PHARMACEUTICAL MICROBIOLOGY

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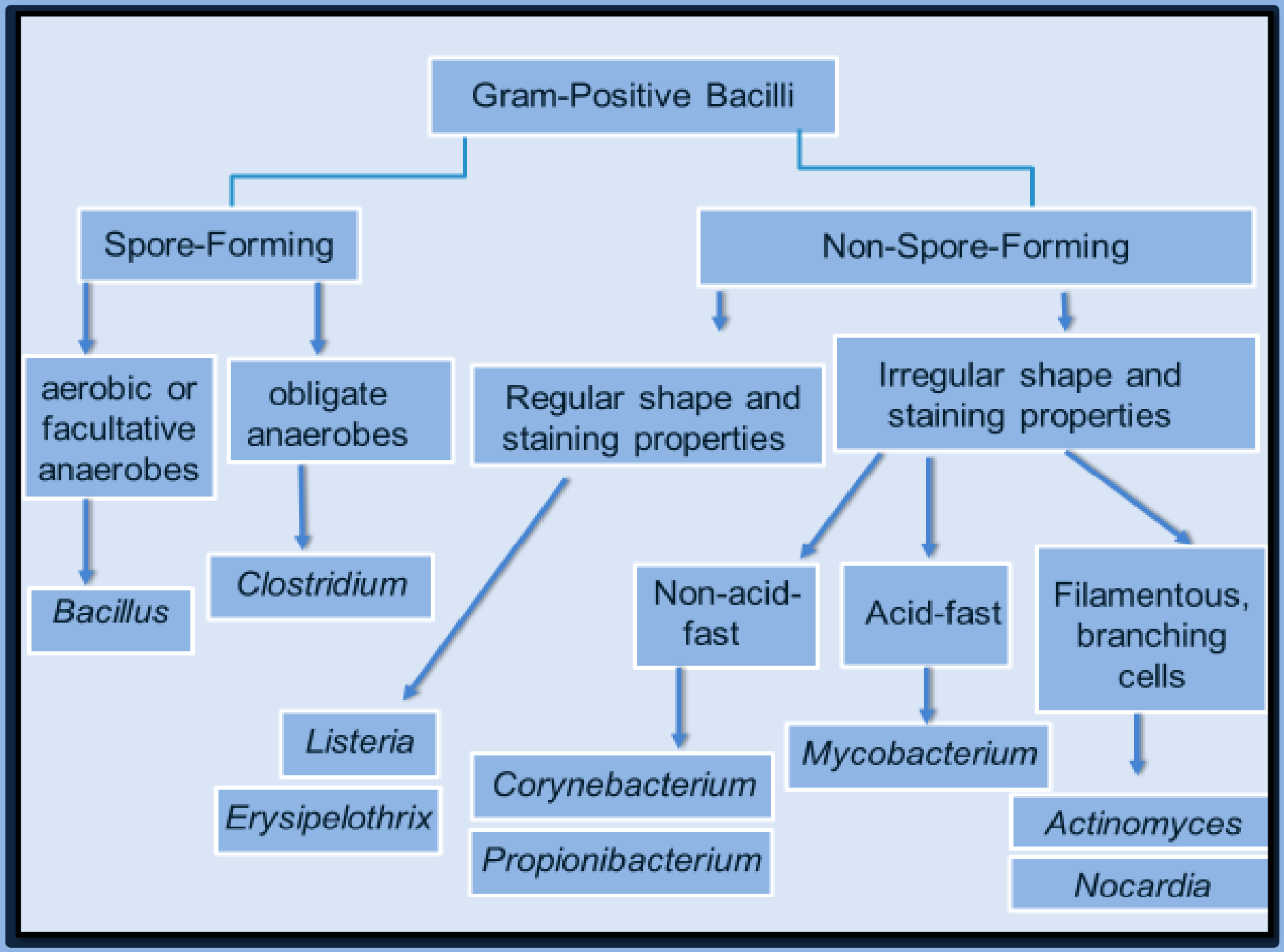

Gram-Positive Bacilli
Spore-Forming
Non-Spore-Forming
aerobic or facultative anaerobes
obligate anaerobes
Regular shape and staining properties
Irregular shape and staining properties
Bacillus
Clostridium
Listeria
Erysipelothrix
Non-acid-fast
Acid-fast
Filamentous, branching cells
Corynebacterium
Propionibacterium
Mycobacterium
Actinomyces
Nocardia

OBJECTIVES

Spore-Forming Gram-Positive Bacilli:

- **Bacillus**
  - *Bacillus anthracis*
  - *Bacillus cereus*

Spore-Forming Gram-Positive Bacilli

The family Bacillaceae comprises the rod-shaped bacteria which form endospores. The two principal subdivisions are the anaerobic spore-forming bacilli of the genus Clostridium and the aerobic or facultatively anaerobic endospore formers constituting the genus Bacillus.

Bacillaceae

Bacillus
aerobic or facultative anaerobic spore-forming bacilli

Clostridium
anaerobic spore-forming bacilli

Spore-Forming Gram-Positive Bacilli

- Bacterial spores are made of tough outer layers that are resistant to chemicals, staining and heat.

- The spores are resistant to heat, cold, radiation, desiccation, and disinfectants. They can survive in the environment for many years.

- The spore allows the bacterium to remain dormant for years, protecting it from external factors, including temperature differences, absence of air, water and nutrients.

Bacillus

- The genus Bacillus includes large aerobic, gram-positive rods occurring in chains.

- Most members of this genus are saprophytic organisms prevalent in soil, water, and air and on vegetation, such as *Bacillus subtilis*.

- Some are insect pathogens, such as *B. thuringiensis*. This organism is also capable of causing disease in humans.

Bacillus

- *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* may 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. Because of its potent toxins, it is a major potential agent of bioterrorism and biologic warfare.

Bacillus anthracis

- *B. anthracis* is a large (4-8 x 1-1.5 µm), **non-motile**, gram-positive rod that exists in two forms:
    - **the vegetative bacillus**
    - **the spore**

- The **spores** of *B. anthracis* are **oval and central in position**.

- The temperature range for growth is 12-45 ºC (optimum 35 ºC); it grows as typical colonies with a wavy margin and small projections, the so-called ‘medusa head’ appearance.

Bacillus anthracis

- It can be grown in an ordinary nutrient medium under aerobic or anaerobic conditions.

- The pathogenicity of *B. anthracis* depends primarily on two major virulence factors:
  - **protein capsule (poly-γ-D-glutamic acid)**
  - **the toxin complex comprising three proteins;**
    - protective antigen (PA)
    - edema factor (EF)
    - lethal factor (LF)

Bacillus anthracis

- The capsule appears to enhance the virulence of *B. anthracis* by inhibiting the phagocytosis of vegetative cells in the extracellular environment of the lymphatic system and bloodstream.

- It is mainly the action of the toxin that mediates damage to the host.

Bacillus anthracis

- **Protective Antigen (PA)** is a protein that binds to specific cell receptors, and after proteolytic activation, it forms a membrane channel that mediates entry of Edema Factor (EF) and Lethal Factor (LF) into the cell.

- **EF** is an adenylate cyclase; with **PA**, it forms a toxin known as **Edema Toxin. Edema toxin is responsible for cell and tissue edema.**

- **LF plus PA form Lethal Toxin**, which is a major virulence factor and **cause of death in infected animals and humans.**

Bacillus anthracis

- *B. anthracis* has a single chromosome which is a circular DNA molecule.
- It also has **two circular, extrachromosomal,** double-stranded DNA **plasmids, pXO1 and pXO2.** Both the pXO1 and pXO2 plasmids are **required for full virulence.**
- **The pXO1 plasmid** contains the genes that **encode** for the **anthrax toxin components: protective antigen (PA), lethal factor (LF), edema factor (EF)**
- **pXO2 encodes** a five-gene operon which synthesizes a **poly-γ-D-glutamic acid (polyglutamate) capsule.** This capsule allows *B. anthracis* to evade the host immune system by protecting itself from phagocytosis.

Bacillus anthracis

- When conditions are not conducive to growth and multiplication of vegetative bacilli, *B. anthracis* tends to form spores.

- Sporulation requires **a nutrient poor environment** and the **presence of free oxygen**.

- The spore form is the predominant phase in the environment, can survive for decades in soil, and it is through the uptake of spores that **anthrax** is contracted.

- **Anthrax is a zoonosis** (a disease of animals transmissible secondarily to man).

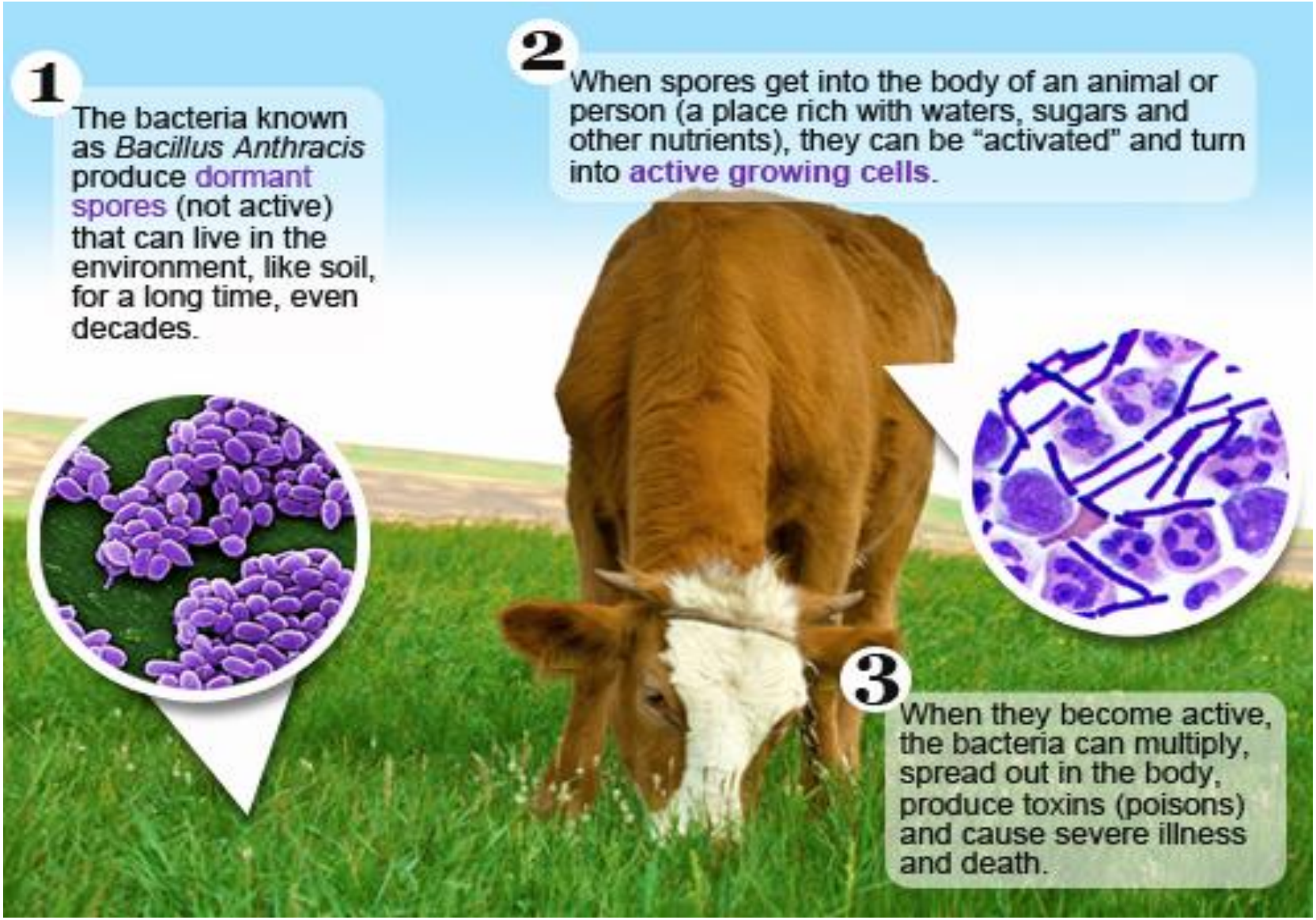
Bacillus anthracis

- Within an infected host, spores germinate to produce the vegetative forms which eventually kill the host.

- These bacilli are released by the dying or dead animal into the environment (usually soil under the carcass). There they sporulate, ready to be taken up by another animal.

- Spores from contaminated soil find easy access when ingested with spiny or irritating vegetation.

Bacillus anthracis



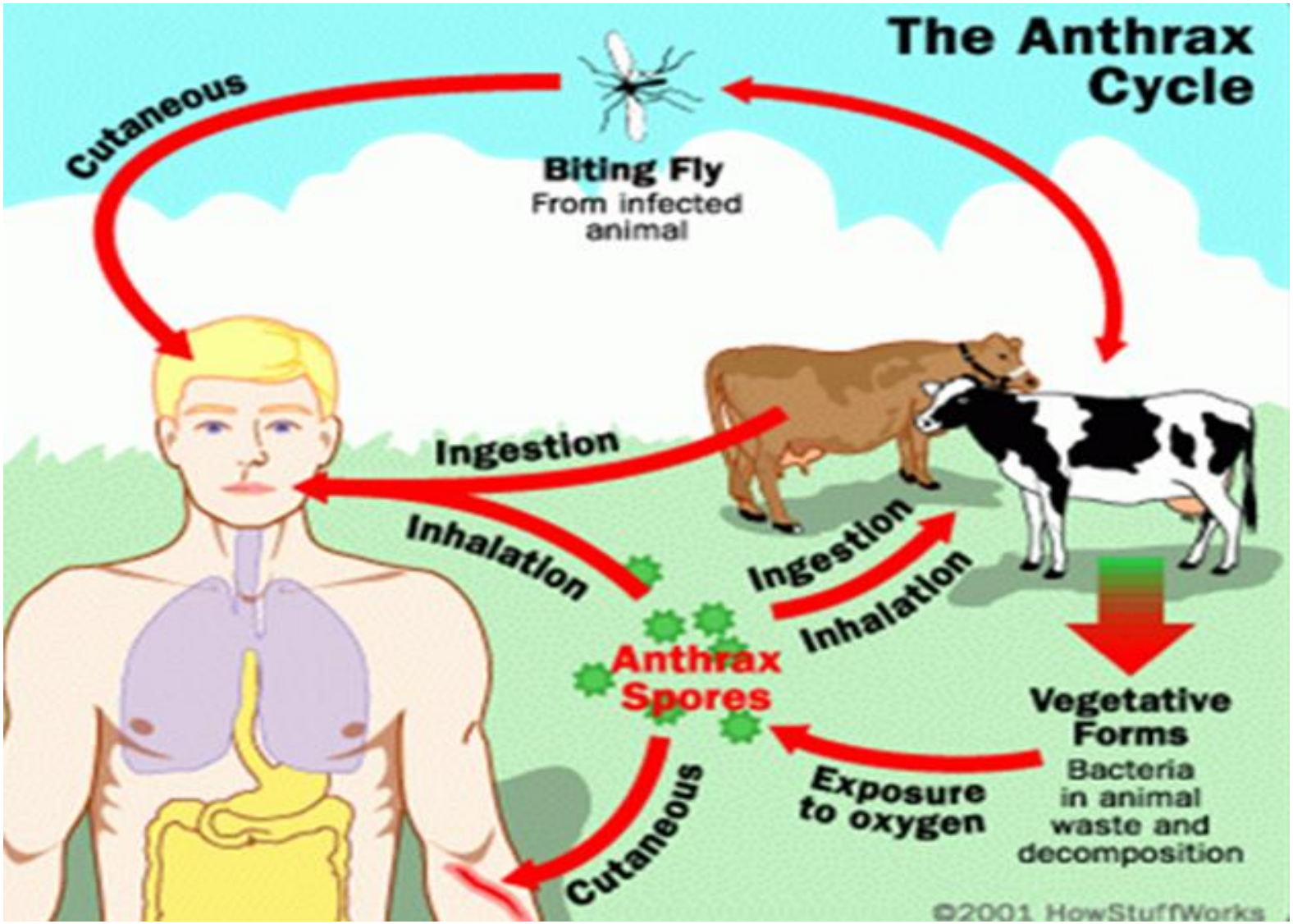
https://www.cdc.gov/anthrax/basics/index.htm

Bacillus anthracis

- People can get sick with anthrax if they come in contact with infected animals or contaminated animal products.

- There are four main routes of anthrax transmission in humans.
    - Cutaneous Anthrax
    - Inhalation Anthrax
    - Gastrointestinal Anthrax
    - Injection Anthrax

Bacillus anthracis



https://science.howstuffworks.com/anthrax1.htm

Bacillus anthracis

Cutaneous Anthrax

- When anthrax spores get into the skin, usually through a cut or scrape, a person can develop cutaneous anthrax. This can happen when a person handles infected animals or contaminated animal products like wool, hides, or hair.

- Biting flies are also suspected of being mechanical vectors of anthrax to humans under certain conditions.

- Cutaneous anthrax is most common on the head, neck, forearms, and hands.

Bacillus anthracis

Cutaneous Anthrax

- It affects the skin and tissue around the site of infection.

- Cutaneous anthrax is the most common form of anthrax infection, and it is also considered to be the least dangerous. Infection usually develops from 1 to 7 days after exposure. Without treatment, up to 20% of people with cutaneous anthrax may die. However, with proper treatment, almost all patients with cutaneous anthrax survive.

Bacillus anthracis

Inhalation Anthrax

- When a person breathes in anthrax spores, they can develop inhalation anthrax. People who work in places such as wool mills, slaughter houses, and tanneries may breathe in the spores when working with infected animals or contaminated animal products from infected animals.

- Inhalation anthrax starts primarily in the lymph nodes in the chest before spreading throughout the rest of the body, ultimately causing severe breathing problems and shock.

Bacillus anthracis

Inhalation Anthrax

- Inhalation anthrax is considered to be the most deadly form of anthrax. Infection usually develops within a week after exposure, but it can take up to 2 months.

- Without treatment, only about 10 – 15% of patients with inhalation anthrax survive. However, with aggressive treatment, about 55% of patients survive.

Bacillus anthracis

Gastrointestinal Anthrax

- When a person eats raw or undercooked meat from an animal infected with anthrax, they can develop gastrointestinal anthrax. Once ingested, anthrax spores can affect the upper gastrointestinal tract (throat and esophagus), stomach, and intestines.
- Infection usually develops from 1 to 7 days after exposure. Without treatment, more than half of patients with gastrointestinal anthrax die. However, with proper treatment, 60% of patients survive.

Bacillus anthracis

Injection Anthrax

- Recently, another type of anthrax infection has been identified in heroin-injecting drug users in northern Europe.

- Symptoms may be similar to those of cutaneous anthrax, but there may be infection deep under the skin or in the muscle where the drug was injected. Injection anthrax can spread throughout the body faster and be harder to recognize and treat.

Bacillus anthracis

- A number of anthrax vaccines have been developed for preventive use in livestock and humans.

- Anthrax vaccine adsorbed may protect against cutaneous and inhalation anthrax.

- However, this vaccine is only used for at-risk adults before exposure to anthrax and has not been approved for use after exposure.

Bacillus anthracis

- Anyone who is at increased risk of being exposed to anthrax, including certain military personnel, laboratory workers, and some people who handle animals or animal products (such as veterinarians who handle infected animals), may get the vaccine.

- These people should get 3 doses of vaccine: the first dose when risk of a potential exposure is identified, and the remaining doses at 1 and 6 months after the first dose.

Bacillus anthracis

- Infections with *B. anthracis* can be treated with **β-lactam antibiotics such as penicillin**, and others which are active against Gram-positive bacteria.

- **Penicillin-resistant *B. anthracis*** can be treated with **fluoroquinolones such as ciprofloxacin or tetracycline antibiotics such as doxycycline.**

Bacillus anthracis

- *Bacillus anthracis*, the bacteria that causes anthrax, is one of the most likely agents to be used in a **biological attack (bioterrorism).**

- Anthrax has been used as a weapon around the world for nearly a century. In 2001, powdered anthrax spores were deliberately put into letters that were mailed through the U.S. postal system. Twenty-two people, including 12 mail handlers, got anthrax, and five of these 22 people died.

Summary of *Bacillus anthracis*

- Spore-forming, gram positive rods
- Non-motile, facultative anaerobe
- Nonfastidious* growth of nonhemolytic colonies that are firmly adherent to the agar surface
- Polypeptide capsule consisting of poly-γ-D-glutamic acid
- Capsule is present in virulent strains
- Virulent strains also produce three exotoxins that combine to form edema toxin (combination of protective antigen and edema factor) and lethal toxin (protective antigen with lethal factor)

(*Nonfastidious bacteria are able to grow without special nutritional supplements or conditions applied to agar growth plates)

Summary of *Bacillus anthracis*

- Spores can survive in soil for years
- *B. anthracis* primarily infects herbivores with humans as accidental hosts.
- Rarely isolated in developed countries but is prevalent in impoverished areas where vaccination of animals is not practiced.
- Individuals at risk include people in endemic areas in contact with infected animals or contaminated soil.
- The greatest danger of anthrax in industrial countries is the use of *B. anthracis* as an agent of bioterrorism.

Summary of *Bacillus anthracis*

- Preliminary identification is based on microscopic (gram positive, nonmotile rods) and colonial (nonhemolytic adherent colonies) morphology.
- Confirmed by demonstrating capsule.
- Ciprofloxacin is the drug of choice; penicillin, doxycyline, erythromycin, or chloramphenicol may be used (if susceptible), but the bacteria are resistant to sulfonamides and extended-spectrum cephalosporins.
- Animal vaccination is effective, but human vaccines have limited usefulness.

Bacillus

Bacillus species other than *B. anthracis* are primarily opportunistic pathogens that have relatively low capacities for virulence.

Microorganism	Diseases
B. anthracis	Anthrax (cutaneous, gastrointestinal, inhalation)
B. cereus	Gastroenteritis (emetic, diarrheal), ocular infections, catheter related sepsis, opportunistic infections
B. mycoides	Gastroenteritis, opportunistic infections
B. thuringiensis	Gastroenteritis, opportunistic infections
Other Bacillus species	Opportunistic infections

Bacillus cereus

- Although most of these species have been found to cause disease, *B. cereus* is clearly the most important pathogen, with gastroenteritis, ocular infections, and intravenous catheter-related sepsis the diseases most commonly observed.

- Food poisoning caused by *B. cereus* has two distinct forms, the **emetic type**, which is associated with fried rice, milk, and pasta, and the **diarrheal type**, which is associated with meat dishes and sauces.

Bacillus cereus

Gastroenteritis caused by *B. cereus* is mediated by one of two enterotoxins.

	Emetic Form	Diarrheal Form
Implicated food	Rice	Meat, vegetables
Incubation period (hours)	<6 (mean, 2)	>6 (mean, 9)
Symptoms	Vomiting, nausea, abdominal cramps	Diarrhea, nausea, abdominal cramps
Duration (hours)	8-10 (mean, 9)	20-36 (mean, 24)
Enterotoxin	Heat stable	Heat labile

Bacillus cereus

- The **heat-stable, proteolysis-resistant enterotoxin** causes the **emetic form** of the disease, and the **heat-labile enterotoxin** causes the **diarrheal form** of the disease.

- The **heat-labile enterotoxin** is similar to the enterotoxins produced by *Escherichia coli* and *Vibrio cholerae*; **each stimulates the adenylate cyclase-cyclic adenosine monophosphate system** in intestinal epithelial cells, leading to profuse watery **diarrhea.**

- The mechanism of action of the heat-stable enterotoxin is unknown.

Bacillus cereus

- *B. cereus* is an important cause of eye infections, such as severe keratitis and endophthalmitis.

- Typically, the organisms are introduced into the eye by foreign bodies associated with trauma but infections can also occur after surgery.

- At least three toxins have been implicated from the ocular infections:
  - necrotic toxin (a heat-labile enterotoxin)
  - cereolysin (a potent hemolysin)
  - phospholipase C (a potent lecithinase; cleaves phospholipids)

Bacillus cereus

- It is likely that the rapid destruction of the eye that is characteristic of *B. cereus* infections results from the interaction of these toxins and other unidentified factors.

- *B. cereus* has also been associated with localized infections, such as wound infections, and with systemic infections, including endocarditis, catheter-associated bacteremia, central nervous system infections, osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use predisposes to these infections. Outbreaks of bacteremia in neonatal intensive care units and other hospital units during construction in health care facilities have been reported.

Bacillus cereus

- As mentioned previously, *B. cereus* is responsible for two forms of food poisoning:
    - vomiting disease (emetic form)
    - diarrheal disease (diarrheal form)

- The emetic form results from the consumption of contaminated rice. Most bacteria are killed during the initial cooking of the rice, but the heat-resistant spores survive.

Bacillus cereus

- If the cooked rice is not refrigerated, the spores germinate, and the bacteria can multiply rapidly.
- The heat-stable enterotoxin that is released is not destroyed when the rice is reheated.
- After ingestion of the enterotoxin and a 1 to 6 hour incubation period, a disease of short duration (less than 24 hours) develops.
- Symptoms consist of vomiting, nausea, and abdominal cramps. Fever and diarrhea are generally absent.

Bacillus cereus

- The diarrheal form of *B. cereus* food poisoning results from the consumption of contaminated meat, vegetables or sauces.

- There is a longer incubation period, during which the organism multiplies in the patient's intestinal tract and produces the heat-labile enterotoxin.

- This enterotoxin is responsible for the diarrhea, nausea and abdominal cramps. This form of disease generally lasts 1 day or longer.

Bacillus cereus

- Like *B. anthracis*, other Bacillus species can be readily grown in the laboratory.

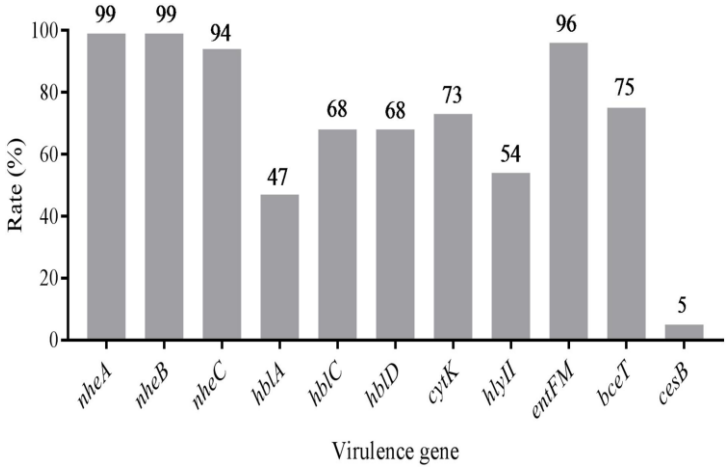
- For confirmation of the existence of foodborne disease, the implicated food (e.g. rice, meat, vegetables) should be cultured.

- Isolation of the organism from the patient should not be attempted, because fecal colonization is common.

- However, isolation of the organism from the stools of a cluster of epidemiologically related patients is strong evidence implicating *B. cereus* as the causal agent.

Bacillus cereus

- They can be easily detected with Gram stain and culture of specimens collected from infected eyes, intravenous culture sites, and other locations.
- Because the course of *B. cereus* gastroenteritis is short and uncomplicated, symptomatic treatment is adequate.
- The treatment of other *Bacillus* infections is complicated by the fact that they have a rapid and progressive course and a high incidence of multiple-drug resistance (e.g. *B. cereus* carries genes for resistance to penicillins and cephalosporins).



Distribution of virulence genes in *Bacillus cereus* isolated from pasteurized milk in China

Bacillus cereus

- **Penicillins and cephalosporins are ineffective**.

- Vancomycin, clindamycin, ciprofloxacin and gentamicin can be used to treat infections. Serious non–food borne infections should be treated with vancomycin or clindamycin with or without an aminoglycoside. Ciprofloxacin has been useful for the treatment of wound infections.

- Rapid consumption of foods after cooking and proper refrigeration of uneaten foods can prevent food poisoning.

Summary of *Bacillus cereus*

- Spore-forming, motile gram positive rods
- Facultative anaerobe
- Nonfastidious growth requirements: β-hemolytic on sheep blood agar
- Heat-stable and heat-labile enterotoxin
- Spores can survive in soil
- Tissue destruction is mediated by cytotoxic enzymes, including cereolysin and phospholipase C.

Summary of *Bacillus cereus*

- People at risk include those who consume food contaminated with the bacterium (e.g., rice, meat, vegetables, sauces), those with penetrating injuries (e.g., to eye), and those who receive intravenous injections.

- Isolation of the organism in implicated food product or nonfecal specimens (e.g. eye, wound) is used for diagnosis.

- Gastrointestinal infections are treated symptomatically.

Summary of *Bacillus cereus*

Ocular infectious or other invasive diseases require removal of foreign bodies and treatment with vancomycin, clindamycin, ciprofloxacin or gentamicin.

Gastrointestinal disease is prevented by proper preparation of food (e.g. foods should be consumed immediately after preparation or refrigerated)

***Bacillus* sp.**

Bacillus subtilis, *B. pumilis* and *B. licheniformis* have been implicated in causing food poisoning similar to that due to *B. cereus.*

They do not appear to form toxins, but some strains produce antibacterial peptides, such as the antibiotic bacitracin, which may facilitate growth in the intestinal tract.

Bacillus polymyxa is the source of the antibiotic polymyxin.