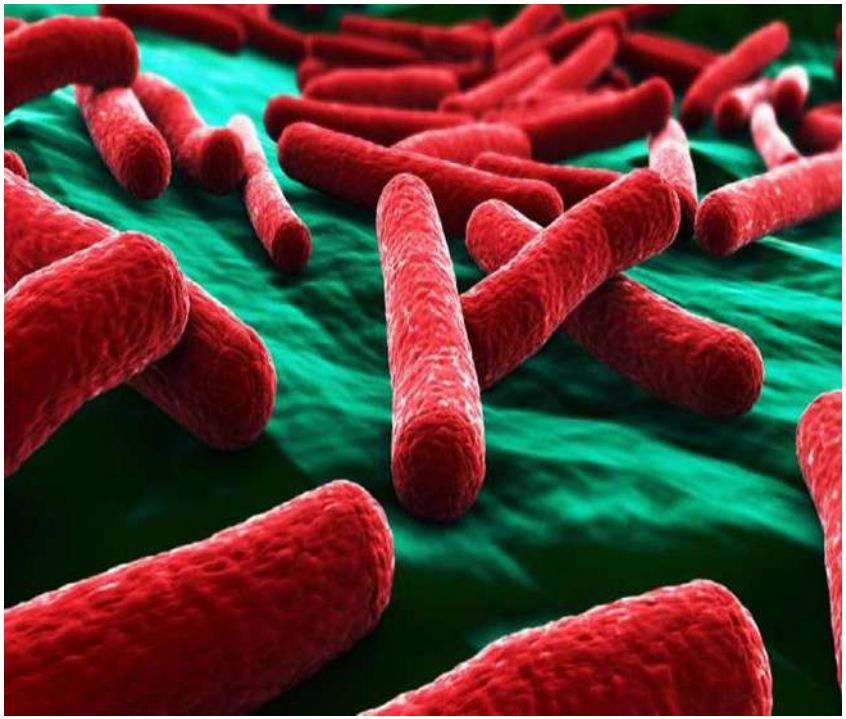
- Plague still occurs endemically in wild rodents over large areas of Asia, Africa, South America, and North America.
- Human plague infections have been reduced to sporadic instances. The sources of infection are mainly diseased rodents. Transmission of the disease is mainly via direct contact with such animals.

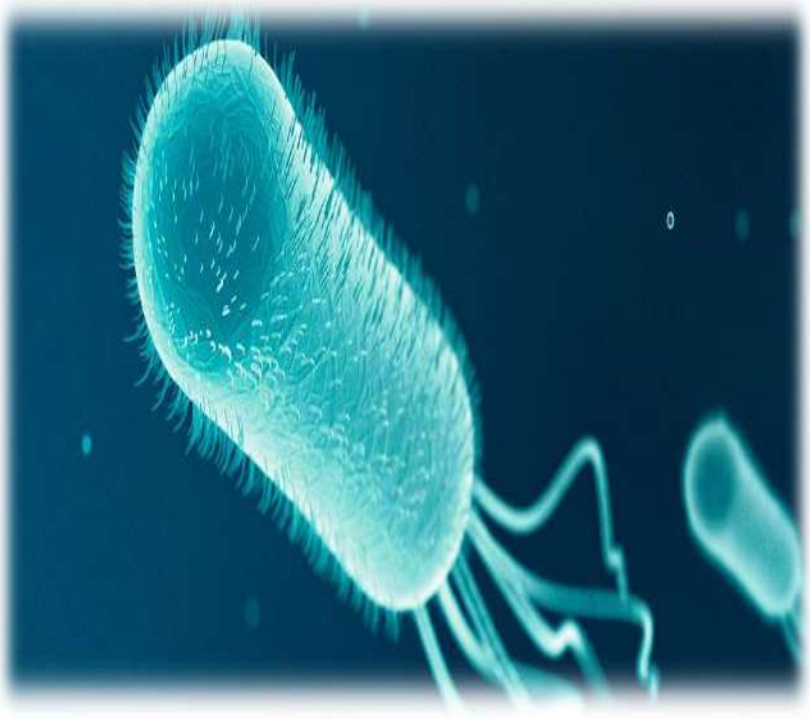
Prevention

- Prevention involves exposure prophylactic measures. Persons with manifest disease, in particular the pulmonary form, must be isolated.

- Contact persons must be quarantined for six days (= incubation period). Cases of plague infection must be reported to health authorities.

4- Escherichia coli





Escherichia coli

- The natural habitat of E. coli is the intestinal tract of humans and animals. E. coli is the most frequent causative pathogen in human bacterial infections.
- Extra intestinal infections include urinary tract infections, The most important other coli infections are cholecystitis, appendicitis, peritonitis, postoperative wound infections, and sepsis.

General characteristics.

- It is therefore considered an indicator organism for fecal contamination of water and foods. Guideline regulations: 100 ml of drinking water must not contain any E. coli.
- Surface water approved for bathing should not contain more than 100 (guideline value) E. coli bacteria per 100 ml.

Morphology, culture, and antigen structure

- They Gram-negative, straight rods are peritrichously flagellated. Lactose is broken down rapidly. The complex antigen structure of these bacteria is based on O, K, and H antigens. Fimbrial antigens have also been described. Specific numbers have been assigned to the antigens, e.g., serovar O18:K1:H7.

Urinary tract infection

- Such an infection manifests either solely in the lower urinary tract (urethritis, cystitis, urethrocystitis) or affects the renal pelvis and kidneys (cystopyelitis, pyelonephritis).
- In acute urinary tract infections, E. coli is the causative organism in 70–80% of cases and in chronic, persistent infections in 40–50% of cases.

E. coli causes about 15% of all cases of nosocomial sepsis (S. aureus20%).

Other E. coli infections.

Wound infections, infections of the gallbladder and bile ducts, appendicitis, peritonitis, meningitis in premature infants, neonates, and very elderly patients.

Pathogenesis and clinical pictures of intestinal infections.

E. coli that cause intestinal infections are nowclassified in five pathovars with differing pathogenicity and clinical pictures:

- Enteropathogenic E. coli (EPEC).
- Enterotoxic E. coli (ETEC).
- Enteroaggregative E. coli (EAggEC).
- Enteroinvasive E. coli (EIEC).
- Enterohemorrhagic E. coli (EHEC).

Therapy.

- Antibiotic therapy must take into consideration the resistance pattern of the pathogen. **Aminopenicillins, ureidopenicillins, cephalosporins, 4-quinolones, or cotrimoxazole are useful agents.**
- **Severe diarrhea necessitates oral replacement of fluid and electrolyte losses. When required, intestinal activity is slowed down with loperamide.**

Epidemiology and prevention

- Transmission of intestinal infections is usually indirect via food, drinkingwater, or surface water. 50% of travelers' diarrhea cases are caused by E. coli, in most cases ETEC.
- The most effective preventive measures against intestinal infections, e.g., when travelling in countries with warm climates, is to eat only thoroughly cooked foods and drink only disinfected water.

The end of chapter 9

Pharmaceutical microbiology

Chapter 10

Gram negative

Bacilli

B- Opportunistic

Enterobacteriaceae

Dr. Mohammed Hussein Taleb

Lecture 18

Opportunistic Enterobacteriaceae

- Many Enterobacteriaceae with minimum pathogenicity are classic opportunists.
- The most frequent opportunistic infections caused by them are: urinary tract infections, respiratory tract infections, wound infections, dermal and subcutaneous infections, and sepsis.
- Another reason why opportunistic Enterobacteriaceae have become so important in hospital medicine is the frequent development of resistance to anti-infective agents, Occurrence of multiple resistance in Enterobacteriaceae is due to the impressive genetic variability of these organisms

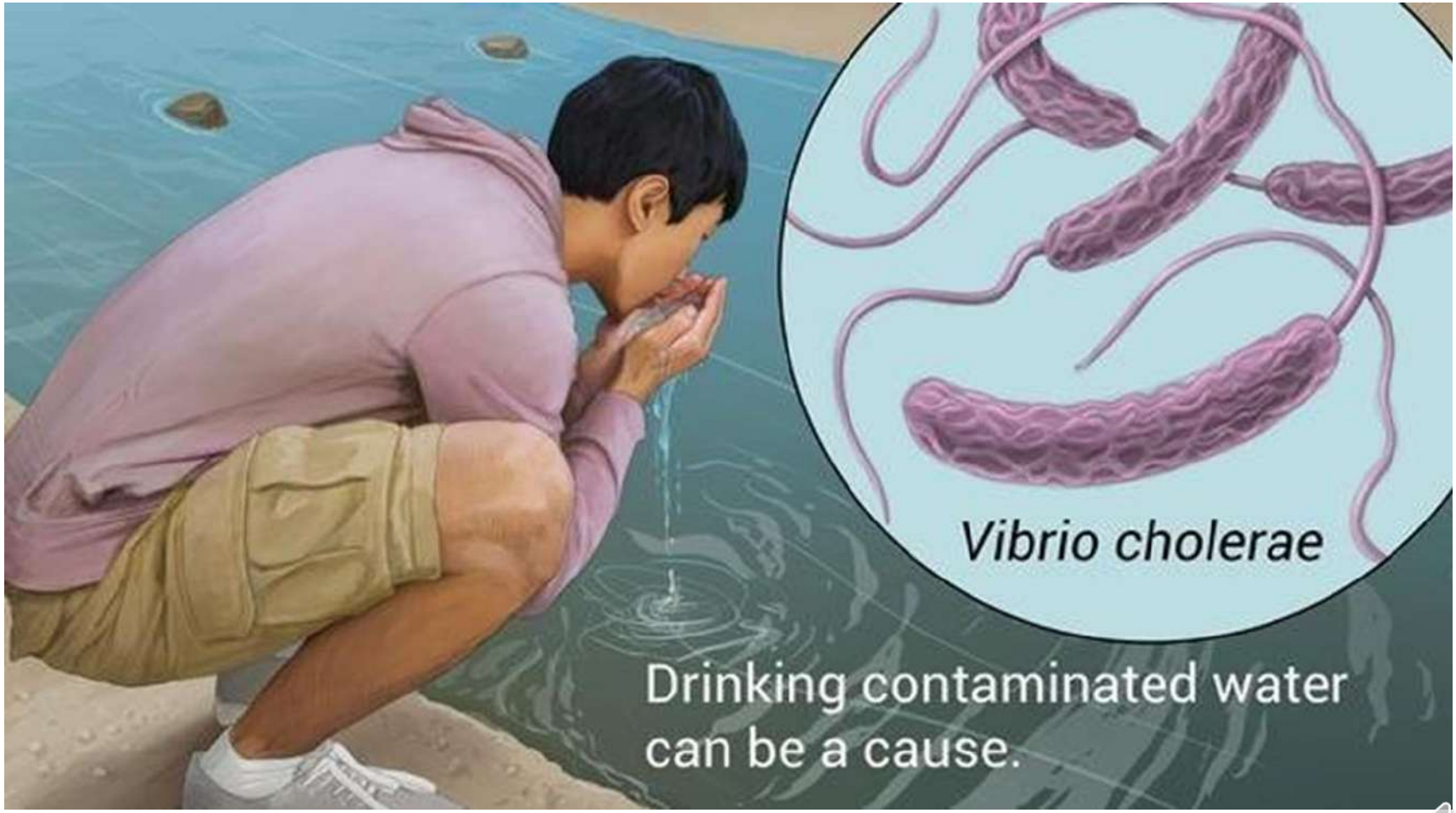
Vibreo cholera

Vibreo cholera

- **Cholera** is an infection of the small intestine by some strains of the bacterium Vibrio cholerae.
- Symptoms may range from none, to mild, to severe.
- The classic symptom is large amounts of watery diarrhea that lasts a few days. Vomiting and muscle cramps may also occur.
- Diarrhea can be so severe that it leads within hours to severe dehydration and electrolyte imbalance. This may result in sunken eyes, cold skin, decreased skin elasticity, and wrinkling of the hands and feet.
- Dehydration can cause the skin to turn bluish.
- Symptoms start two hours to five days after exposure.

- **Cholera** is caused by a **number of types of Vibrio cholerae,** with some types producing more severe disease than others.
- It is **spread mostly by unsafe water and unsafe food that has been contaminated with human feces containing the bacteria.**
- **Undercooked seafood is a common source. Humans are the only animal affected. Risk factors for the disease include poor sanitation, not enough clean drinking water, and poverty.**
- **V. cholerae bacteria are subdivided into serovars based on their O antigens (lipopolysaccharide antigens).**

Cholera can be caused by unsafe water


Vibrio cholerae
Drinking contaminated water
can be a cause.

- **Cholera toxin.** Cholera toxin is the sole cause of the clinical disease. This substance induces the enterocytes to increase secretion of electrolytes, above all Cl– ions, whereby passive water loss also occurs. The toxin belongs **to the group of AB toxins**

-

- Subunit B of the toxin binds to enterocyte receptors, the active toxin subunit A causes the AC in the enterocytes to produce cAMP continuously and in large amounts .
- cAMP in turn acts as a second messenger to activate protein kinase A, which then activates the specific cell proteins that control secretion of electrolytes.

- Pathogenesis and clinical picture.
- Infection results from oral ingestion of the pathogen.
- The infective dose must be large (> 108), since many vibrios are killed by the hydrochloric acid in gastric juice.
- Based on their pronounced stability in alkaline environments, vibrios are able to colonize the mucosa

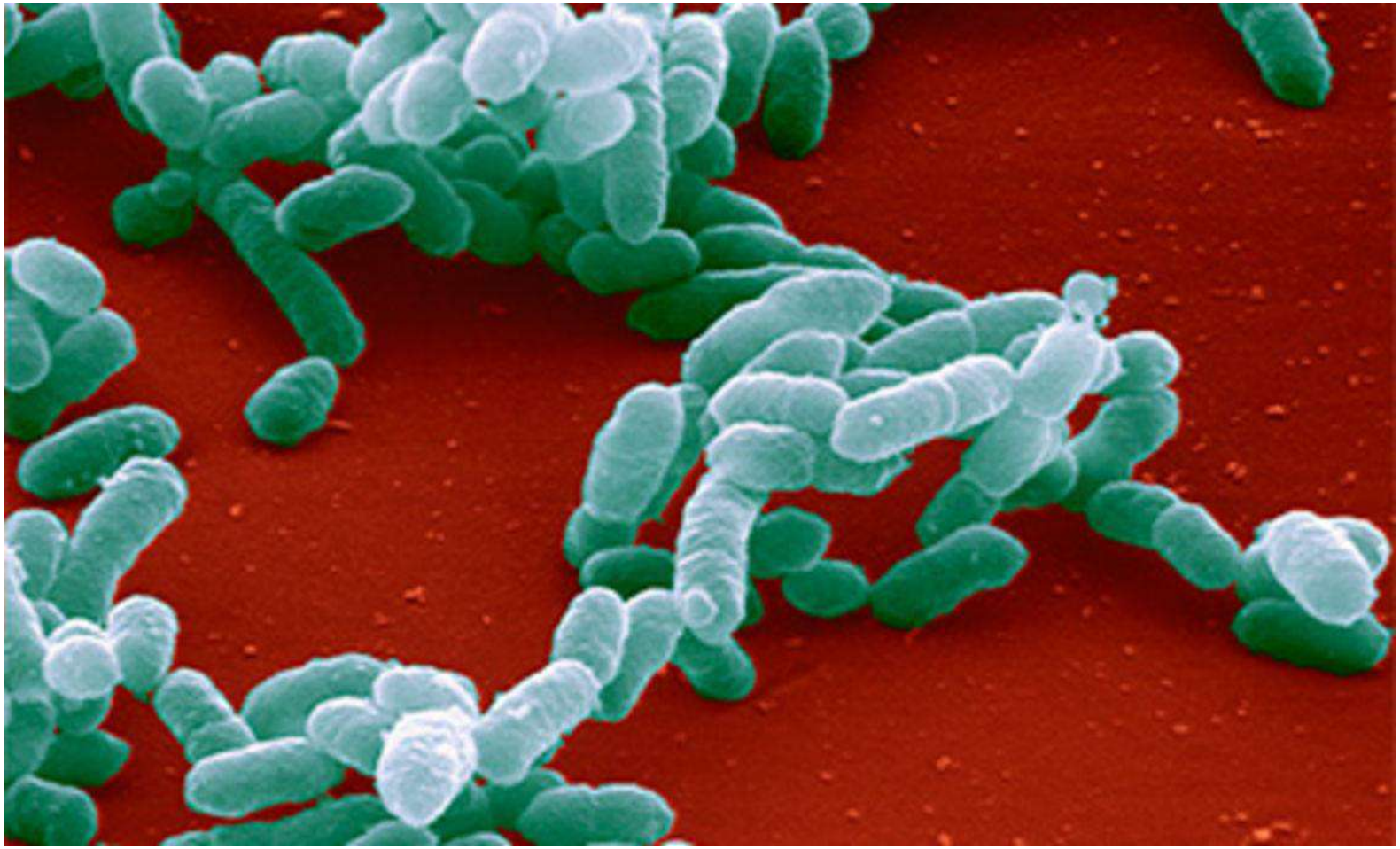
The pathogen **does not invade the mucosa**.

- The incubation period of cholera is 2-5 days. The clinical picture is characterized by voluminous, watery diarrhea and vomiting. The amount of fluids lost per day can be as high as 20L– day
- Lethality can be as high as 50% in untreated cases.

• Therapy.

- The most important measure is restoration of the disturbed water and electrolyte balance in the body.
- Secondly, tetracyclines and cotrimoxazole can be used, above all to reduce fecal elimination levels and shorten the period of pathogen secretion.
- **Epidemiology and prevention**.. Transmission of the disease is usually via foods, and in particular drinking water. This explains why cholera can readily spread to epidemic proportions in countries with poor hygiene standards.

2-H. influenzae



2- Haemophilus

- The most important human pathogen in this genus is H. influenzae. Haemophilus influenzae is a nonmotile, Gram-negative rod that is encapsulated.
- Capsule serovar b is the main pathogenic form. H. influenzae is a typical parasite of the respiratory tract mucosa. It occurs only in humans. It causes infections of the upper and lower respiratory tract in individuals with weakened immune defenses and in children under the age of four or five. Invasive infections meningitis and sepsis are also observed in small children.

- A betalactamase stable beta lactam antibiotic is required for treatment since the number of beta lactamase-producing strains observed is increasing.

- Conjugate vaccines in which the capsule polysaccharide is coupled with proteins are available for prophylactic immunization.

Pathogenesis and clinical pictures

H. influenzae is a mucosal parasite of the upper respiratory tract present in 30–50% of healthy persons. The capsule protects the cells from phagocytosis and is thus the primary determinant of pathogenicity.

Others include the affinity of H. influenzae to respiratory tract mucosa and meninges . H. influenzae infections are seen frequently in children aged from 0.5 to 4 years of age due to the low levels of anticapsule antibodies in this age group.

Maternal antibodies still protect children during the first months of life. The body has built up a sufficient store of antibodies by the age of four.

- **Therapy.** In view of the increasing number of betalactamase-producing H. influenzae strains observed in recent years, penicillinase-stable betalactam antibiotics should be used to treat these infections.

- The likehood that a strain produces betalactamase is 5–30% in most countries. 4-quinolones are an alternative to betalactams that should not, however, be used in children.

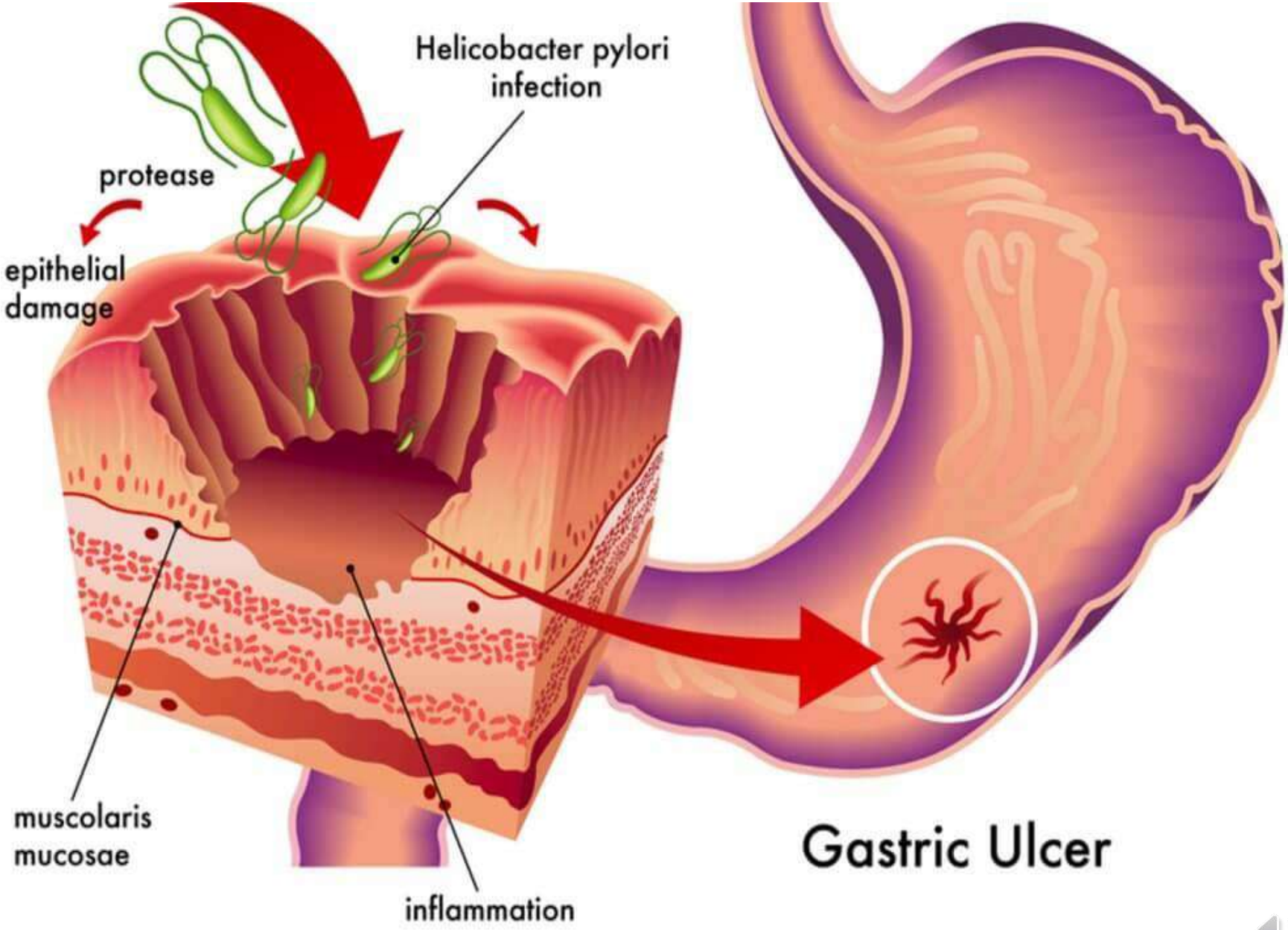
- The agent of choice in meningitis is ceftriaxone

3-H. pylori

Helicobacter pylori

H. pylori are spirally shaped, Gram-negative rods with lophotrichous flagellation. H. pylori occurs only in humans and is transmitted by the fecal-oral pathway. The pathogen colonizes and infects the stomach mucosa. The pathogenicity factors include

- pronounced motility for efficient target cell searching,
- adhesion to the surface epithelial cells of the stomach,
- urease that releases ammonia from urea to facilitate survival of the cells in a highly acidic environment
- a vacuolizing cytotoxin (VacA) that destroys epithelial cells.


Helicobacter pylori
infection
protease
epithelial
damage
muscolaris
mucosae
inflammation
Gastric Ulcer

Therapy.

- In patients with ulcers and/or gastritis symptoms, a triple combination therapy with omeprazole (proton pump blocker), metronidazole, and clarithromycin lasting seven days is successful in 90% of cases.

- **Epidemiology.** Based on sero epidemiological studies we know that H. pylori occur worldwide. Generalized contamination of the population begins in childhood and may reach 100% in adults in areas with poor hygiene. Transmission is by the fecal-oral route.

4-Pseudomonas aeruginosa

4-Pseudomonas

- Pseudomonads are Gram-negative, aerobic, rod-shaped bacteria with widespread occurrence in nature, especially in damp biotopes.

- The most important species from a medical point of view is **Pseudomonas aeruginosa**.

- The pathogenesis of Pseudomonas infections is complex. The organism can use its attachment pili to adhere to host cells.
- The relevant virulence factors are: exotoxin A, exoenzyme S, cytotoxin, various metal proteases, and two types of phospholipase C.
- Of course, the lipopolysaccharide (LPS) of the outer membrane also plays an important role in the pathogenesis. Pseudomonas infections occur only in patients with weakened immune defense systems,

Pseudomonas aeruginosa

- All pseudomonads are widespread in nature.
- **They are regularly found in soils, surface water, including the ocean, on plants and, in small numbers, in human and animal intestines.**

- The most important species in this group from a medical point of view is P. aeruginosa, which causes infections in person with immune defects.
- P. aeruginosa possesses an outer membrane as part of its cell wall. The architecture of this membrane is responsible for the natural resistance of this bacterium to many antibiotics.
- . Colonies on nutrient agar often have a metallic sheen (P. aeruginosa; Latin: aes = metal ore).

Pathogenesis and clinical pictures.

- The pathomechanisms involved are highly complex. P. aeruginosa usually enters body tissues through injuries.
- It 1- attaches to tissue cells using specific attachment fimbriae. The most important virulence factor is 2-exotoxin A ,which blocks translation in protein synthesis by inactivating the elongation factor eEF2.

3-The exoenzyme S inactivates cytoskeletal proteins and GTP-binding proteins in eukaryotic cells.

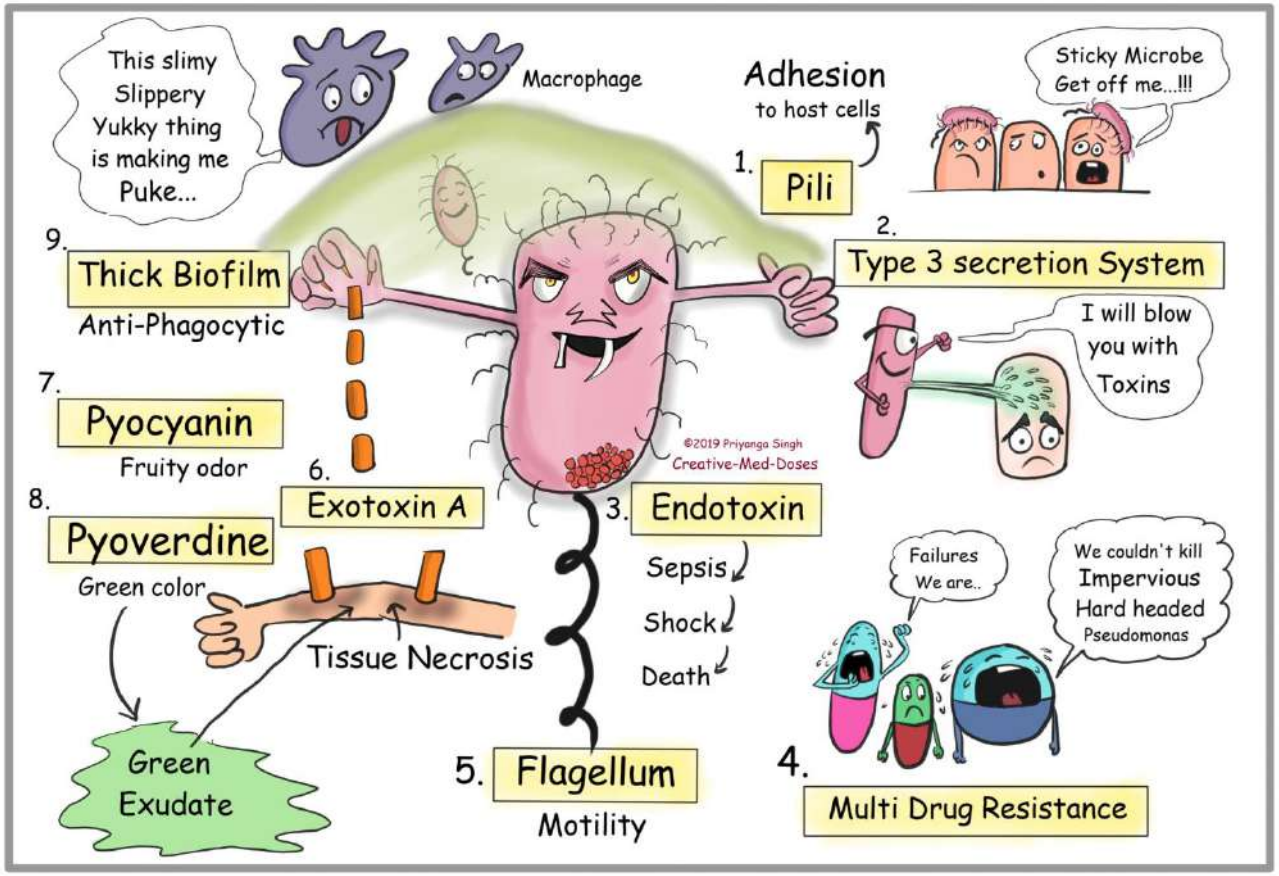
4- The so-called cytotoxin damages cells by creating transmembrane pores.

5-Various different metalloproteases hydrolyze elastin, collagen, or laminin.

6-Two type C phospholipases show membrane activity.

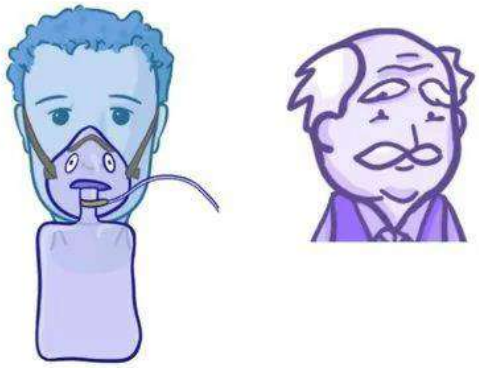
Psuedomonas auroginosa

Patient 1: Ten days after admission, continued darkening and necrosis of the overlying skin are apparent.


This slimy Slippery Yukky thing is making me Puke...
Macrophage
Adhesion
to host cells
Sticky Microbe Get off me...!!!
1. Pili
2. Type 3 secretion System
I will blow you with Toxins
9. Thick Biofilm
Anti-Phagocytic
7. Pyocyanin
Fruity odor
6. Exotoxin A
8. Pyoverdine
Green color
Tissue Necrosis
Green Exudate
©2019 Priyanga Singh
Creative-Med-Doses
3. Endotoxin
Sepsis
Shock
Death
5. Flagellum
Motility
Failures We are..
We couldn't kill Impervious Hard headed Pseudomonas
4. Multi Drug Resistance

PSEUDOMONAS AERUGINOSA

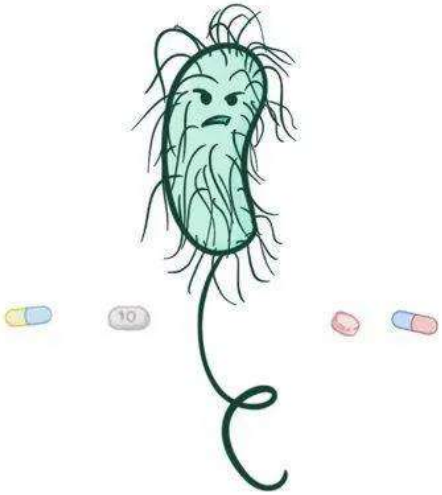
* GRAM-NEGATIVE BACTERIUM
* ABUNDANT in the ENVIRONMENT
* OPPORTUNISTICALLY INFECT HIGH-RISK INDIVIDUALS

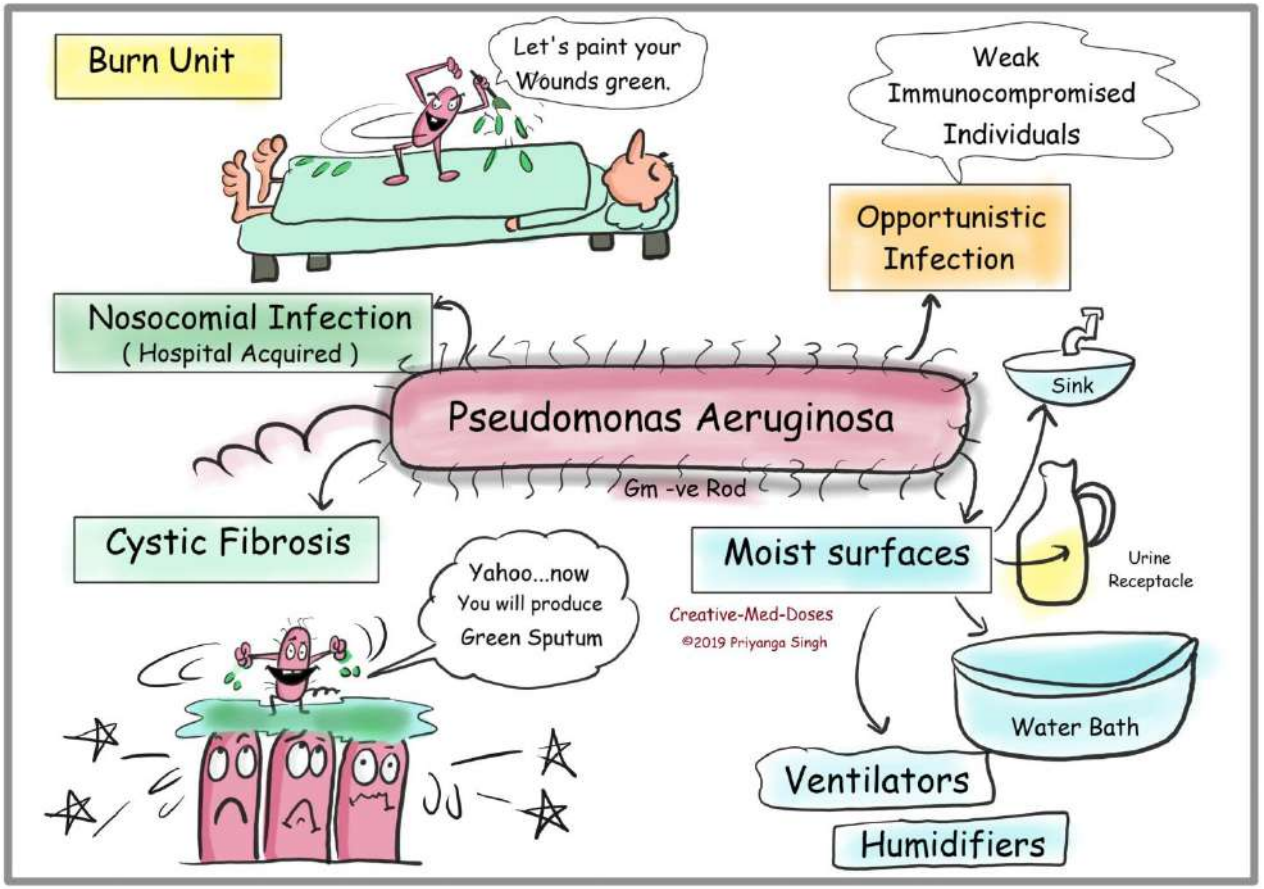


* MULTI-DRUG RESISTANCE

↓

HARD to TREAT




Burn Unit
Let's paint your Wounds green.
Weak Immunocompromised Individuals
Opportunistic Infection
Nosocomial Infection
(Hospital Acquired)
Pseudomonas Aeruginosa
Gm -ve Rod
Sink
Cystic Fibrosis
Yahoo...now You will produce Green Sputum
Moist surfaces
Urine Receptacle
Creative-Med-Doses
©2019 Priyanga Singh
Water Bath
Ventilators
Humidifiers

- The main **infections are** pneumonias in cystic fibrosis or in patients on respiratory equipment, infections of burn, wounds, postoperative wound infections, chronic pyelonephritis, endocarditis in drug addicts, sepsis, and malignant otitis externa.
- P. aeruginosa frequently causes nosocomial infections

Therapy

- The antibiotics that can be used to treat *P. aeruginosa* infections are aminoglycosides (gentamycin), acarboxylpenicillins, group 3rd cephalosporin,
- Combination of an aminoglycoside with a betalactam is indicated in severe infections.
- Susceptibility tests are necessary due to frequent resistance.

5-Klebsiella

Klebsiella

- ***Klebsiella*** **is a genus of Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule.**
- They can be found in **water, soil, plants, insects and other animals including humans. The species are aerobic but facultatively anaerobic.**
- *Klebsiella* species are routinely found in the **human nose, mouth, and gastrointestinal tract as normal flora**; however, they can also behave as opportunistic human pathogens.

- *K. pneumoniae* (type-species)*K. p.* subsp. *ozaenae*
- *K. p.* subsp. *pneumoniae*
- *K. p.* subsp. *Rhinoscleromatis*
- **Klebsiella organisms** can lead to a wide range of disease states, **notably pneumonia, urinary tract infections, sepsis, meningitis, diarrhea, and soft tissue infections.**

- This antimicrobial resistance is thought to be attributable mainly to multidrug efflux pumps.
- The ability of ***K. pneumoniae*** to **colonize the hospital environment, including carpeting, sinks, flowers, and various surfaces, as well as the skin of patients and hospital staff,** has been identified as a major factor in the spread of hospital-acquired infections.

- Members of the genus produce a prominent capsule, or slime layer, which can be used for serologic identification, but molecular serotyping may replace this method.
- Members of the genus *Klebsiella* typically express two types of antigens on their cell surfaces.

- The first, **O antigen, is a component of the lipopolysaccharide (LPS), of which 9 varieties exist.**
- The **second is K antigen, a capsular polysaccharide with more than 80 varieties.Both contribute to pathogenicity and form the basis for serogrouping**

Pharmaceutical microbiology

Chapter 11

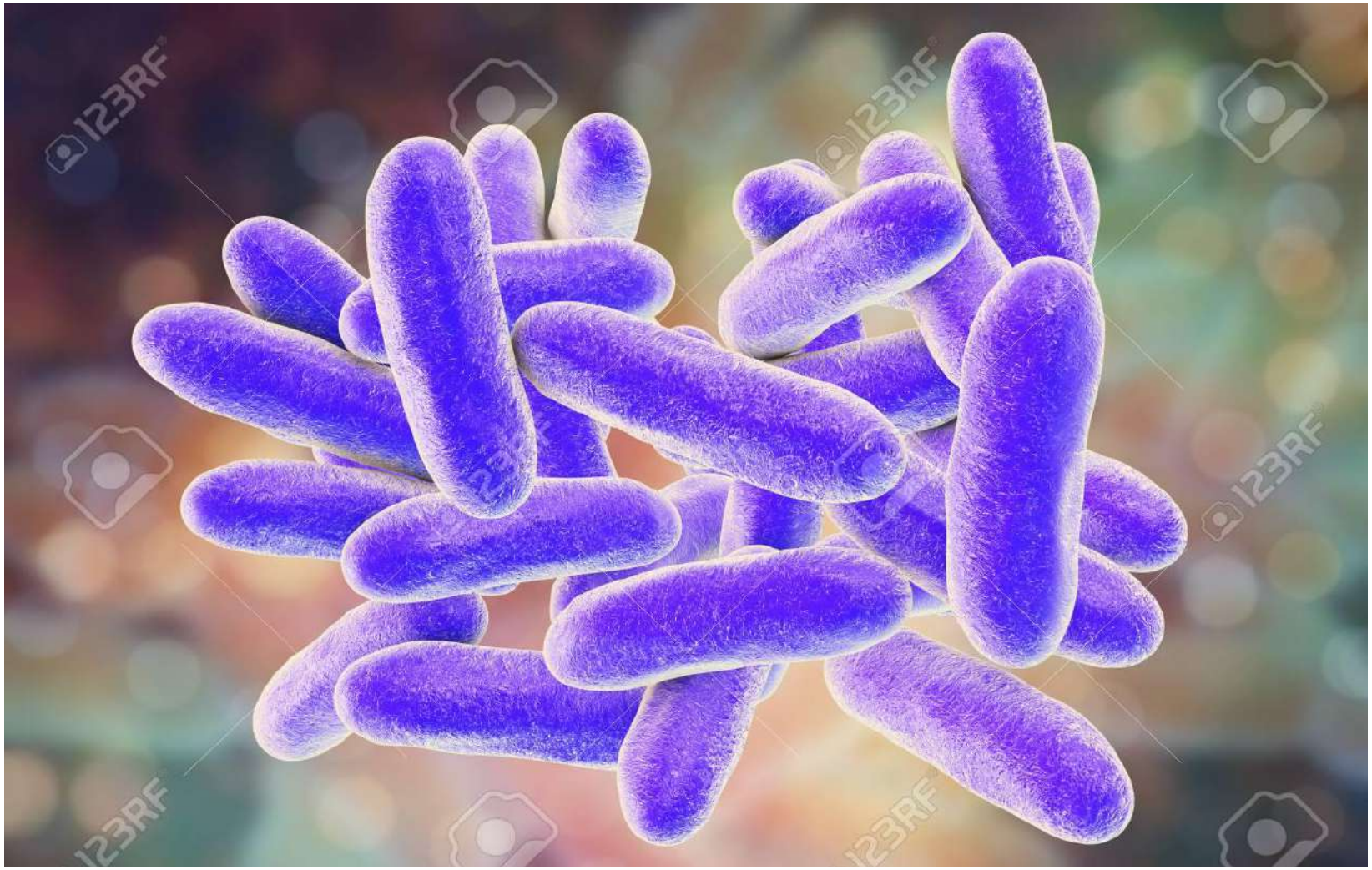
Gram negative Bacilli

Opportunistic Enterobacteriaceae

Dr. Mohammed Hussein Taleb

Lecture 19

6-Legionella



6-Legionella (Legionnaire's Disease)

- **Legionella is the only genus in the family Legionellaceae.**
- The species **Legionella pneumophila is responsible for most legionelloses in humans.**
- **They are Gram-negative, aerobic rod bacteria.**
- **Infections with Legionella occur when droplets containing the pathogens are inhaled. Two clinically distinct forms are on record: legionnaire's disease leading to multifocal pneumonia and nonpneumonic legionellosis or Pontiac fever.**

Legionnaire's disease.

- Infection results from inhalation of droplets containing the pathogens. The incubation period is 2 to 10 days. The clinical picture is characterized by a multifocal, sometimes necrotizing pneumonia. Occurrence is more likely in patients with cardiopulmonary primary diseases or other immunocompromising conditions.
- Lethality >20%.

Therapy.

- Macrolide antibiotics are now the agent of choice, having demonstrated clinical efficacy.
- Alternatively, 4-quinolones can be used.

7-Brucella

- The genera Brucella, Gram negative rods.
- Brucella abortus, B. melitensis, and B. suis cause brucellosis, a classic zoonosis that affects cattle, goats, and pigs. The pathogens can be transmitted to humans directly from diseased animals or indirectly in food. (in particular unpasteurized milk and dairy products.).
- They cause characteristic granulomas in the organs of the RES. The primary clinical symptom is the undulant fever.

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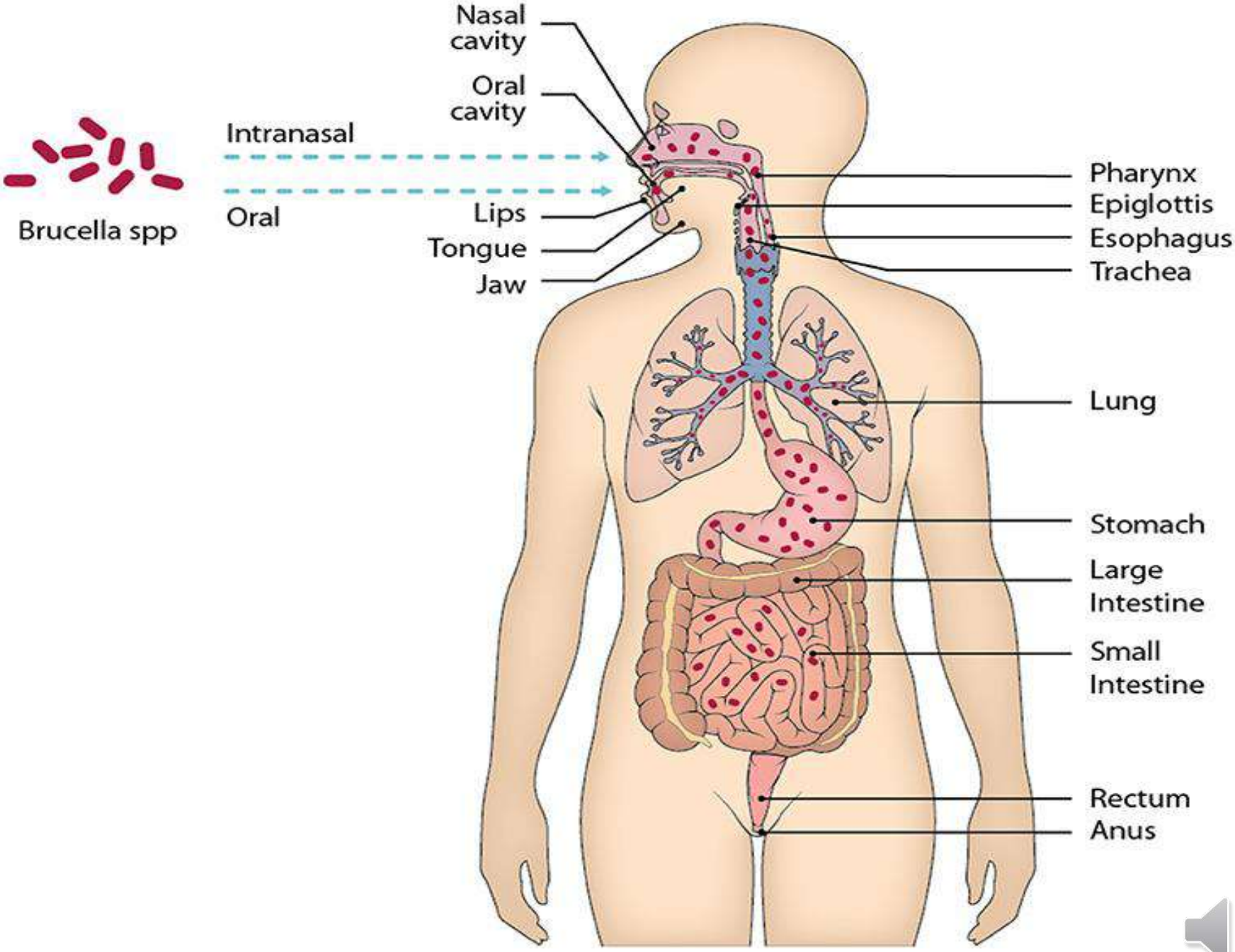

Brucella spp
Intranasal
Oral
Nasal cavity
Oral cavity
Lips
Tongue
Jaw
Pharynx
Epiglottis
Esophagus
Trachea
Lung
Stomach
Large Intestine
Small Intestine
Rectum
Anus

- **Diagnosis.** Antibody detection is done using the agglutination reaction according to Gruber-Widal in a standardized method. In doubtful cases, the complement binding reaction and direct Coombs test can be applied to obtain a serological diagnosis.
- **Therapy.**
- Doxycycline is administered in the acute phase, often in combination with gentamicin.
- A therapeutic alternative is cotrimoxazole. The antibiotic regimen must be continued for three to four weeks.

8-Bordetella

(Whooping Cough, Pertussis)

- The genus **Bordetella, includes species B. pertussis, B. parapertussis, and B. bronchiseptica**. Of the three, the pathogen responsible for **whooping cough**, B. pertussis, is of greatest concern for humans.
- **B. pertussis bacteria are small, coccoid, nonmotile, Gram-negative rods that can be grown aerobically on special culture mediums at 37 C for 3 to 4 days.**

Whooping Cough

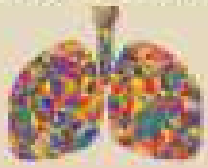
Also known as Pertussis.
An upper respiratory tract infection caused by Bordetella Pertussis

Pregnant women should get vaccinated during each pregnancy to protect their newborns

Symptoms:
1 week after exposure - Low fever, runny nose, mild cough, vomiting, diarrhea, apnea (in infants)

After 10 to 12 days severe coughing episodes ending with "whooping"

Complications:



-Pneumonia

30-40% of infant infections come from their mother

Spread by coughing, sneezing and other close contacts

-Encephalopathy
-Seizures
-Cerebral hypoxia

- Fractured ribs

-Ear infection

300,000 annual number of death

92% of deaths occur in infants under 4 months of age

30-50 million cases annually (worldwide)

Vaccination - The best defence

#roypath histopathology-india.net

Whooping Cough

- Bordetella pertussis is the causative organism of whooping cough, which affects only humans.
- The pathogens are transmitted by aerosol droplets.
- The organism is not characterized by specific invasive properties, although it is able to cause epithelial and subepithelial necroses in the mucosa of the lower respiratory tract.
-

Pathogenicity Factors of Bordetella pertussis

- Adhesion factors.
- Exotoxins.
- Endotoxins.
- Lipopolysaccharide:
-

•Therapy.

- Antibiotic treatment can only be expected to be effective during the catarrhal and early paroxysmal phases before the virulence factors are bound to the corresponding cell receptors.
- Macrolides are the agents of choice.

9-Treponema pallidum(Syphilis)

- **Syphilis affects only humans. The disease is normally transmitted by sexual intercourse.**
- Stage I (primary syphilis).
- Stage II (secondary syphilis).
- Latent syphilis.
- Stage III (tertiary or late syphilis).
- Syphilis connata.
- **Therapy**. **Penicillin G is the antibiotic agent of choice.**
- **Dosage and duration of therapy depend on the stage of the disease and the galenic formulation of the penicillin used.**

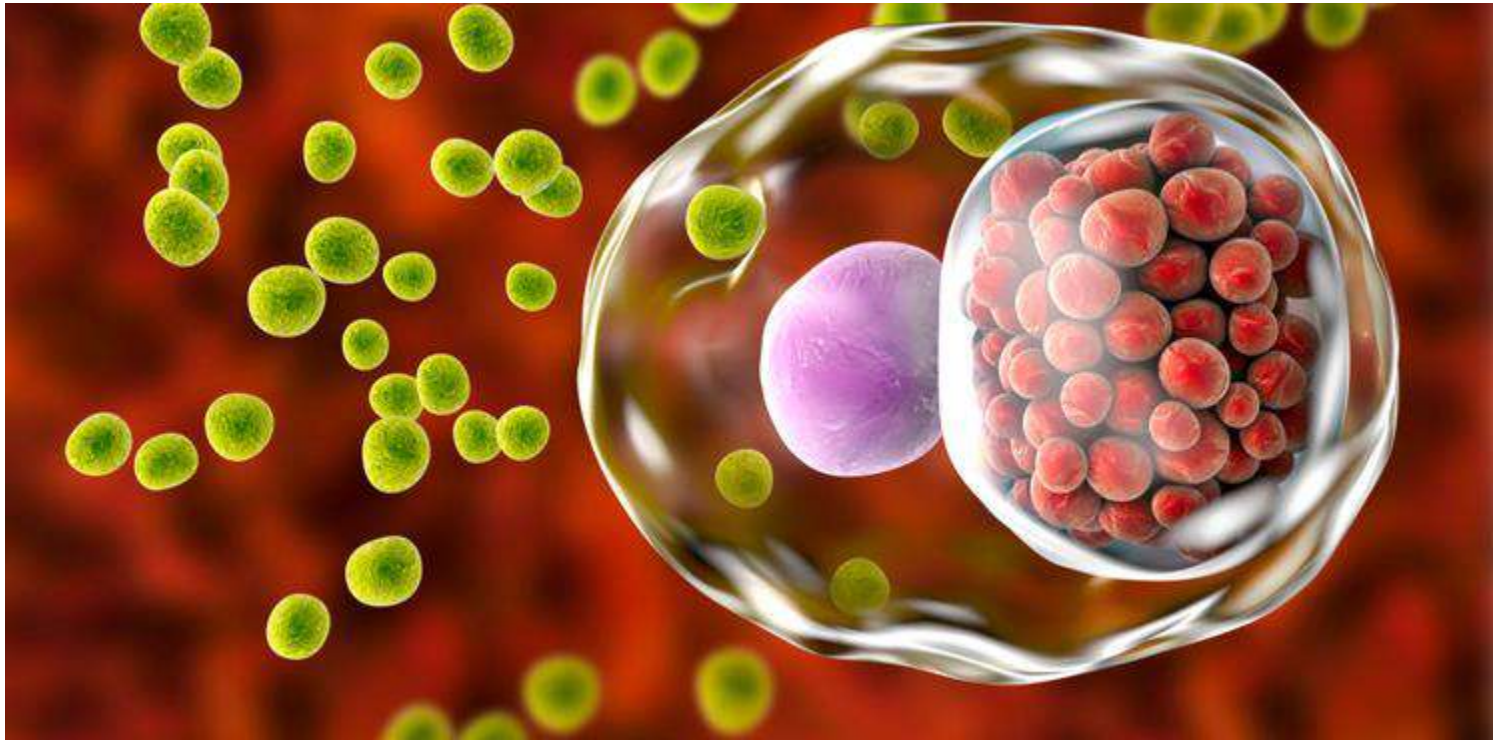
10-Rickettsia, Coxiella

(Typhus, Spotted Fever, Q Fever,)

- **The genera of the Rickettsiaceae and Coxelliaceae contain short, coccoid, small rods that can only reproduce in host cells.**
- With the exception of Coxiella (aerogenic transmission), they **are transmitted to humans via the vectors lice, ticks, fleas, or mites.**
- R. prowazekii and R. typhi cause typhus, a disease characterized by high fever and a spotty exanthem. Several rickettsiae species cause spotted fever, a milder typhuslike disease.
- **. Coxiella burnetii is responsible for Q fever, an infection characterized by a pneumonia with an atypical clinical course**

- Tetracyclines represent the antibiotic of choice for all of these infections.
- Typhus and spotted fever no longer occur in Europe.
- Q fever infections are reported from all over the world. Sources of infection include diseased sheep, goats, and cattle.
-

11- Chlamydia



11- Chlamydia

- Chlamydiae are obligate cell parasites.
- The three human pathogen species of chlamydiae are C. psittaci, C. trachomatis, and C. pneumoniae.
- Tetracyclines and macrolides are suitable for treatment of all chlamydial infections.

- **C. psittaci** is the cause of psittacosis or ornithosis. This zoonosis is a systemic disease of birds. The pathogens enter human lungs when dust containing chlamydiae is inhaled.

- After an incubation period of one to three weeks, pneumonia develops that often shows an atypical clinical course.

- C. pneumoniae

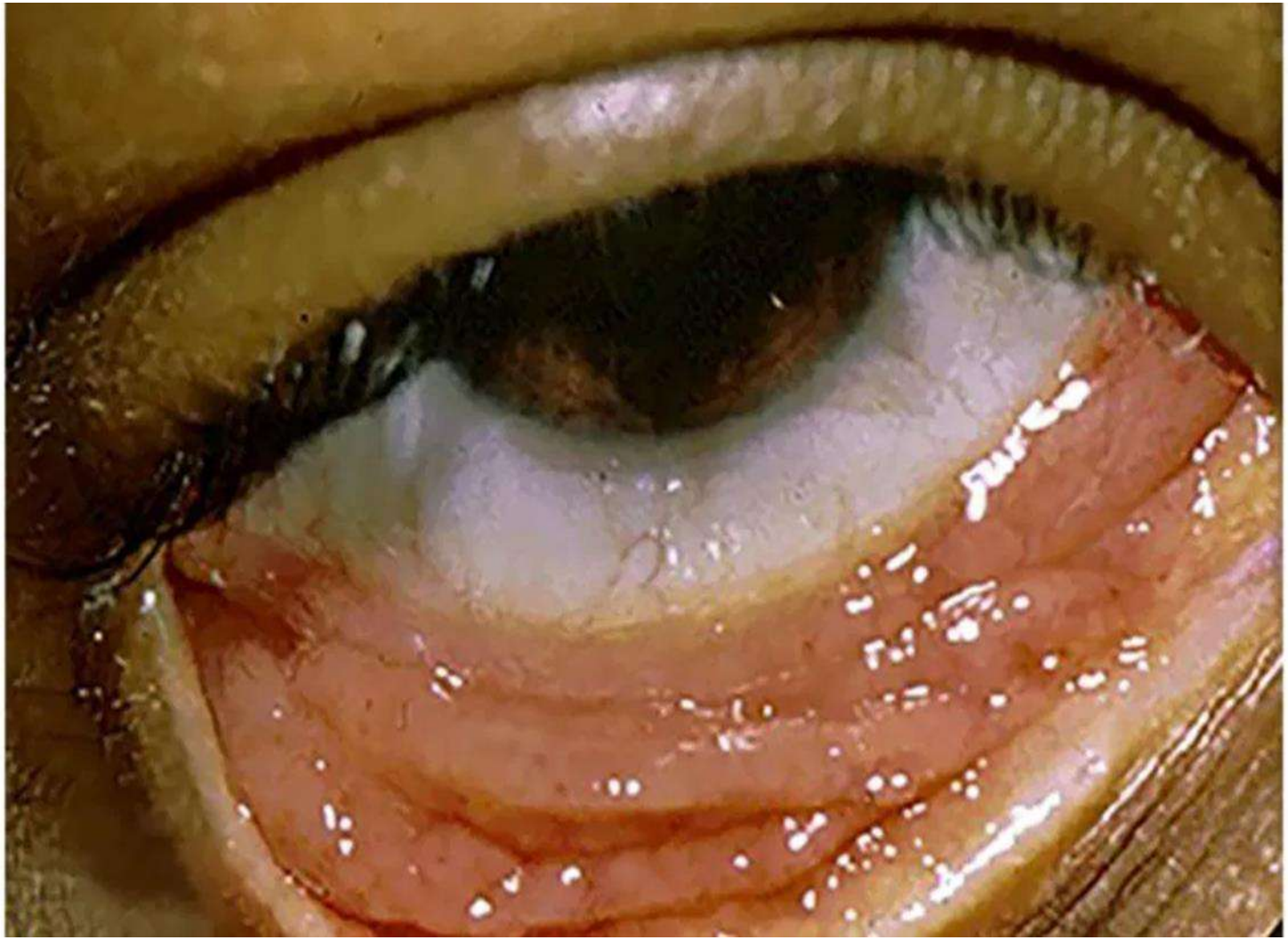
- C. pneumoniae is responsible for infections of the upper respiratory tract as well as for a mild form of pneumonia..

• C. trachomatis

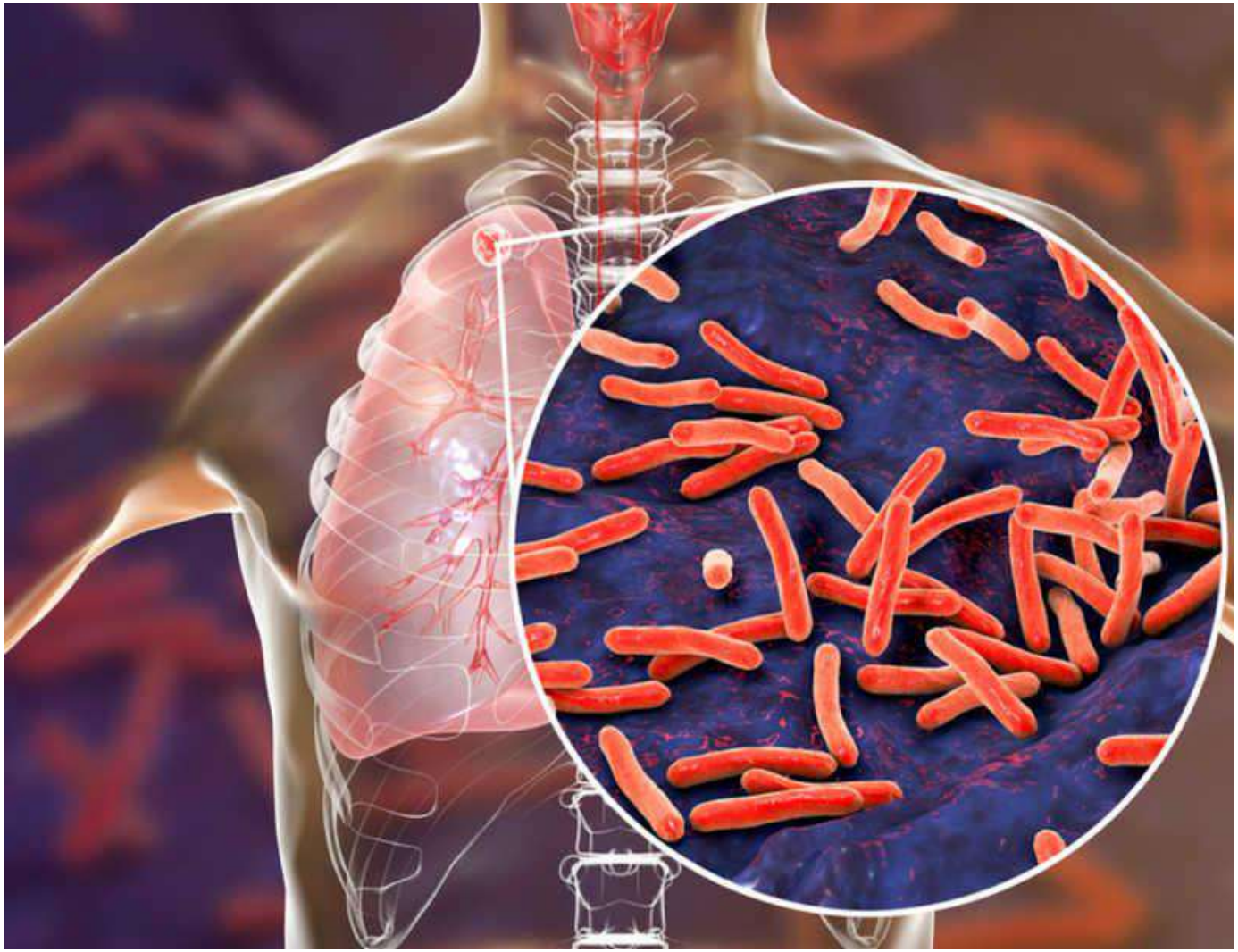
- is found only in humans.
- This species causes the following diseases:

1. Trachoma, a chronic follicular keratoconjunctivitis. The pathogens are transmitted by smear infection.
2. Inclusion conjunctivitis in newborn and swimming-pool conjunctivitis.
3. Nonspecific urogenital infections (urethritis, cervicitis, salpingitis, etc.).
4. Lymphogranuloma venereum, a venereal disease

C. trachomatis



12-Mycobacterium tuberculosis



12-Mycobacterium tuberculosis

- **Mycobacterium tuberculosis (M. tb)** is a species of pathogenic bacteria in the **family Mycobacteriaceae** and **the causative agent of tuberculosis.**
- First discovered in **1882 by Robert Koch**, M. tuberculosis has an unusual, **waxy coating on its cell surface primarily due to the presence** of **mycolic acid.**
- This **coating makes the cells impervious to Gram staining, and as a result, M. tuberculosis can appear either Gram-negative or Gram-positive.**
- **Acid-fast stains** such as **Ziehl-Neelsen,** or **fluorescent stains such as auramine** are used instead to **identify M. tuberculosis with a microscope**

- The **physiology of M. tuberculosis is highly aerobic and requires high levels of oxygen.**

- Primarily a **pathogen of the mammalian respiratory system, it infects the lungs.**

- The most frequently used diagnostic methods for tuberculosis are the **tuberculin skin test, acid-fast stain, culture, and polymerase chain reaction.**

- **M. tuberculosis is part of a complex that has at least 9 members**: M. tuberculosis sensu stricto, M. africanum, M. canetti, M. bovis, M. caprae, M. microti, M. pinnipedii, M. mungi, and M. orygis.

- It requires oxygen to **grow, does not produce spores, and is nonmotile**.
- M. tuberculosis divides **every 15–20 hours**. This is **extremely slow** compared with other bacteria, which tend to have division times measured in minutes (**Escherichia coli can divide roughly every 20 minutes)**
- . **It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks**. Its unusual cell wall is rich in lipids such as mycolic acid and is likely responsible **for its resistance to desiccation and is a key virulence factor.**

Pathophysiology

- **Humans are the only known reservoirs of *M. tuberculosis*.**
- A misconception is that *M. tuberculosis* can be spread by shaking hands, making contact with toilet seats, sharing food or drink, sharing toothbrushes, or kissing.
- It can **only be spread** **through air droplets** **originating from a person who has the disease either coughing, sneezing, speaking, or singing**

TB bacteria become lighter in weight due to water loss
(The size of a bacteria after water loss will be several microns)

TB bacteria

Sneezing and coughing

Droplet nuclei of TB bacteria
(several microns in size)

The healthy person
will not become infected
if the TB bacteria are
destroyed inside the nasal
passage or throat

Lung cavity

Lungs

Moisture in mucus

Patient

People close to the patient

Japan Anti-Tuberculosis Association:Common sense of Tuberculosis 2007,2,2007

Treatment of TB

- You'll be prescribed **at least a six-month course of a combination** of antibiotics if **you're diagnosed with active pulmonary TB**, where your lungs are affected and you have symptoms.

The usual treatment is:

- **Two antibiotics** (isoniazid and rifampicin) **for six months**
- **Two additional antibiotics** (pyrazinamide and ethambutol) for **the first two months of the six-month treatment period**
- It may be several weeks before you start to feel better. The exact length of time will depend on your overall health and the severity of your TB.
- After taking antibiotics for two weeks, most people are no longer infectious and feel better.

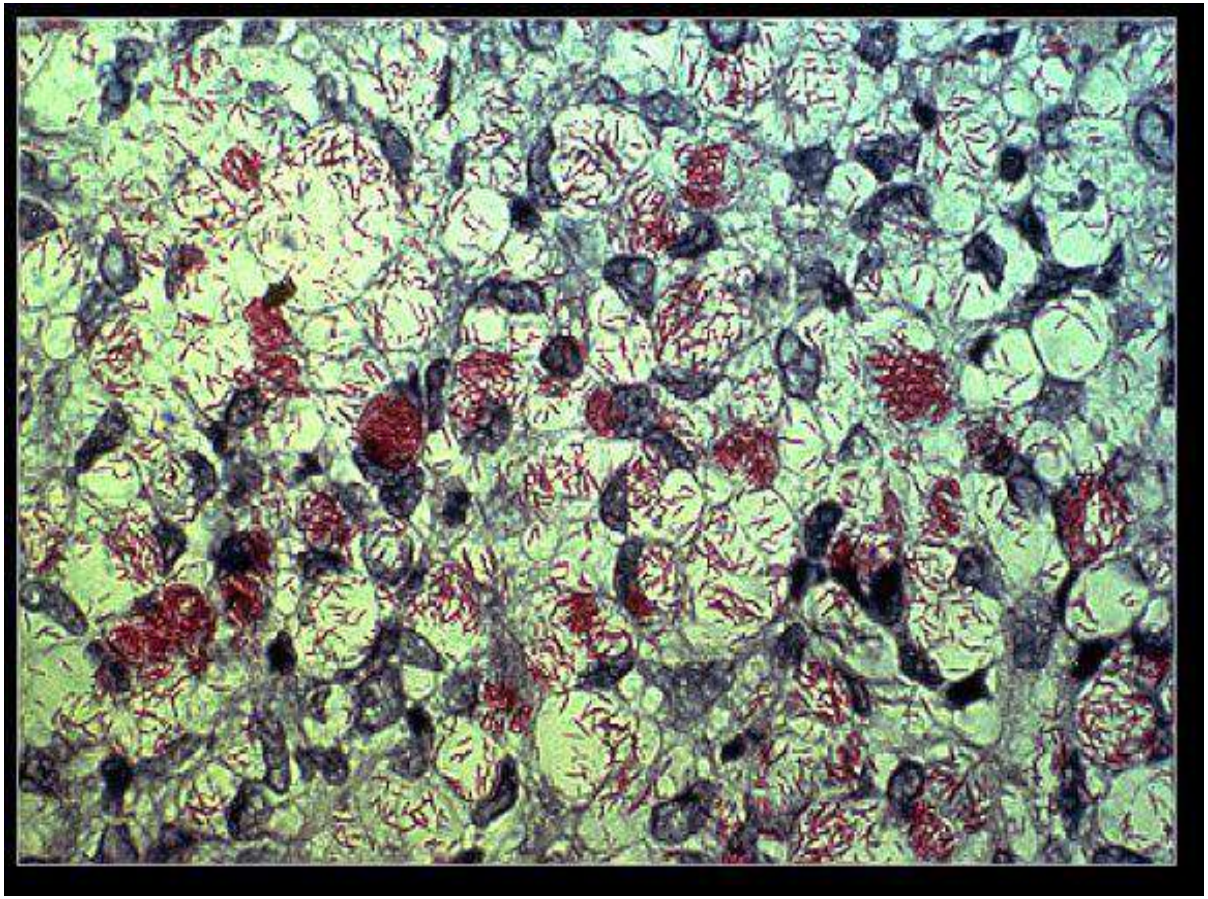
Vaccinations

- The BCG vaccine (bacille Calmette-Guerin), which was derived from M. bovis, has had limited success in preventing tuberculosis.

- This vaccine is used in countries that are notorious for having cases of M. tuberculosis, therefore it is not a recommended vaccine in the United States due to the low risk of infection.

- To receive this vaccine, the individual is required to go through a consultation process with an expert in M. tb and is only given to those who meet the specific criteria.

13-Mycobacterium lepra



Leprosy

- **Leprosy**, also known as Hansen's disease (HD), is **a long-term infection by the bacteria Mycobacterium leprae**
- Initially, a **person who is infected does not have symptoms and typically remains this way for 5 to 20 years.**
- Infection can **lead to damage of the nerves, respiratory tract, skin, and eyes. Approximately 30% of people affected with leprosy experience nerve damage.** The nerve damage sustained is irreversible, even with treatment of the infection **which can lead to the loss of parts of a person's extremities from repeated injuries or infection due to unnoticed wounds.**

Mode of transmission &Diagnosis

- Spread is thought to occur through a cough or contact with fluid from the nose of a person infected by leprosy.
- **Leprosy is not spread during pregnancy to the unborn children or through sexual contact.**
- **Leprosy occurs more commonly among people living in poverty.**
- The diagnosis is confirmed by finding acid-fast bacilli in a biopsy of the skin or by detecting the bacteria's DNA using polymerase chain reaction.

Treatment

- Leprosy is curable with multidrug therapy. Treatment of paucibacillary leprosy is with the medications dapsone, rifampicin, and clofazimine for six months

A
B

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168911152

The end

of medical

Bacteriology

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