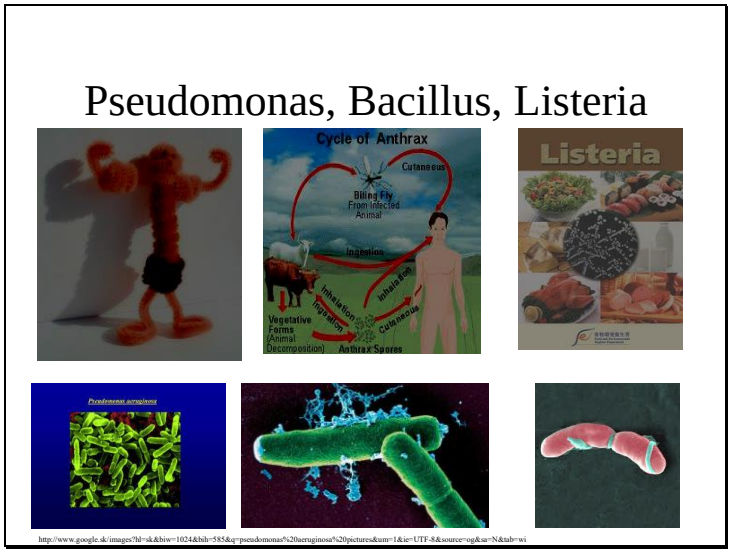
Snímka 1



Pseudomonas is a type of bacteria (germ) that is found commonly in the environment, like in soil and in water. Of the many different types of *Pseudomonas*, the one that most often causes infections in humans is called *Pseudomonas aeruginosa*, which can cause infections in the blood, lungs (pneumonia), or other parts of the body after surgery. P*seudomonas aeruginosa* can be spread to people in healthcare settings when they are exposed to water or soil that is contaminated with these germs. Resistant strains of the germ can also spread in healthcare settings from one person to another through contaminated hands, equipment, or surfaces.

Bacillus is a very diverse genus with more than 200 species, and the identification and diagnosis of potential disease-causing *Bacillus* species from patient material by the clinical lab can be challenging. *Bacillus* includes both free-living (nonparasitic) species, and two parasitic pathogenic species. These two *Bacillus* species are medically significant: *B. anthracis* causes anthrax; and *B. cereus* causes food poisoning.

Listeria is a genus of bacteria that acts as an intracellular parasite in mammals. *Listeria* can be found in soil, which can lead to vegetable contamination. Animals can be carriers. *Listeria* has been found in uncooked meats, uncooked vegetables, fruits including cantaloupe[12] and apples,[13] pasteurized or unpasteurized milk, foods made from milk, and processed foods. Pasteurization and sufficient cooking kill *Listeria*; however, contamination may occur after cooking and before packaging.

Snímka 2

Pseudomonas, Bacillus, Listeria

- Hospital infections
- Antrax a bioterrorism
- Microscopy - Gram - *P. aeruginosa, Bacillus anthracis, Bacillus cereus, Listeria monocytogenes*
- Wirtz Conklin- *B. anthracis*,
- Cultivation- *P. aeruginosa* -blood agar and Endo agar,
  - *Bacillus anthracis, Bacillus cereus*, Listeria – blood agar
- Biochemical properties - *P. aeruginosa*
- ATB therapy of Ps. aeruginosa

Snímka 3

Hospital infections

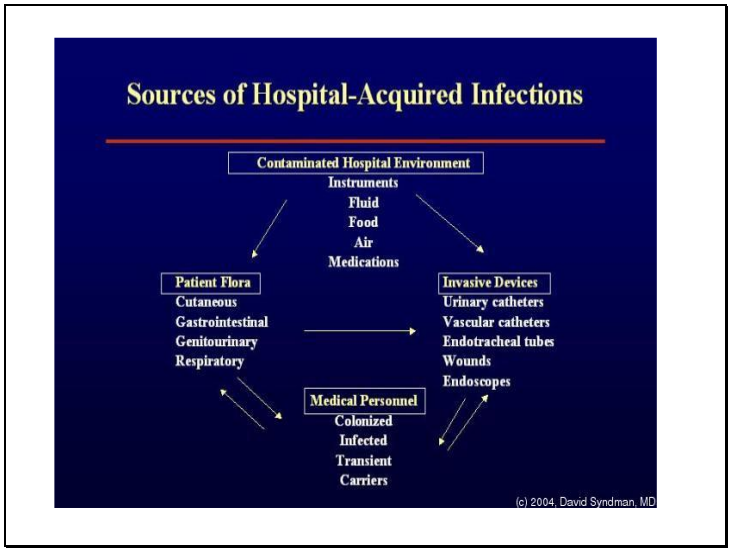
- infections acquired in the hospital due to the exposure to the pathogen in the hospital. It sometimes called as nosocomial infections or hospital-acquired infections (HAI)
- development of infections 48 hours after admission.

Hospital-acquired infections, also known as healthcare-associated infections (HAI), are nosocomially acquired infections that are typically not present or might be incubating at the time of admission. These infections are usually acquired after hospitalization and manifest 48 hours after admission to the hospital.

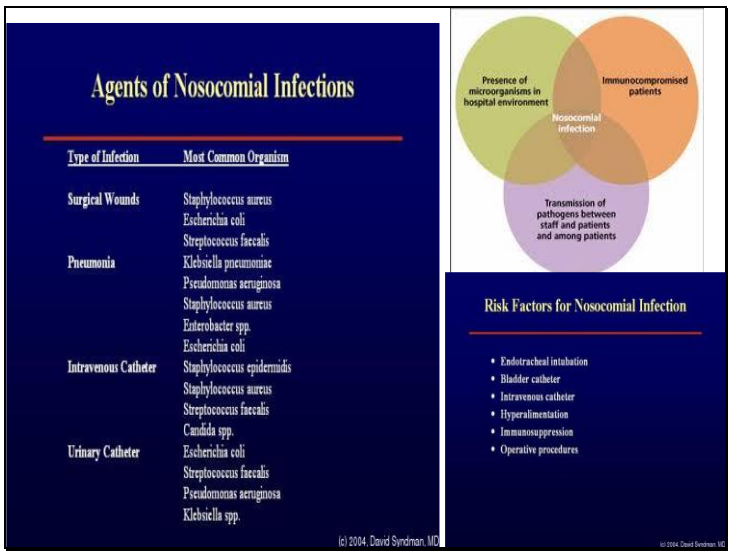
For the last few decades, hospitals have taken the hospital-acquired infections seriously. Several hospitals have established infection tracking and surveillance systems in place, along with prevention strategies to reduce the rate of hospital-acquired infections.

The impact of hospital-acquired infections is seen not just at an individual patient level, but also at the community level as they have been linked to multidrug-resistant infections. Identifying patients with risk factors for hospital-acquired infections and multidrug-resistant infections is very important in the prevention and minimization of these infections.

Hands of HCW's are the most common transmitter of disease in healthcare facilities•A disease transmitted from a HCW to a patient is known as a Healthcare Associated Infection (HAI) or a nosocomial infection•Pathogens can be transmitted to patients, HCWs and yourself by poorly sanitized hands •Gloves are NOT a substitute for hand hygiene



Transmission of pathogens in a health care environment is complex and can occur through direct contact with the healthcare workers or the surrounding contaminated environment.
Risk factors for the development of CDI are well known.
These include recent antibiotic use, gastric acid suppresants, nonselective non-steroidal anti-inflammatory drugs (NSAIDs), and some comorbidities.
Risk factors for SSI include both patient factors such as age, diabetes, obesity, nutritional status, colonization, co-existing infections, and operative factors such as duration of the procedure, skin antisepsis, surgical technique, antimicrobial prophylaxis.



These infections can have significant morbidity and mortality and have a large financial impact on hospital resources. They lead to increased stay length of infected patients, resulting in decreased total throughput of patients.
Multidrug-resistant (MDR) pathogens are significant cause of infections in hospitals, particularly in the intensive care unit. Infections with MDR organisms are associated with an increase in the length of stay (LOS), mortality indicators, and increased costs of care. Some pathogens possess a tendency to colonize in areas with warmth and moisture. These areas are typically located in the inguinal and perineal region, axilla, and trunk. Certain bacteria and fungi thrive in such environments.

MDR pathogens are resistant to at least one antibiotic from three different classes or with different mechanisms of action.
MDR organisms are often suspected of HAP.
The use of intravenous antibiotics within the past 90 days is an important risk factor for MDR infections.
Other risk factors for MDR include the presence of septic shock and duration of hospitalization
The three most common types of nosocomial infection are urinary tract infections, wound and soft tissue infections [including those resulting after procedures on the gastrointestinal (GI) tract], and nosocomial pneumonia, which frequently occurs due to aspiration of bacteria colonizing the oropharynx or upper gastrointestinal tract. However, nosocomial infections of the gastrointestinal tract are also a significant cause of morbidity and sometimes mortality in hospitals and nosocomial gastrointestinal infections can lead to multiple ward closures.
All hospitalized patients are susceptible to contracting a nosocomial infection. Some patients are at greater risk than others-young children, the elderly, and persons with compromised immune systems are more likely to get an infection. Other risk factors are long hospital stays, the use of indwelling catheters, failure of healthcare workers to wash their hands, and overuse of antibiotics.

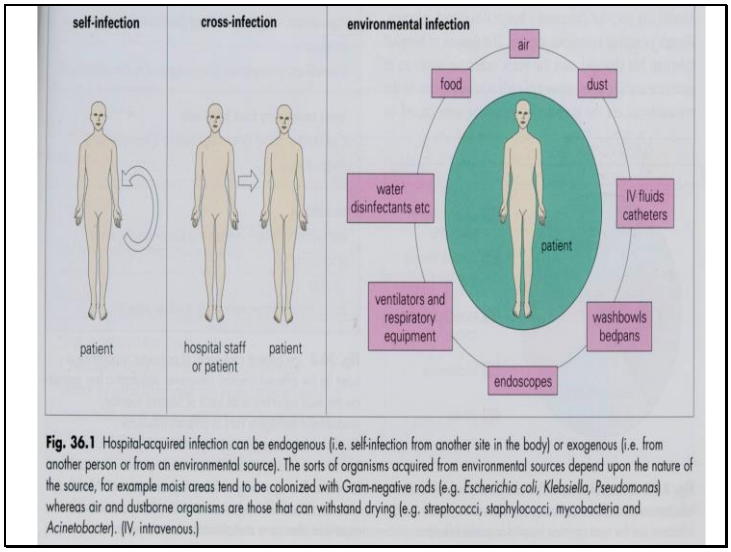


Fig. 36.1 Hospital-acquired infection can be endogenous (i.e. self-infection from another site in the body) or exogenous (i.e. from another person or from an environmental source). The sorts of organisms acquired from environmental sources depend upon the nature of the source, for example moist areas tend to be colonized with Gram-negative rods (e.g. *Escherichia coli, Klebsiella, Pseudomonas*) whereas air and dustborne organisms are those that can withstand drying (e.g. streptococci, staphylococci, mycobacteria and *Acinetobacter*). (IV, intravenous.)

Pathogens originate from several sources
Endogenous sources —most commonly, patient´s skin, mucous membranes or mucous membranes
Exogenous sources —contact of wound with contaminated environment, operating room personnel, air, surgical instruments
Hematogenous or lymphatic sources

Role of microbes in hospital infections

- Staphylococcus aureus - problems of per secundam healing wounds Virulence, colonisation capacitiy, resistence - MRSA - methicilin resistent *Staphylococcus aureus*
- G-rods (60% of HAI) - urinary tract infections, respiratory infections, wound infection, GIT
- Opportunistic pathogens - *Pseudomonas aeruginosa* (Hospital environment – food, cut flowers, water, towels, mops, respiration devices, desinfection solutions. Persistent carriage in less than 6% helathy, 38% in hospitalised, 78% imunocompromised) and other non fermenting G- rods – *Acinetobacter sp., Stenotrophomonas maltophilia, Burkholderia cepacia…* - present in environment (Legionella pneumophila - climatisation)
- PC negative staphylococci - colonisation of plastic material, indwelling catheters
- Viruses - blood borne infection agenses HIV, VHB, VHC, CMV….

Virtually every pathogen has the potential to cause infection in hospitalized patients but only limited number of both gram positive and gram negative bacteria are responsible for the majority of nosocomial infection. Among them Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Enterococci takes the leading

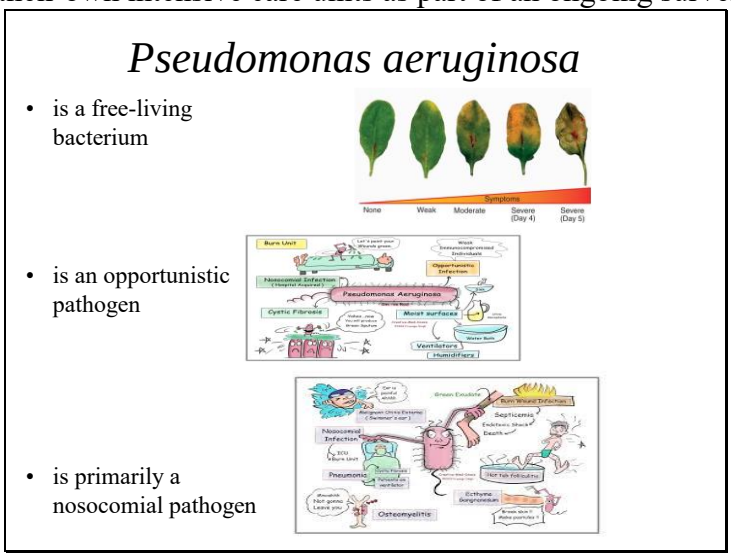
The clinical spectrum caused by nosocomial pathogens depend on body site of infection, the involving pathogen and the patient's underlying condition.
Structural and non structural virulence factors associated with the bacteria are responsible for the observed clinical manifestation.
Bacteria isolation and characterization from appropriate clinical materials with antimicrobial susceptibility testing is the standard of laboratory diagnosis.

ATB therapy in hospital infections

- **Overuse of ATB** - resistence and multiresistence (selection pressure of ATB and transmission in hospital environment), toxic side effects, economic burden, deterioration of physiological microflora
- **Racional indication** - preventive in spread of HI
- **Prophylaxis** - oriented to anaerobic infection - perioperative preparation for GIT and UGT surgery, in instrumental examination of patients with bacteriuria
- **ATB surveys** - monitoring of ATB susceptibility
- **Restrictive policy** - time restricted contraindication of some ATB
- **Rotation of atb** - periodical changes of used - decreasing of selection pressure
- **Combination of atb** - agains possible resistent mutnants, broader antimicrobial spectrum

Resistance to antimicrobial agents is emerging in a wide variety of pathogens, particularly those that cause nosocomial infections.
As a consequence of this increasing resistance, morbidity and mortality in nosocomial infections is also increasing. It is therefore critical to treat nosocomial infections appropriately by starting antimicrobial treatment early in the course of infection, using the correct agent, at the most appropriate dose, and for an adequate duration.

Selection of appropriate antimicrobial therapy requires a thorough understanding of the likely microbial cause of the infection, including local susceptibility patterns, as well as the properties of the antimicrobials available for treating these infections, namely spectrum of activity and potency (including activity versus known resistance mechanisms), pharmacokinetic profile and tolerability and safety.
With the rise in antimicrobial resistance have come a variety of strategies designed to prevent this problem. In a recent consensus conference, the Centers for Disease Control suggested that the problem could be better controlled through optimization of prophylactic, empiric, and therapeutic antimicrobial use and that this could be accomplished by education about appropriate antibiotic use and by providing data to physicians about the types of resistant organisms seen in their own intensive care units as part of an ongoing surveillance program



- is a free-living bacterium, commonly found in soil and water, it occurs regularly on the surfaces of plants and occasionally on the surfaces of animals. Moist surfaces example sink, water bath, humidifiers, ventilators and urine receptacles are its favorite.

- is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It causes disease when there is compromise in host defense system, mucosal injury or antibiotic mediated suppression of normal flora. Intensive Care Unit (ICU) and Burn unit patients are most commonly affected. Cystic Fibrosis cases harbor pseudomonas in their lungs causing recurrent respiratory infections.

- is primarily a nosocomial pathogen: According to the CDC, the overall incidence of *P. aeruginosa* infections in U.S. hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections

Pseudomonas aeruginosa – virulence factors

Oportunistic: factors of virulence:
Pilli - adherence

Alginat(mucoid exopolysaccharide) capsule – antiphagocytosis, attachment ,
Endotoxin – LPS - sepsis

Exotoxin A – most important, blocs proteosynthesis of eukaryotic cells

Alkalic protease – destruction of tissue

Phospholipase C – destruction of lipids and lecithin, destruction of tissue

Pyocyanin and Pyoverdine – leads to blue green pigmentation

http://www.google.sk/images?hl=sk&biw=1024&bih=585&q=pseudomonas%20aeruginosa%20pictures&um=1&ie=UTF-8&source=og&sa=N&tab=wi

This pathogen penetrates the affected skin or mucosa, invades them locally and produces systemic infection. It can also cause necrotizing pneumonia through contaminated respiratory equipment, necrotizing external otitis in elderly diabetic patients; ocular infection after surgical procedures or wounds; urinary tract infections through the insertion of catheters or irrigation solutions; meningitis after neurosurgery or endorcaditis after surgery or catheter usage. It produces large number of virulence factors which have pivotal role in variety of clinical manifestations. infected wounds and burns produces a greenish-blue exudate which is fluorescent under ultraviolet light and may present a characteristic odor of grapefruit.
Once the bacterium has passed through the affected area, it can penetrate the bloodstream causing septicemia. In this case, it is classical the presence of skin hemorrhagic necrosis characterized by round erythematous lesions, denominated ecthyma gangrenosum.
Pseudomonas aeruginosa's infection frequently affects the urinary tract, respiratory system, skin and mucosa, causing damage to the affected organ.
It produces large number of virulence factors which have important role in variety of clinical manifestations.
Flagellum and pili – flagellum provide motility which is important in host invasion and spread. Flagellum and pili both demonstrate adhesive properties and they adhere best on injured mucosal surfaces.
Endotoxin – it can lead to sepsis, shock and DIC (disseminated intravascular coagulation).
Exotoxin A- it impairs protein synthesis by inactivating elongation factor 2. Reduced protein synthesis causes tissue necrosis and prepare the environment for wound infection.
Thick Biofilm – Biofilm protects bacteria from phagocytosis and makes bacteria resistant to multiple drugs.
Pyocyanin and Pyoverdine – leads to blue green pigmentation and fruity odor to the cultured specimen, wound exudate and sputum.
Multidrug resistance – Beta lactamase and aminoglycoside modifying enzyme makes significant numbers of antibiotics useless on pseudomonas.

Pseudomonas aeruginosa microscopy

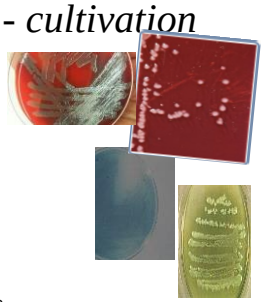
- is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm.
- Almost all strains are motile - single polar flagellum.

- It is identified on the basis of its Gram morphology,
- inability to ferment lactose,
- a positive oxidase reaction,
- its fruity odor,
- its ability to grow at 42°C.

Pseudomonas aeruginosa - cultivation

P. aeruginosa strains produce soluble pigments:
- fluorescent pigment pyoverdin
- blue pigment pyocyanin.

Pyocyanin (from "pyocyaneus") refers to "blue pus", which is a characteristic of suppurative infections caused by *Pseudomonas aeruginosa*.



- ability to grow at 42°C
- fruity odour

ENDO, DCA: inability to ferment lactose, H2S negative

A triple sugar iron agar (TSI) tube inoculated with *Pseudomonas aerugi*
incubated at 37°C for 24 hours results in an unchanged slant and butt.

OXIDASE TEST positive

http://www.google.sk/images?hl=sk&biw=1024&bih=585&q=pseudomonas%20aeruginosa%20pictures&um=1&ie=UTF-8&source=og&sa=N&tab=wi

Blood agar: Pseudomonas aeruginosa has very simple nutritional requirements. It is often observed "growing in distilled water", which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of Pseudomonas aeruginosa consists of acetate as a source of carbon and ammonium sulfate as a source of nitrogen. P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samplesproduces two colony types. One type has a fried-egg appearance which is large, smooth, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.
Dexycholate – citrate agar, Endo agar: P. aeruginosa does not ferment lactose, is H2S negative. Strains produce soluble pigments, the fluorescent pigment pyoverdin and the blue pigment pyocyanin. Pyocyanin (from "pyocyaneus") refers to "blue pus", which is a characteristic of suppurative infections caused by Pseudomonas aeruginosa.
A triple sugar iron agar (TSI) tube inoculated with *Pseudomonas aeruginosa* and incubated at 37°C for 24 hours results in an unchanged slant and butt.

Pseudomonas aeruginosa is oxidase positive - The oxidase test is a biochemical reaction that assays for the **presence of cytochrome oxidase**, an enzyme sometimes called indophenol oxidase.
In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product.

The oxidase test often uses a reagent, **tetra-methyl-*p*-phenylenediamine dihydrochloride (or KOVÁCS reagent**), as an artificial electron donor for cytochrome *c*.

When the reagent is oxidized by cytochrome *c*, it changes from colorless to a dark blue or purple compound, **indophenol blue.** On the left side in the picture is oxidase-positivePseudomonas aeruginosaand on the right is oxidase-negativeEscherichia coli.Both organisms were rubbed on a filter that had been dipped in Kovács oxidase reagent and allowed to dry

ATB susceptibility of Pseudomonas

- **Therapy** - Frustrating – immunocompromised defence of patient, typical ATB rezistence
  induction of ATB inactivating enzymes
  resistence transmission via plasmids
  Isolation without signs of infection is not indication for ATB therapeutic intervention
- Combination of ATB – beta lactams + aminoglycosides, Meronem, Imipenem, Sulperason, Cefoperason
- **Preventive approaches -** Interruption of contamination of steril materials and cross contamination. Decontamination of fits and and tubes and catheters and environment - nosocomial strains in intensive care units. Broad spectrum ATB use - very carefully - suppression of normal flora and overgrowth of resistent bacteria and mutants

Pseudomonas aeruginosa can develop fast resistance to antibiotics, so it is not possible to combat with only one drug. It is recommended the realization of antibiograms in order to choose the best antibiotic therapy.
The antibiotics that are most mostly used in PA infection's treatment are penicilins such as Piperacillin and Ticarcillin, aminoglycosides like Gentamicin and Amikacin, Imipenem, Quinolones, Aztreonam and Cephalosporines are also used

Listeria

- The genus *Listeria* consists of 10 species, with
- ***Listeria monocytogenes***

and

- ***Listeria ivanovii***

the only recognized pathogens.

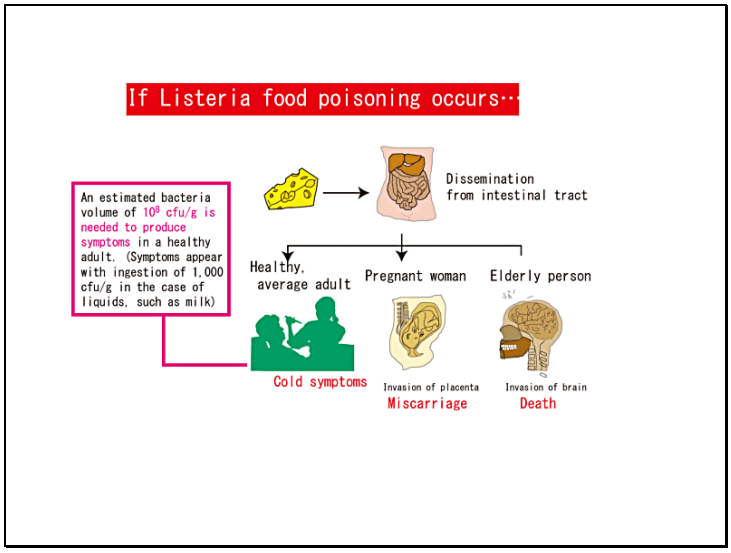
The genus *Listeria* is currently comprised of 17 species, including 10 *Listeria* species newly described since 2009. After going through several name changes since the bacteria were first described in 1911, the name was officially changed to *Listeria monocytogenes* in 1940 to honor Joseph Lister, the British surgeon who discovered that sterilizing surgical instruments before operations reduced the risk of infections.

L. monocytogenes is widespread in the environment, especially in soil and water. The bacteria can survive in soil for many months. Animals, particularly cattle, can carry *L. monocytogenes* without appearing sick and shed the bacteria in their feces.

The bacteria are able to live in a wide range of conditions and environments—they can tolerate both acidic and salty conditions, both high and low temperatures, and a fairly low moisture content. These characteristics allow *L. monocytogenes* to survive a long time in a variety of food products and food processing plants. Because the bacteria can multiply and persist in food processing plants for years.

L. monocytogenes is especially hard to control and can result in intermittent contamination of food.

Unlike most bacteria, *L. monocytogenes* can grow and multiply at low temperatures, making the bacteria a potential problem even in properly refrigerated food. One study also found a relatively high percentage of frozen raw beef products contaminated with *L. monocytogenes*.



People become infected with *L. monocytogenes* by eating contaminated food. People can also become infected by handling contaminated food, such as contaminated pet food, or touching contaminated surfaces and utensils and then accidentally transferring the bacteria from their hands to their mouths. Babies can become infected in utero or at birth if their mothers ate contaminated food during pregnancy.

The bacteria can contaminate a variety of food, such as:

Raw meat; Ready-to-eat processed meat such as hot dogs and deli meat (both factory-sealed packages and products sold at deli counters); Raw vegetables; Refrigerated pates; Ready-to-eat smoked seafood and raw seafood; Prepared or stored salads, including coleslaw and fresh fruit salad; Melons; Soft cheeses made with unpasteurized milk; and Unpasteurized milk and milk products.

Pasteurization, cooking, and most disinfecting agents kill *L. monocytogenes*.

Listeriosis occurs almost exclusively in pregnant women, newborns, the elderly, and people with weakened immune systems (such as those with cancer or other diseases). Pregnant women are 18 times more likely to get listeriosis than other healthy adults, and 16 to 27% of all *L. monocytogenes* infections are in pregnant women. Scientists don't know why pregnant women are so susceptible to the disease.

After a person ingests *L. monocytogenes*, the bacteria grow quickly in the liver and then move into the bloodstream and can invade many places in the body, including the: Brain; Membranes surrounding the brain and spinal cord; Gastrointestinal, or digestive, tract (the stomach and intestines); and Bloodstream.

L. monocytogenes

- **Granulomatosis infantiseptica** - severe form of early-onset listeriosis,
- abscesses and granulomas in multiple organs
- high mortality rate unless treated promptly

Granulomatosis infantiseptica is an intrauterine infection with a high fetal or neonatal mortality but a very low mortality for the mother. In the fetus the infection is generalized but is characterized by extensive focal necrosis with a monocytic infiltration of the liver, spleen, and more rarely of the lungs and intestines. There is associated meningitis in some cases

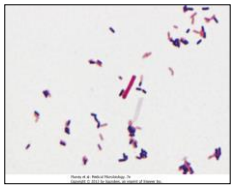
L. Monocytogenes - diagnosis

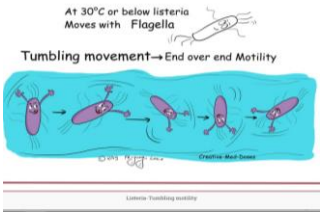
- The diagnosis is typically made by microscopy and culture of :
- Blood
- cerebrospinal fluid (CSF)
- amniotic fluid.

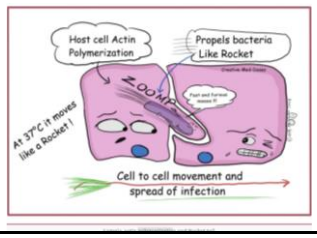
Symptoms of listerial infection are nonspecific and similar to other infectious diseases.
Listeriosis occasionally affects healthy, young, nonpregnant individuals.
Illness should be considered in groups at risk -
Pregnant women
Elderly person
Neonates
Individuals immunocompromised by organ transplantation, cancer, or treatment with tumor necrosis factor antagonists or glucocorticoids
The diagnosis is typically made by culture of blood, cerebrospinal fluid (CSF), or amniotic fluid.

L. monocytogenes - microscopy

- significant human pathogen,
- **facultative intracellular pathogen** gram-positive rod
- The **short rods** appear singly, in pairs, or in short chains
- Motile : „end over end“ tumbling motion







Listeria monocytogenes is a facultatively intracellular, gram-positive rod that grows over a broad temperature range, including ***refrigeration*** temperatures. This organism is motile during growth at low temperatures, it demonstrates ***tumbling*** motility at low temperatures.
At body temperature (37°C) it induces actin polymerization in host cell actin and forms “***rocket tails***” that facilitates intracellular movement and cell-to-cell spread across cell membranes, this way it ***avoids antibodies*** too.

L. Monocytogenes - culture

- blood agar - hemolysis
- Mueller-Hinton agar
- cultures : 1-2 days

Treatment

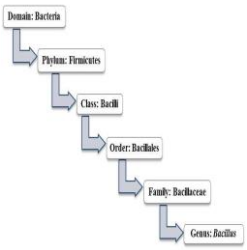
- **Ampicillin**

Listeria monocytogenes can be isolated readily on routine media, but care must be taken to distinguish this organism from other Gram-positive rods, particularly diphtheroids.
Listerias pecies grow on media such as Mueller-Hinton agar. On blood agar the characteristic small zone of hemolysis can be observed around and under colonies, the cultures will take 1-2 days for growth.

Treatment

Antibiotic therapy is the treatment of choice. Bacteremia should be treated for 2 weeks if the patient is immunocompetent. Longer courses may be required in the immunocompromised patient. Meningitis should be treated for 3 weeks. Ampicillin is generally considered the preferred agent, gentamicin is added frequently for synergy.

Bacillus

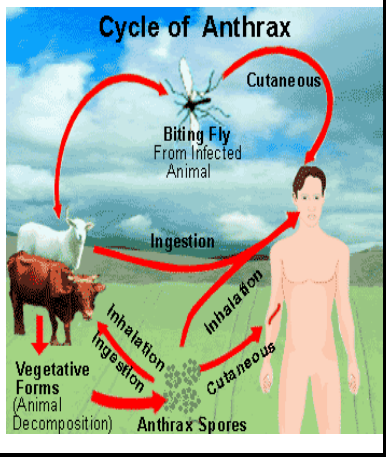


Species	Host(s)	Diseases
Bacillus anthracis	Cattle and sheep	Septicaemic form of anthrax; usually presents as sudden death
	Carnivores	Comparatively resistant to disease; pattern similar to that in pigs; a massive dose from eating anthrax-infected carcasses can lead to septicaemia
	Horses	Oral route: septicaemia with colic and enteritis Wound infections: localized oedema and lymphadenitis
	Pigs	Subacute anthrax with oedematous swelling in pharyngeal tissues and regional lymphadenitis or intestinal form with a higher mortality
	Human	Intestinal, cutaneous and inhalational forms
Bacillus cereus	Cattle	Rarely mastitis
	Dogs and cats	Rarely food poisoning
	Human	Food-borne diarrhoeal illness
Bacillus licheniformis	Cattle and sheep	Abortion and mastitis
	Human	Food-borne diarrhoeal illness
Bacillus subtilis	Cattle and sheep	Ovine abortion and bovine mastitis

The Bacillaceae are a family of Gram-positive, heterotrophic, rod-shaped bacteria that may produce endospores. Motile members of this family are characterized by peritrichous flagella. Some Bacillaceae are aerobic, while others are facultative or strict anaerobes. Most are not pathogenic, but Bacillus species are known to cause disease in humans. A familyof Bacilli can producecylindric ,ellipsoid,or spheric endospores situated terminally,subterminally,or centrally.These cells are chemoheterotrophic and mostly saprophytic,commonly appearingin soil. Some are parasitic on insects and animals.

Bacillus anthracis.

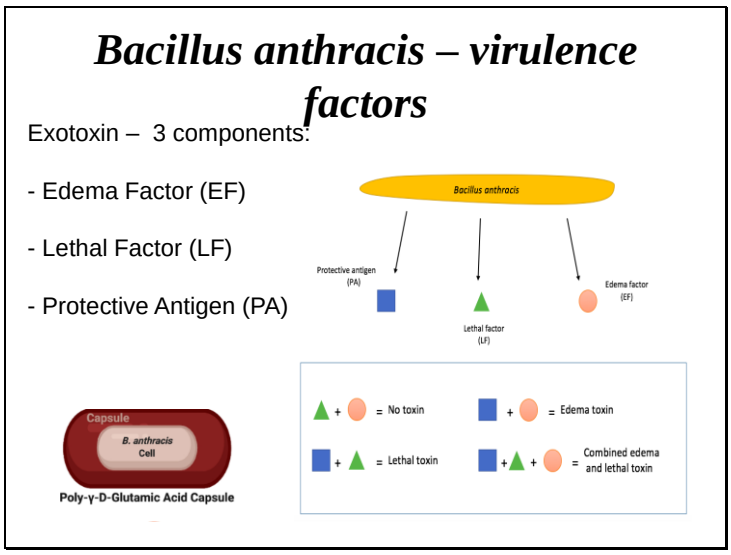
- Anthrax - fatal disease - ***Bacillus anthracis.***
- anthrax spores are
- inhaled,
- ingested,
- contact with the skin lesion



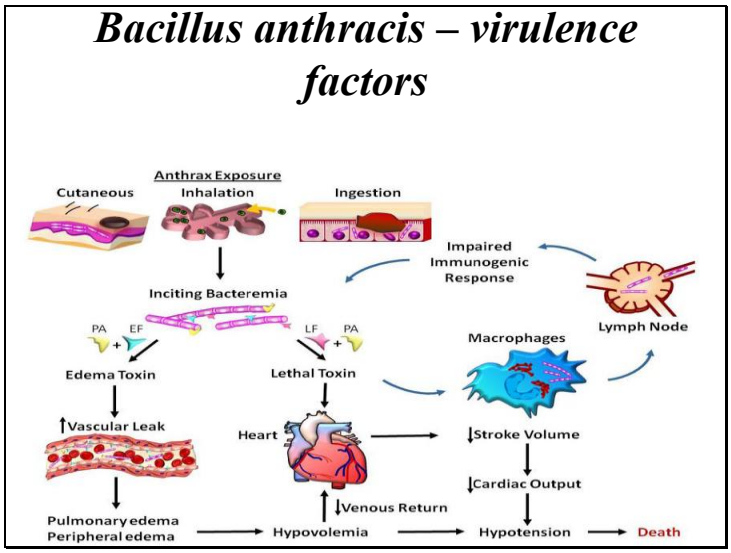
Anthrax - is an infectious and potentially fatal disease caused by the bacterium ***Bacillus anthracis.***

It spreads when the anthrax spores are inhaled, ingested, or come into contact with the skin lesion on a host. Anthrax commonly affects warm-blooded animals, especially cattle and sheep; but can also occasionally affect human population. Infection in humans involves the skin, gastrointestinal tract lungs. Humans can become infected with anthrax by handling products from infected animals or by breathing in anthrax spores from infected animal products (like wool).

People also can become infected with gastrointestinal anthrax by eating undercooked meat from infected animals

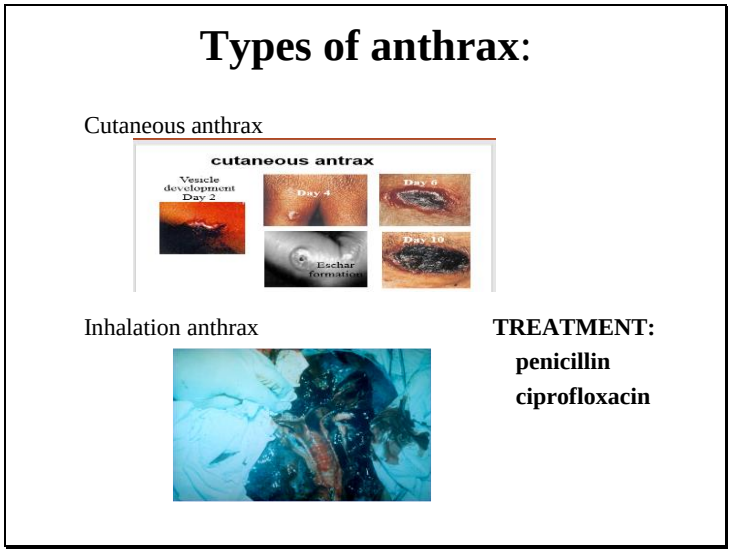


The virulence factors of *B. anthracis* include a number of exotoxins and the capsule. Exotoxin is plasmid-encoded, heat-labile, heterogeneous protein complex made up of 3 components: Edema Factor (EF),Lethal Factor (LF), Protective Antigen (PA). These three factors act synergistically (for toxic effects). In vivo, the secretion of inactive molecules by *B. anthracis* lead to the production of toxins. The binding of LF and EF alone do not create a toxin. The binding of LF and PA create an active lethal toxin, and the binding of PA and EF create an edema toxin. PA bound with both LF and EF have lethal and edema effects.
The capsule consists of a polypeptide of D-glutamic acid which is encoded by a plasmid and is anti-phagocytic. It is not a good immunogen and, even if any antibodies produced, they are not protective against the disease. When covered in this capsule, the bacteria are less likely to attract attention and can safely multiply.



The damaging nature of *B. anthracis* is revealed when large numbers of these bacteria begin to release exotoxins. Bacteria release protective antigen, edema factor, and lethal factor as three separate molecules that are not active by themselves. When the edema factor binds to the protective antigen, the edema factor becomes activated and causes fluid to rush out from cells and collect in the tissue. When the lethal factor binds to the protective antigen, a lethal toxin is created that helps kill macrophages and other cells of the immune system. It also

changes the signals cells receive from each other, severely disrupting vital processes that allow for basic functions of cells and leading to cell death.



An infection develops in humans after exposure to *B. anthracis* in specific locations in the body depending on the route of exposure.
Cutaneous anthrax occurs when bacteria enter skin through a wound or break in the skin, creating black lesions. More than 95% of all anthrax cases develop from cutaneous exposure. Cutaneous exposure can have a mortality rate around 20%.

Infection can also result from inhaling endospores released into the air from manufacturing wool or hides. 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.

Less commonly, anthrax infection can occur in the digestive system from eating contaminated food like undercooked and tainted meat. 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.

Anthrax infection is not as easily treated and is often not recognized until the disease is in its later stages, leading to high mortality rates.
Antibiotic treatments are available and useful when administered early, but they aren't able to reverse the serious damage done by toxins. Infections can usually be treated with penicillin, an antibiotic that causes cell death by disrupting the links of peptidoglycan on a bacteria's cell wall. Other antibiotics like ciprofloxacin has been used in recent infections caused by the *B. anthracis.* Penicillin can also be used to treat animals, like cattle, that have contracted anthrax. Though treatments are available, anthrax vaccines are available to humans and animals to offer the best protection against *B. anthracis*. Human vaccines are not available to the general population, but they are given to people working directly with animals or animal products that are at risk for infection. Currently administered human anthrax vaccines include: acellular (USA) and live spores (Russia) varieties. All currently used anthrax vaccines show local and general raectogenicity (erythema, induration, soreness, fever) and require a six-dose primary series at 0, 2, 4 weeks and 6, 12, 18 months, with annual boosters to maintain immunity.

http://www.youtube.com/watch?v=ig1Cz2tdVjY

Anthrax can also be used as a weapon.
in the United States in 2001- Anthrax was deliberately spread through the postal system by sending letters with powder containing anthrax. This caused 22 cases of anthrax infection. B. anthracis can be use as bioterroristic weapon, because it is very virulent (toxin), easy spread by air - aerosol, bombes, and spores are resistent to drying. In 1942, during the Second World War, a biolgical test was carried out on Gruinard by British military scientists from the Biology Department. The British government was investigating the feasibility of a bioweapons attack using anthrax. It was recognised that tests would cause long-lasting contamination of the immediate area by anthrax spores.

Bacillus cereus

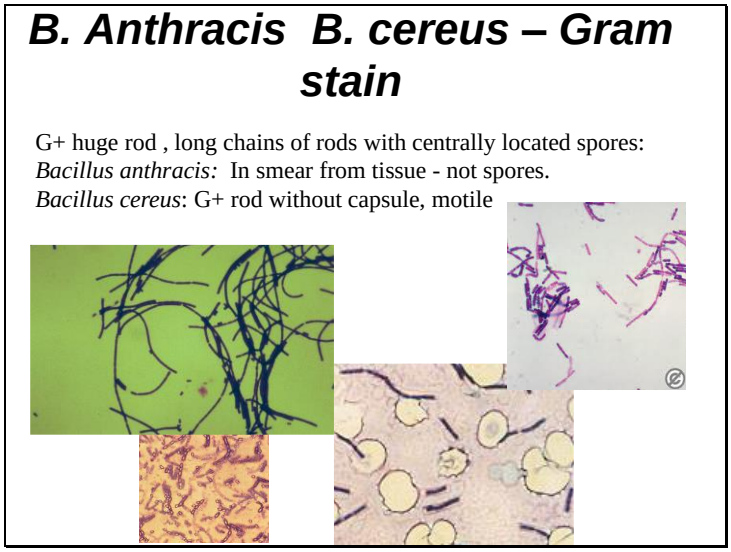
- opportunistic pathogen
- relatively low capacities for virulence
- Gastroenteritis
- ocular infections
- intravenous catheter-related sepsis

https://www.sciencedirect.com/science/article/pii/S0014483519307493

While *B. cereus* is associated mainly with food poisoning, it is being increasingly reported to be a cause of serious and potentially fatal non-gastrointestinal-tract infections. The pathogenicity of *B. cereus*, whether intestinal or nonintestinal, is intimately associated with the production of tissue-destructive exoenzymes. Among these secreted toxins are four hemolysins, three distinct phospholipases, an emesis-inducing toxin, and proteases.

This bacterium has been incriminated in a multitude of other clinical conditions such as food poisoning and severe eye infections, anthrax-like progressive pneumonia, fulminant sepsis,

and devastating central nervous system infections, particularly in immunosuppressed individuals, intravenous drug abusers, and neonates. Its role in nosocomial acquired bacteremia and wound infections in postsurgical patients has also been well defined, especially when intravascular devices such as catheters are inserted. Primary cutaneous infections mimicking clostridial gas gangrene induced subsequent to trauma have also been well documented.
The symptoms of B. cereus diarrheal type food poisoning mimic those of Clostridium perfringens food poisoning. The onset of watery diarrhea, abdominal cramps, and pain occurs 6-15 hours after consumption of contaminated food. Nausea may accompany diarrhea, but vomiting (emesis) rarely occurs. Symptoms persist for 24 hours in most instances.
The emetic type of food poisoning is characterized by nausea and vomiting within 0.5 to 6 h after consumption of contaminated foods. Occasionally, abdominal cramps and/or diarrhea may also occur. Duration of symptoms is generally less than 24 h. The symptoms of this type of food poisoning parallel those caused by Staphylococcus aureus foodborne intoxication.



Bacillus anthracis is very large, Gram-positive, sporeforming rod (1 -1.2µm in width x 3 - 5µm in length) arranged in chains. It is very similar to Bacillus cereus, the species have the same cellular size and morphology. Also characteristic is the square-ended (“box-car” shaped) appearance associated with *B. anthracis* vegetative cells
Bacillus cereus is a Gram-positive aerobic or facultatively anaerobic, motile, spore-forming, rod-shaped bacterium

Culture

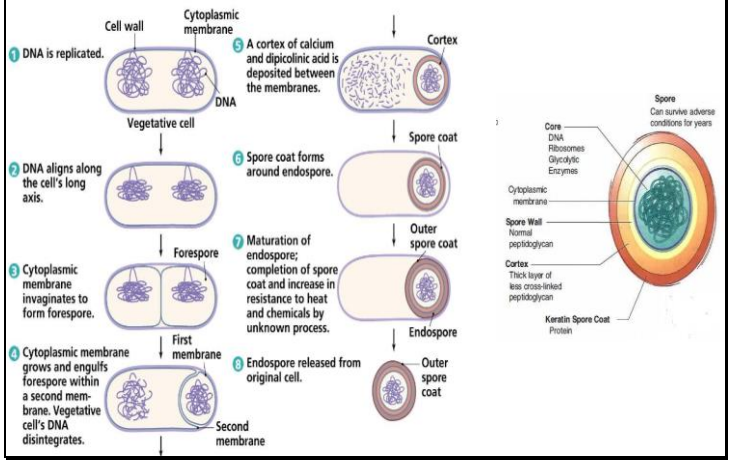
- ***B. anthracis* on blood agar**- aerobe, facult. anaerobe rod, small adherent colonies lining in paralel way - growing in picture of *caput medusae*, colonies with wave edges.
- colonies are rough,
- Older colonies growing in aerobic environment contain spores – dry, wrinkled look.
- Growth on medium with PNC - chain of pearls

- ***B. cereus* on blood agar** –
- capsules are not present,
- gray colonies,
- Beta-haemolysis

B. anthracis ***B. cereus***

B. anthracis is a non-fastidious, facultatively anaerobic bacterium organism which grows readily on simple laboratory media. The optimum temperature for growth is 35–37 °C. After overnight incubation at 35–37 °C colonies are large, 2–3 mm in diameter, irregular, raised, dull, opaque and greyish-white with “frosted glass” (ground glass) appearance. Occasionally the colonies may have fringed edges or put out curled protrusions (tailing). This is the so-called “Medusa head appearance” or Caput medusae. Capsules are formed during growth in bicarbonate agar containing serum under a 5%–20% carbon dioxide atmosphere, or in defibrinated blood or in serum (defibrinated horse blood seems to work best).
B. cereus - When grown under aerobic conditions on 5% sheep blood agar at 37°C, *B. cereus* colonies are dull gray and opaque with a rough matted surface. Colony perimeters are irregular and represent the configuration of swarming from the site of initial inoculation, perhaps due to *B. cereus* swarming motility. Zones of beta-hemolysis surround and conform to the colony morphology.

Sporulation



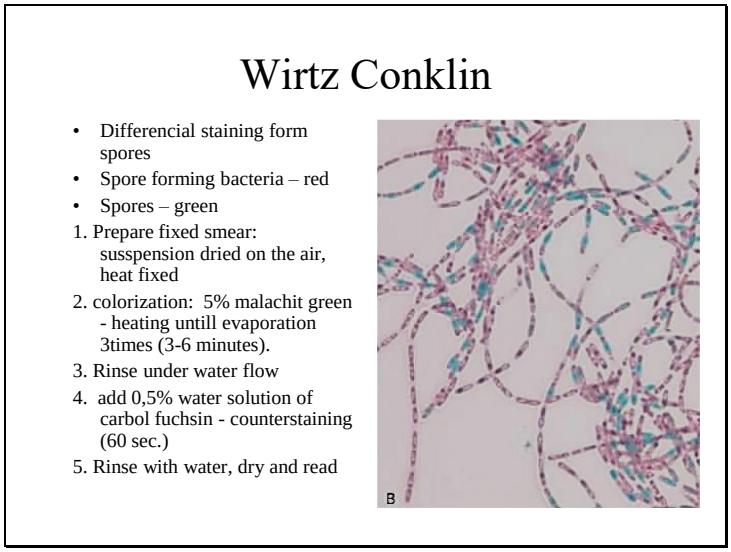
Some bacteria have the ability to enter a state of suspended animation when conditions are unfavorable.
An endospore is an extremely resistant dormant cell structure produced by some bacterial species (term endospore: 'endo-' means 'inside' and '-spore' refers to the 'dormant structure,')

so the endospore is a structure formed inside the cell. There are many examples of endospore-forming bacteria. The two most common are *Clostridium* and *Bacillus*. Bacteria normally grow, matured and reproduce by somatic cells. When there is nutrient depletion or environmental stress (heat, UV radiation, chemical disinfection,desiccation), spore former bacteria begin spore formation. The process of endospore formation is called sporulation. Since spores often survive boilingfor an hour or more therefore, autoclave must be used to sterilize any bacteria.

After return of suitable environmental spores produce vegetative cell, the return of an endospore to its vegetative state is called germination.

Properties of Endospores:

- Core: The core is the spore protoplast. It contains a complete chromosome, all of the components of the protein-synthesizing apparatus, and an energy-generating system based on glycolysis. A number of unique enzymes are formed (eg, dipicolinic acid synthetase). The heat resistance of spores is due in part to their dehydrated state and in part to the presence in the core of large amounts of calcium dipicolinate.
- Spore Wall: The innermost layer surrounding the inner spore membrane is called the spore wall. It contains normal peptidoglycan and becomes the cell wall of the germinating vegetative cell.
- Cortex: The cortex is the thickestlayer of the spore envelope. Cortex peptidoglycan is extremely sensitive to lysozyme, and its autolysis plays a role in spore germination.
- Coat: The coat is composed of a keratin-likeprotein. The impermeability of this layer confers on spores their relative resistance to antibacterial chemical agents.
- Exosporium: The exosporium is a lipoprotein membrane containing carbohydrates.



Bacillus anthracis and *Bacillus cereus* endospores develop at the end of the log phase of multiplication. For diagnostic purposes, they can generally be visualized in smears of standard laboratory agar plate cultures (e.g. blood agar or nutrient agar) after 20–24 hours of incubation at 35 °C to 37 °C. The spores are central or subterminal, ellipsoidal and do not swell the vegetative cell. Spores are not present in smears prepared from clinical specimens. Endospores are stained by Wirtz-Conklin method where malachite green is used for staining of endospores and heat is used to penetrate stain. The rest of the cell is then counterstained a light pink-red with safranin or carbolfuchsin.

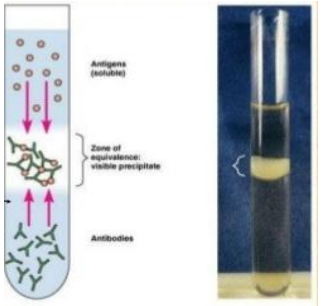
Ascoli termoprecipitation reaction

- Detection of antigens extracted by heat directly from biological material by antibodies in agar

- Serology (Ascoli Thermo Precipitin Test):
  - → Tissues are ground up in saline and boiled for 5 mins and filtered.
  - → Then this extract layered over anti anthrax serum in a narrow tube.

  +ve case :ring of precipitate appears at junction of two liquids with in 5minutes.

- mainly used for rapid diagnosis when sample received is putrid and viable bacilli less likely found



ASCOLI'S THERMOPRECIPITATION TEST : used for diagnosis of anthrax (Bacillus anthracis). It is a ring precipitation test, done when sample is received in putrid form and bacilli are likely to be nonviable. Tissue samples are grounded in saline, boiled and filtered. This antigenic extract from tissues is layered over anthrax
antiserum in a narrow capillary tube. A ring of precipitate appears at the junction of two liquids within
5 minutes. Diagnosis may be established by Ascoli's thermoprecipitin test by demonstration of the anthrax antigen in tissue extracts.

Treatment

- *Bacillus cereus* infections

Treatment, Prevention, and Control

Gastrointestinal infections are treated symptomatically.

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 anthracis*

Treatment & Prophylaxis

- **Treatment**
  - **Penicillin** is drug of choice
  - Erythromycin, chloramphenicol acceptable alternatives
  - **Doxycycline** now commonly recognized as **prophylactic**
- **Vaccine (controversial)**
  - **Laboratory workers**
  - Employees of mills handling goat hair
  - Active duty **military** members

Though treatments are available, anthrax vaccines are available to humans and animals to offer the best protection against *B. anthracis*. Human vaccines are not available to the general population, but they are given to people working directly with animals or animal products that are at risk for infection. Currently administered human anthrax vaccines include: acellular (USA) and live spores (Russia) varieties. All currently used anthrax vaccines show local and general reactogenicity (erythema, induration, soreness, fever) and require a six-dose primary series at 0, 2, 4 weeks and 6, 12, 18 months, with annual boosters to maintain immunity. Risks of a vaccine reaction are: Tenderness, redness, itching, or a lump where the shot is given, muscle aches or short-term trouble moving arm, headaches or fatigue.