PSEUDOMONAS

Pseudomonas aeruginosa has become an important cause of infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week, and it is a frequent cause of nosocomial infections. Pseudomonal infections are complicated and can be life-threatening.

Signs and symptoms

Pseudomonal infections can involve the following parts of the body, with corresponding symptoms and signs:

- Respiratory tract (eg, pneumonia)
- Bloodstream (bacteremia)
- Heart (endocarditis)
- CNS (eg, meningitis, brain abscess)
- Ear (eg, otitis externa and media)
- Eye (eg, bacterial keratitis, endophthalmitis)
- Bones and joints (eg, osteomyelitis)
- GI tract (eg, diarrhea, enteritis, enterocolitis)
- Urinary tract
- Skin

Physical findings depend on the site and nature of the infection, as follows:

- Endocarditis: Fever, murmur, and positive blood culture findings; peripheral stigmata such as Roth spots, Janeway lesions, Osler nodes, splinter hemorrhages, and splenomegaly
- Pneumonia: Rales, rhonchi, fever, cyanosis, retractions, and hypoxia; occasionally shock; with cystic fibrosis, clubbing, increased anteroposterior (AP) diameter, and malnutrition
- GI tract: Fever, signs of dehydration, abdominal distention, and signs of peritonitis; physical findings of Shanghai fever
- Skin and soft tissue infections: Hemorrhagic and necrotic lesions, with surrounding erythema; subcutaneous nodules, deep abscesses, cellulitis, and fasciitis; in burns, black or violaceous discoloration or eschar
- Skeletal infections: Local tenderness and a decreased range of motion; neurologic deficits
- Eye infections: Lid edema, conjunctival erythema and chemosis, and severe mucopurulent discharge
- Malignant otitis externa: Erythematous, swollen, and inflamed external auditory canal; local lymphadenopathy
- Bacteremia: Fever, tachypnea, and tachycardia; hypotension and shock; jaundice

Diagnosis

Laboratory studies that may be helpful include the following:

- Complete blood count (CBC)
- Blood cultures
- In urinary tract infection (UTI), urinalysis
- In pneumonia, culture of sputum and respiratory secretions, as well as blood gas analysis

- Wound and burn cultures and cultures from other body fluids and secretions according to the clinical scenario
- Gram stain and culture of CSF if meningitis is suspected

Pseudomonas aeruginosa is member of the Gamma Proteobacteria class of Bacteria. It is aerobic bacterium belonging to the bacterial family Pseudomonadaceae.

GRAM stain

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

Gram stain of *Pseudomonas aeruginosa.*

Cultivation on 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.

***Pseudomonas aeruginosa* on Blood Agar (typical metallic sheen).**

P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth 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.

Cultivation on Deoxycholate Citrate Agar

P. aeruginosa strains produce soluble pigments, the fluorescent pigment pyoverdin and the blue pigment pyocyanin. The latter is produced abundantly in media of low-iron content and

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

***Pseudomonas aeruginosa* on Deoxycholate Citrate Agar (lactose negative, H$_2$S negative).**
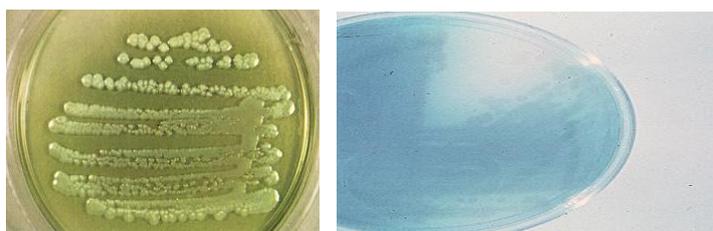
The green pigment pyoverdin and blue pigment pyocyanin are produced by many, but not all, strains of *Pseudomonas aeruginosa.*

Cultivation on TSI (Hajn) Agar

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 **growing on TSI Agar.**

OXIDASE test

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.
There are many method variations to the oxidase test. These include, but are not limited to, the filter paper test, filter paper spot test, direct plate method, and test tube method.

Filter Paper Test Method

1. Soak a small piece of filter paper in 1% Kovács oxidase reagent and let dry.
2. Use a loop and pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and rub onto treated filter paper (please see Comments and Tips section for notes on recommended media and loops).
3. Observe for color changes.
4. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

On the left is oxidase-positive *Pseudomonas aeruginosa* and on the right is oxidase-negative *Escherichia coli*. Both organisms were rubbed on a filter that had been dipped in Kovács oxidase reagent and allowed to dry

BACILLI

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 family of Bacilli of the order Bacillales can produce cylindric, ellipsoid, or spheric endospores situated terminally, subterminally, or centrally. These cells are chemoheterotrophic and mostly saprophytic, commonly appearing in soil. Some are parasitic on insects and animals and are pathogenic. The family includes the genus *Bacillus,* which is aerobic, and the genus *Clostridium,* which is facultatively anaerobic.

Bacillus anthracis

Bacillus anthracis is the causative agent of anthrax. It is a Gram-positive, aerobic, spore-forming large bacillus. Spores are formed in culture, in the soil, and in the tissues and exudates of dead animals, but not in the blood or tissues of living animals. Spores remain viable in soil for decades.

Anthrax is a major disease threat to herbivorous animals (cattle, sheep, and to a lesser extent horses, hogs, and goats). People become infected by the cutaneous route (direct contact with diseased animals, industrial work with hides, wool, brushes, or bone meal), by inhalation (Woolsorter's disease), or by ingestion (meat from diseased animals). It is not contagious.

The virulence factors of *B. anthracis* include a number of exotoxins and the capsule.

Exotoxin

A plasmid-encoded, heat-labile, heterogeneous protein complex made up of 3 components:

- Edema Factor (EF)
- Lethal Factor (LF)
- Protective Antigen (PA).

In vivo, these three factors act synergistically (for toxic effects). The protective antigen binds to surface receptors on eucaryotic cells and is subsequently cleaved by a cellular protease. The larger C-terminal piece of PA remains bound to the receptor and then binds either EF or LF, which enters the cell by endocytosis. Edema Factor, when inside the cells binds calmodulin-dependent and acts as adenylate cyclase. Lethal factor's mechanism of action involves activation of macrophages and production of cytokines which cause necrosis, fever, shock and death. Individually, the three proteins have no known toxic activity. Antibodies to protective antigens prevent PA binding to cells stop EF and LF entry.

Capsule

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.

Clinical diagnosis of anthrax can be confirmed by direct examination or culture. Fresh smears of vesicular fluid, fluid from under the eschar, blood, or spleen or lymph node aspirates are stained with polychrome methylene blue and examined for the characteristic blunt ended, blue-black rods with a pink capsule. In case of a negative finding, the specimen can be cultured on blood agar plates. Cultured organisms stain as Gram-positive long thin rods.

Bacillus cereus

Bacillus cereus is a type of bacteria that produces toxins. These toxins can cause two types of illness: one type characterized by diarrhea and the other, called emetic toxin, by nausea and vomiting. These bacteria are present in foods and can multiply quickly at room temperature. Most *B. cereus* are motile.

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.

Confirmation of *B. cereus* as the etiologic agent in a foodborne outbreak requires either (1) isolation of strains of the same serotype from the suspect food and feces or vomitus of the patient, (2) isolation of large numbers of a *B. cereus* serotype known to cause foodborne illness from the suspect food or from the feces or vomitus of the patient, or (3) isolation of *B. cereus* from suspect foods and determining their enterotoxigenicity by serological (diarrheal toxin) or biological (diarrheal and emetic) tests. The rapid onset time to symptoms in the emetic form of disease, coupled with some food evidence, is often sufficient to diagnose this type of food poisoning.

Examination of Foods for *B. cereus*

1. **Sampling**
   If the quantity of food to be examined is large, take representative samples of 50 g each from different parts of the suspect food because contamination may be unevenly distributed. If the food is a powder or consists of small discrete particles, then it should be thoroughly mixed before taking samples.
2. **Transporting and storage of samples**
   Transport samples promptly in insulated shipping containers with enough gel-type

refrigerant to maintain them at 6°C or below. Upon receipt in the laboratory, store the samples at 4°C and analyze as soon as possible. If analysis cannot be started within 4 days after collection, freeze samples promptly and store at -20°C until examined. Thaw at room temperature and proceed with analysis as usual. Maintain frozen samples at -20°C until examined. Ship on dry ice to avoid thawing. Dehydrated foods may be stored at room temperature and shipped without refrigeration.

GRAM stain

Bacillus anthracis

Bacillus anthracis is very large, Gram-positive, sporeforming rod (1 - 1.2µm in width x 3 - 5µm in length) arranged in chains. Genotypically and phenotypically it is very similar to *Bacillus cereus,* the species have the same cellular size and morphology.
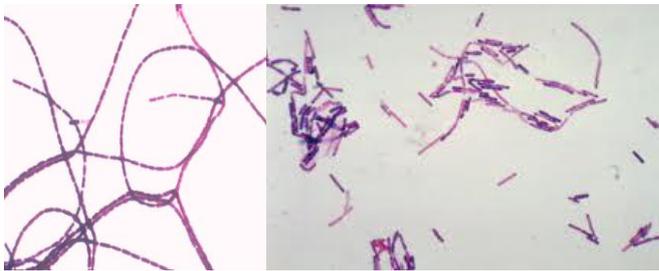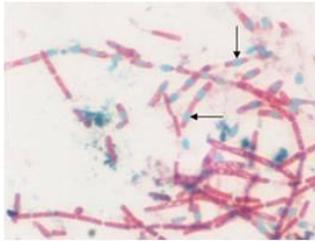

Bacillus anthracis   **Bacillus cereus**

ENDOSPORE staining

Bacilli form oval, central to subterminal spores that do not swell the bacterial cell. Spores are not present in clinical specimens. Endospores produced by *Bacillus* do not stain easily. Endospores are stained by *Wirtz-Conklin* method where malachite green is used for staining and heat is used to penetrate stain. The rest of the cell is then counterstained a light pink-red with safranin.

Wirtz-Conklin's method

1. Prepare a smear and heat gently to fix.
2. Flood the slide with 5 - 10% malachite green solution.
3. Leave the slide to stain for 45min or alternatively, the slide can be heated gently to steaming for 3 - 6min, reapplying stain if it begins to dry out.
4. Rinse under running tap water.
5. Counterstain with 0.5% safranin for 30sec.
6. Rinse and dry.
7. View slide under oil immersion with a light microscop.

Bacillus anthracis -Wirtz-Conklin stain - bacterial spores stain green, vegetative cells stain red.

Endospores

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*. In favorable conditions, these bacteria are actively growing and dividing cells. If a nutrient, such as carbon or nitrogen, becomes scarce or if the population becomes too dense, the bacteria can become stressed. They will enter a stasis phase, which is their equivalent of survival mode. The bacteria can survive in stasis until better growth conditions return.

Sporulation

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 boiling for an hour or more therefore, autoclave must be used to sterilize any bacteria.
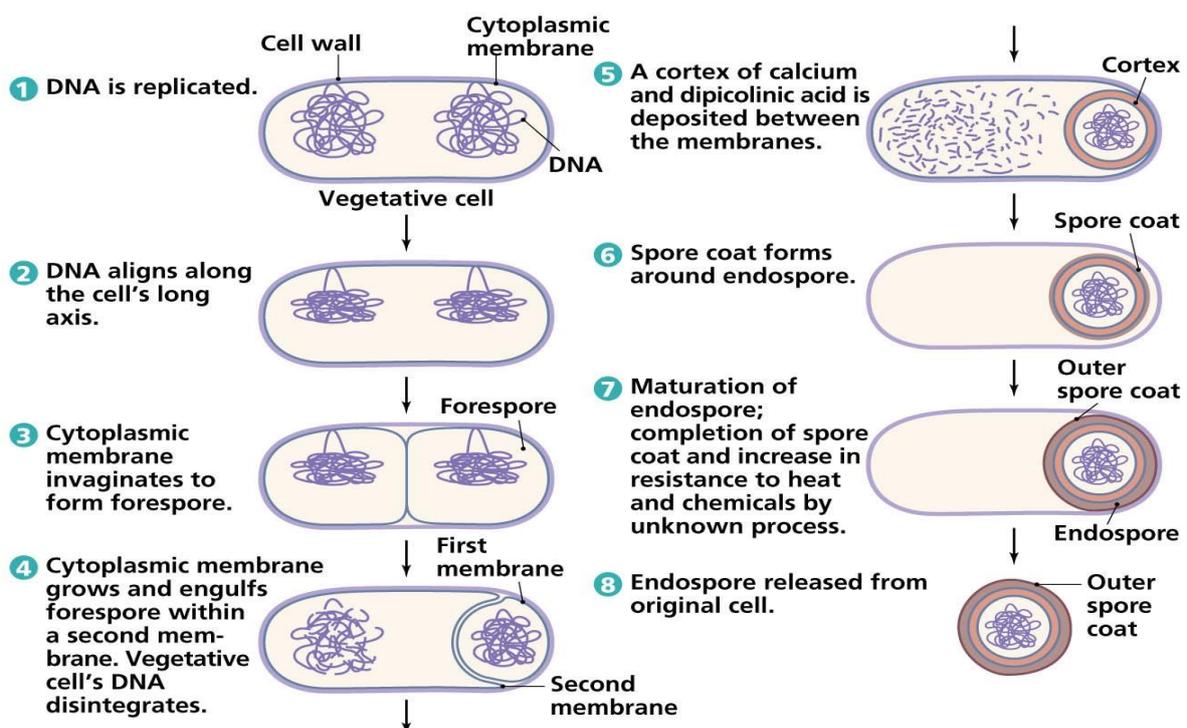

Endospore structure

Properties of Endospores:

1. **Core**: The core is the spore protoplast. It contains a complete nucleus (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). Spores contain no ATP. The energy for germination is stored as 3-phosphoglycerate rather than as ATP. 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**.

2. **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.

3. **Cortex**: The cortex is the **thickest** layer of the spore envelope. Cortex peptidoglycan is extremely sensitive to lysozyme, and its autolysis plays a role in spore germination.

4. **Coat**: The coat is composed of a **keratin-like** protein. The impermeability of this layer confers on spores their relative resistance to antibacterial chemical agents.

5. **Exosporium**: The exosporium is a lipoprotein membrane containing some carbohydrate.

Morphology of endospores
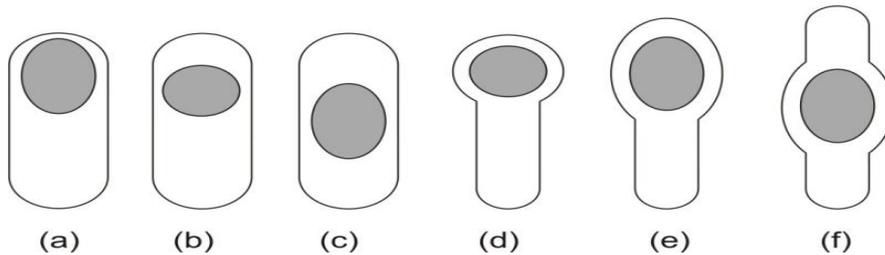
Location: terminal (a, d, e), subterminal (b), central (c, f).

Shape: circular (b, d), ellipsoid (a, c, e, f).

Spore diameter compared with cell diameter: non-deforming (a, b, c), deforming (d, e, f).
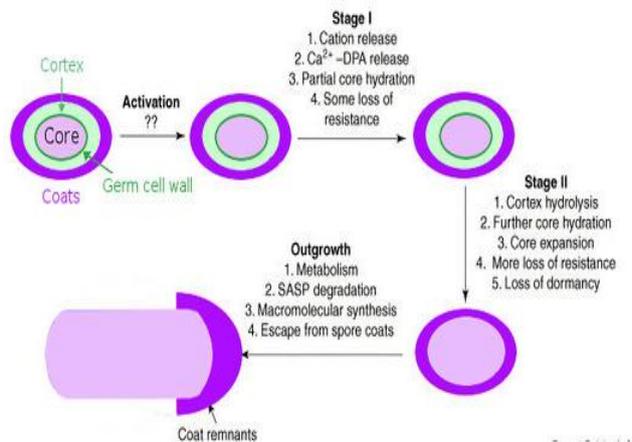
Germination

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

The germination process occurs in three stages: activation, initiation, and outgrowth.

1. **Activation**:Endospores cannot germinate immediately after they have formed, but they can germinate after they have rested for several days. They need certain conditions to be activated.

2. **Initiation**: Once activated, a spore will initiate germination if the environmental conditions are favorable. Autolysin will be activated and it will rapidly degrade the cortex peptidoglycan.

Water is taken up, calcium dipicolinate is released, and a variety of spore constituents are degraded by hydrolytic enzymes.

3. **Outgrowth**: Degradation of the cortex and outer layers results in the emergence of a new vegetative cell consisting of the spore protoplast with its surrounding wall. Now, using nutrients around, the cell can multiply again.

Once activated, a spore will initiate germination if the environmental conditions are favorable.

Different species have evolved receptors recognise different effectors as signaling a rich medium.

Binding of the effector activates an autolysin that rapidly degrades the cortex peptidoglycan. Water is taken up, calcium dipicolinate is released, and a variety of spore constituents are degraded by hydrolytic enzymes.

Cultivation on Blood agar

Bacillus anthracis - **colony morphology:**

- grows well on Blood Agar (BA)
- will not grow on MacConkey (MAC) agar
- 2-5 mm on BA at 24 h
- flat or slightly convex with irregular borders that have comma-shaped protrusions
- colonies have a ground-glass appearance
- non-hemolytic on Blood agar
- tenacious colonies
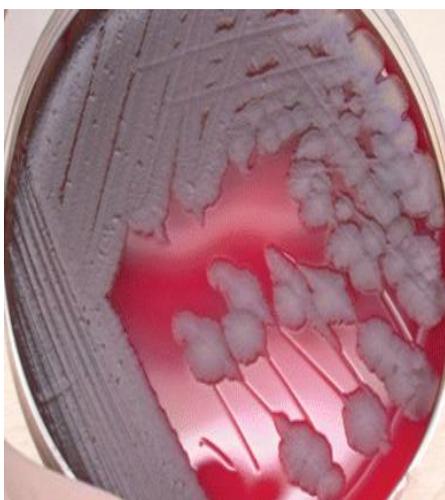
Bacillus anthracis - **comma-shaped protrusions**

Bacillus anthracis

Bacillus cereus

Colony Morphology:

B. cereus **colonies**

- the optimum growth temperatures range from 30°C to 50°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 (rhizoid growth is characteristic of *B. cereus*)
- zones of beta-hemolysis surround and conform to the colony morphology (whereas *B. anthracis* is usually nonhemolytic)

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LISTERIA

Listeria monocytogenes is a Gram-positive rod-shaped bacterium. *L. monocytogenes* can be isolated in soil, wood, and decaying matter in the natural environment. The principal route of acquisition of *Listeria* is through the ingestion of contaminated food products. *Listeria* has been isolated from prepared meat (eg, hot dogs, deli meat), dairy products, unwashed raw vegetables, and seafood. Soft cheeses and unpasteurized milk have been the most frequently incriminated dairy products. Invasive infection by *L. monocytogenes* causes the disease listeriosis. When the infection is not invasive, any illness as a consequence of infection is termed febrile gastroenteritis. Listeriosis is relatively rare and occurs primarily in newborn infants, elderly patients, and patients who are immunocompromised. The two main clinical manifestations are sepsis and meningitis. Meningitis is often complicated by encephalitis, a pathology that is unusual for bacterial infections. The manifestations of listeriosis include septicemia, meningitis or meningoencephalitis), encephalitis, corneal ulcer, pneumonia, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (second to third trimester) or stillbirth. Surviving neonates may suffer granulomatosis infantiseptica (pyogenic granulomas distributed over the whole body) and physical retardation.

MICROSCOPY - GRAM STAIN, WET MOUNT

Listeria monocytogenes is a Gram-positive, non spore-forming, motile, facultatively anaerobic, rod-shaped bacterium. It is catalase-positive and oxidase-negative.

Listeria monocytogenes

This bacterium exhibits characteristic tumbling motility when viewed with light microscopy. *L. monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20−25 °C), the organism does not synthesize flagella at body temperatures (37 °C).

LISTERIA MONOCYTOGENES - CULTURE

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. *Listeria* species 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.

Listeria monocytogenes – Blood agar culture.

LISTERIA MONOCYTOGENES – 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.

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