Listeria

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Listeria is a genus of bacteria that contains ten species. Named after the English pioneer of sterile surgery Joseph Lister, the genus received its current name in 1940. Listeria species are facultatively anaerobic, Gram-positive bacilli. The major human pathogen in the Listeria genus is *L. monocytogenes*. It is usually the causative agent of the relatively rare bacterial disease, listeriosis, a serious infection caused by eating food contaminated with the bacteria. The disease affects primarily pregnant women, newborns, adults with weakened immune systems, and the elderly.
Listeriosis is a serious disease for humans; the overt form of the disease has a mortality rate of about 20 percent. The two main clinical manifestations are sepsis and meningitis. Meningitis is often complicated by encephalitis, a pathology that is unusual for bacterial infections. *Listeria ivanovii* is a pathogen of mammals, specifically ruminants, and has rarely caused listeriosis in humans.
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<th>Kingdom:</th>
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<td><em>L. grayi</em></td>
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<td>Order:</td>
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<td>Family:</td>
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<td><em>L. ivanovii</em></td>
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Eventually, the genus *Listeria* was proposed and accepted. All species within the *Listeria* genus are Gram-positive, non-sporeforming, catalase-positive rods. The genus *Listeria* was classified in the family Corynebacteriaceae through the seventh edition of *Bergey's Manual of Systematic Bacteriology*. The 16S rRNA cataloging studies of Stackebrandt, et al. demonstrated that *L. monocytogenes* is a distinct taxon within the Lactobacillus-Bacillus branch of the bacterial phylogeny constructed by Woese. In 2004, the genus was placed in the newly created Family Listeriaceae. The only other genus in the family is *Brochothrix*. 
Under the microscope, *Listeria* species appear as small, Gram-positive rods, which are sometimes arranged in short chains. In direct smears, they may be coccoid, so they can be mistaken for *streptococci*. Longer cells may resemble *corynebacteria*. Flagella are produced at room temperature, but not at 37 °C. Hemolytic activity on blood agar has been used as a marker to distinguish *L. monocytogenes* among other *Listeria* species, but it is not an absolutely definitive criterion. Further biochemical characterization may be necessary to distinguish between the different species of *Listeria*. 
Listeria can be found in soil, which can lead to vegetable contamination. Animals can also be carriers. Listeria has been found in uncooked meats, uncooked vegetables, fruit such as cantaloupes, 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. For example, meat-processing plants producing ready-to-eat foods, such as hot dogs and deli meats, must follow extensive sanitation policies and procedures to prevent Listeria contamination.
*Listeria monocytogenes* is commonly found in soil, stream water, sewage, plants, and food. *Listeria* is responsible for *listeriosis*, a rare but potentially lethal food-borne infection. The case fatality rate for those with a severe form of infection may approach 25% (*Salmonella*, in comparison, has a mortality rate estimated at less than 1%). Although *Listeria monocytogenes* has low infectivity, it is hardy and can grow in temperatures from 4 °C (39.2 °F) (the temperature of a refrigerator), to 37 °C (98.6 °F), (the body's internal temperature). Listeriosis is a serious illness, and the disease may manifest as meningitis, or affect newborns due to its ability to penetrate the endothelial layer of the *placenta*. 
The Center for Science in the Public Interest has published a list of foods that have sometimes caused outbreaks of *Listeria*: hot dogs, deli meats, pasteurized or unpasteurized milk, cheeses (particularly soft-ripened cheeses like feta, Brie, Camembert, blue-veined, or Mexican-style *queso blanco*), raw and cooked poultry, raw meats, ice cream, raw vegetables, and raw and smoked fish.
Listeria monocytogenes, for example, encodes virulence genes that are thermoregulated. The expression of virulence factor is optimal at 39°C, and is controlled by a transcriptional activator, PrfA. The majority of Listeria bacteria are targeted by the immune system before they are able to cause infection. Those that escape the immune system's initial response, however, spread through intracellular mechanisms and are, therefore, guarded against circulating immune factors (AMI).
To invade, *Listeria* induces macrophage phagocytic uptake by displaying D-galactose in their teichoic acids that are then bound by macrophage's polysaccharide receptors. Other important adhesins are the internalins. Once phagocytosed, the bacterium is encapsulated by the host cell's acidic phagolysosome organelle. *Listeria*, however, escapes the phagolysosome by lysing the vacuole's entire membrane with secreted hemolysin, now characterized as the exotoxin listeriolysin O with a listeriophospholipases-c system. The bacteria then replicate inside the host cell's cytoplasm.
Listeria must then navigate to the cell's periphery to spread the infection to other cells. Outside the body, Listeria has flagellar-driven motility, sometimes described as a "tumbling motility". However, at 37 °C, flagella cease to develop and the bacterium instead usurps the host cell's cytoskeleton to move. Listeria, inventively, polymerizes an actin tail or "comet", from actin monomers in the host's cytoplasm with the promotion of virulence factor ActA. The comet forms in a polar manner and aids the bacteria's migration to the host cell's outer membrane.
Gelsolin, an actin filament severing protein, localizes at the tail of *Listeria* and accelerates the bacterium's motility. Once at the cell surface, the actin-propelled *Listeria* pushes against the cell's membrane to form protrusions called *filopods* or "rockets". The protrusions are guided by the cell's leading edge to contact adjacent cells, which then engulf the listeria rocket and the process is repeated, perpetuating the infection. Once phagocytosed, the bacterium is never again extracellular: it is an intracytoplasmic parasite like *Shigella flexneri* and *Rickettsia*.
Preventing listeriosis as a food illness requires effective sanitation of food contact surfaces. **Alcohol** is an effective topical sanitizer against *Listeria*. **Quaternary ammonium** can be used in conjunction with alcohol as a food contact safe sanitizer with increased duration of the sanitizing action. Refrigerated foods in the home should be kept below 4 °C (39.2 °F) to discourage bacterial growth. Preventing listeriosis also can be done by carrying out an effective sanitation of food contact surfaces.
In non-invasive listeriosis, the bacteria will often remain within the digestive tract, causing mild symptoms lasting only a few days and requiring only supportive care. Muscle pain and fever in mild cases can be treated with over-the-counter pain relievers, and diarrhoea and gastroenteritis can be treated with over-the-counter medications if needed.

**Treatment**
In invasive listeriosis, the bacteria has spread to the bloodstream and central nervous system. Treatment includes intravenous delivery of high-dose antimicrobials and in-patient hospital care. Duration of hospital care will vary depending on how widespread the infection is, but is usually no less than 2 weeks. Ampicillin, penicillin, or amoxicillin are often given for invasive listeriosis, and gentamicin is often added in patients with compromised immune systems. Trimethoprim-sulfamethoxazole, vancomycin, and fluoroquinolones can be used in cases of allergy to penicillin. For treatment to be effective, the antibiotic must penetrate the host cell and bind to penicillin-binding protein 3 (PBP3). Cephalosporins are not effective for treatment of listeriosis.
Prompt treatment of *listeria* infections in pregnancy is critical to prevent the bacteria from infecting the fetus, and antibiotics may be given to pregnant women even in non-invasive listeriosis. These oral therapies in less severe cases can include amoxicillin or *erythromycin*. In addition to *antibiotic therapy*, it often recommended that infected pregnant women receive *ultrasounds* to monitor the health of the fetus. Higher doses of antibiotics are sometimes given to pregnant women to ensure penetration of the umbilical cord and placenta. 

Asymptomatic patients who have been exposed to *listeria* are not recommended for treatment. It is recommended that these patients be informed of the signs and symptoms of the disease and to return for medical care if symptoms present.
Research

Listeria is an opportunistic pathogen: It is most prevalent in the elderly, pregnant mothers, and AIDS patients. With improved healthcare leading to a growing elderly population and extended life expectancies for AIDS patients, physicians are more likely to encounter this otherwise-rare infection (only 7 per 1,000,000 healthy people are infected with virulent Listeria each year). Better understanding the cell biology of Listeria infections, including relevant virulence factors, may lead to better treatments for listeriosis and other intracytoplasmic parasite infections. Researchers are now investigating the use of Listeria as a cancer vaccine, taking advantage of its "ability to induce potent innate and adaptive immunity."
Listeria monocytogenes is the bacterium that causes the infection listeriosis. It is a facultative anaerobic bacterium, capable of surviving in the presence or absence of oxygen. It can grow and reproduce inside the host's cells and is one of the most virulent food-borne pathogens, with 20 to 30 percent of clinical infections resulting in death. Responsible for an estimated 1,600 illnesses and 260 deaths in the United States (U.S.) annually, listeriosis is the leading cause of death among foodborne bacterial pathogens, with fatality rates exceeding even Salmonella and Clostridium botulinum.
"L. monocytogenes is a Gram-positive bacterium, in the division Firmicutes, named after Joseph Lister. Motile via flagella at 30°C and below, but usually not at 37°C, L. monocytogenes can instead move within eukaryotic cells by explosive polymerization of actin filaments (known as comet tails or actin rockets). Studies suggest up to 10% of human gastrointestinal tracts may be colonized by L. monocytogenes. Nevertheless, clinical diseases due to L. monocytogenes are more frequently recognized by veterinarians, especially as meningoencephalitis in ruminants. Due to its frequent pathogenicity, causing meningitis in newborns (acquired transvaginally), pregnant mothers are often advised not to eat soft cheeses such as Brie, Camembert, feta, and queso blanco fresco, which may be contaminated with and permit growth of L. monocytogenes. It is the third-most-common cause of meningitis in newborns."
Listeria monocytogenes is well equipped to survive food possessing technologies and host defense strategies due to quorum sensing behavior, thus mimicking behavior of this super talent pathogen is important for reducing their adverse effects on man and animals, through verification of HACCP plans in farms, foods, feeds and hospitals by using of new and rapid technologies of Chromogenic selective media and diagnostic tools such as Microbact technology aids in precise identification of this pathogen.
Classification

*L. monocytogenes* is a Gram-positive, non spore-forming, motile, facultatively anaerobic, rod-shaped bacterium. It is catalase-positive and oxidase-negative, and expresses a beta hemolysin, which causes destruction of red blood cells. This bacterium exhibits characteristic tumbling motility when viewed with light microscopy. Although *L. monocytogenes* actively motile by means of peritrichous flagella at room temperature (20–25°C), the organism does not synthesize flagella at body temperatures (37°C).
Both *L. ivanovii* and *L. monocytogenes* are pathogenic in mice, but only *L. monocytogenes* is consistently associated with human illness. There are 13 serotypes of *L. monocytogenes* that can cause disease, but more than 90 percent of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b. *L. monocytogenes* serotype 4b strains are responsible for 33 to 50 percent of sporadic human cases worldwide and for all major foodborne outbreaks in Europe and North America since the 1980s.
L. monocytogenes was first described by E.G.D. Murray in 1926 based on six cases of sudden death in young rabbits. Murray referred to the organism as *Bacterium monocytogenes* before Harvey Pirie changed the genus name to *Listeria* in 1940. Although clinical descriptions of *L. monocytogenes* infection in both animals and humans were published in the 1920s, not until 1952 in East Germany was it recognized as a significant cause of neonatal sepsis and meningitis.
Listeriosis in adults would later be associated with patients living with compromised immune systems, such as individuals taking immunosuppressant drugs and corticosteroids for malignancies or organ transplants, and those with HIV infection. Not until 1981, however, was *L. monocytogenes* identified as a cause of foodborne illness. An outbreak of listeriosis in Halifax, Nova Scotia involving 41 cases and 18 deaths, mostly in pregnant women and neonates, was epidemiologically linked to the consumption of coleslaw containing cabbage that had been treated with *L. monocytogenes*-contaminated raw sheep manure. Since then, a number of cases of foodborne listeriosis have been reported, and *L. monocytogenes* is now widely recognized as an important hazard in the food industry.
Pathogenesis

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. The manifestations of listeriosis include septicemia, meningitis (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 of fetomaternal listeriosis may suffer granulomatosis infantiseptica a pyogenic granulomas distributed over the whole body — and may suffer from physical retardation. Influenza-like symptoms, including persistent fever, usually precede the onset of the aforementioned disorders. Gastrointestinal symptoms, such as nausea, vomiting, and diarrhoea, may precede more serious forms of listeriosis or may be the only symptoms expressed. Gastrointestinal symptoms were epidemiologically associated with use of antacids or cimetidine. The onset time to serious forms of listeriosis is unknown, but may range from a few days to three weeks.
gastrointestinal symptoms is unknown but probably exceeds 12 hours. An early study suggested that \textit{L. monocytogenes} is unique among \textbf{Gram-positive} bacteria in that it might possess \textit{lipopolysaccharide}, which serves as an \textit{endotoxin}. Later it was found to not be a true endotoxin. \textit{Listeria} cell walls consistently contain \textit{lipoteichoic acids}, in which a glycolipid moiety, such as a galactosyl-glucosyl-diglyceride, is covalently linked to the terminal phosphomonoester of the teichoic acid.
This lipid region anchors the polymer chain to the cytoplasmic membrane. These lipoteichoic acids resemble the lipopolysaccharides of Gram-negative bacteria in both structure and function, being the only amphipathic polymers at the cell surface. *L. monocytogenes* has D-Galactose residues on its surface that can attach to D-Galactose receptors on the host cell walls. These host cells are generally M cells and Peyer's patches of the intestinal mucosa. Once attached to this cells, *L. monocytogenes* can translocate past the intestinal membrane and into the body.
The infective dose of *L. monocytogenes* varies with the strain and with the susceptibility of the victim. From cases contracted through raw or supposedly pasteurized milk, one may safely assume that, in susceptible persons, fewer than 1,000 total organisms (log 2) may cause disease. *L. monocytogenes* may invade the gastrointestinal epithelium. Once the bacterium enters the host's monocytes, macrophages, or polymorphonuclear leukocytes, it becomes blood-borne (septicemic) and can grow. Its presence intracellularly in phagocytic cells also permits access to the brain and probably transplacental migration to the fetus in pregnant women.
The pathogenesis of *L. monocytogenes* centers on its ability to survive and multiply in phagocytic host cells. It seems that Listeria originally evolved to invade membranes of the intestines, as an intracellular infection, and developed a chemical mechanism to do so. This involves a bacterial protein "internalin" which attaches to a protein on the intestinal cell membrane "cadherin". These adhesion molecules are also to be found in two other unusually tough barriers in humans — the blood brain barrier and the feto–placental barrier, and this may explain the apparent affinity that Listeria has for causing meningitis and affecting babies in-utero.
Stages in the intracellular life-cycle of *Listeria monocytogenes*. (Center) Cartoon depicting entry, escape from a vacuole, actin nucleation, actin-based motility, and cell-to-cell spread. (Outside) Representative electron micrographs from which the cartoon was derived. LLO, PLCs, and ActA are all described in the text. The cartoon and micrographs were adapted from Tilney and Portnoy (1989).
Detection

Anton Test: A test used in the identification of *Listeria monocytogenes*; instillation of a culture into the conjunctival sac of a rabbit or guinea pig causes severe keratoconjunctivitis within 24 hours. Culture Characteristics: *Listeria* grows on media such as Mueller-Hinton agar. Identification is enhanced if the primary cultures are done on agar containing sheep blood, because the characteristic small zone of haemolysis can be observed around and under colonies. Isolation can be enhanced if the tissue is kept at 4 °C for some days before inoculation into bacteriologic media. The organism is a facultative anaerobe and is catalase-positive and motile. *Listeria* produces acid but not gas in a variety of carbohydrates. The motility at room temperature and hemolysin production are primary findings that help differentiate *listeria* from *coryneform* bacteria.
The methods for analysis of food are complex and time-consuming. The present U.S. FDA method, revised in September 1990, requires 24 and 48 hours of enrichment, followed by a variety of other tests. Total time to identification takes from five to seven days, but the announcement of specific non-radiolabelled DNA probes should soon allow a simpler and faster confirmation of suspect isolates. Recombinant DNA technology may even permit two- to three-day positive analysis in the future. Currently, the FDA is collaborating in adapting its methodology to quantitate very low numbers of the organisms in foods.
L. monocytogenes has been associated with such foods as raw milk, pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (of all types), and raw and smoked fish. Its ability to grow at temperatures as low as 0°C permits multiplication in refrigerated foods. At refrigeration temperature, such as 4°C, the amount of ferric iron can affect the growth of L. monocytogenes.
The primary site of infection is the intestinal epithelium, where the bacteria invade non-phagocytic cells via the "zipper" mechanism. Uptake is stimulated by the binding of listerial internalins (Inl) to **E-cadherin**, a host cell adhesion factor, or **Met (c-Met)**, hepatocyte growth factor. This binding activates certain Rho-GTPases, which subsequently bind and stabilize Wiskott Aldrich syndrome protein (WASp). WASp can then bind the **Arp2/3 complex** and serve as an **actin** nucleation point. Subsequent actin polymerization creates a "phagocytic cup", an actin-based structure normally formed around foreign materials by phagocytes prior to endocytosis. The net effect of internalin binding is to exploit the junction-forming apparatus of the host into internalizing the bacterium. **L. monocytogenes** can also invade phagocytic cells (e.g., **macrophages**), but requires only internalins for invasion of non-phagocytic cells.
Following internalization, the bacterium must escape from the vacuole/phagosome before fusion with a lysosome can occur. Three main virulence factors that allow the bacterium to escape are listeriolysin O (LLO-encoded by hly) phospholipase A (encoded by plcA) and phospholipase B (plcB). Secretion of LLO and PlcA disrupts the vacuolar membrane and allows the bacterium to escape into the cytoplasm, where it may proliferate. Once in the cytoplasm, L. monocytogenes exploits host actin for the second time. ActA proteins associated with the old bacterial cell pole (being a bacillus, L. monocytogenes septates in the middle of the cell and thus has one new pole and one old pole) are capable of binding the Arp2/3 complex, thereby inducing actin nucleation at a specific area of the bacterial cell surface. Actin polymerization then propels the bacterium unidirectionally into the host cell membrane. The protrusion that is formed may then be internalized by a neighboring cell, forming a double-membrane vacuole from which the bacterium must escape using LLO and PlcB. This mode of direct cell-to-cell spread involves a cellular mechanism known as paracytophagy.
When listeric meningitis occurs, the overall mortality may reach 70%, from septicemia 50%, and from perinatal/neonatal infections greater than 80%. In infections during pregnancy, the mother usually survives. Reports of successful treatment with parenteral penicillin or ampicillin exist. Trimethoprim-sulfamethoxazole has been shown effective in patients allergic to penicillin.

A bacteriophage, *Listeria phage P100*, has been proposed as food additive to control *Listeria monocytogenes*. Bacteriophage treatments have been developed by several companies. EBI Food Safety and Intralytix both have products suitable for treatment of the bacterium. The U.S. Food and Drug Administration (FDA) approved a cocktail of six bacteriophages from Intralytix, and a one type phage product from EBI Food Safety designed to kill *L. monocytogenes*. Uses would potentially include spraying it on fruits and ready-to-eat meat such as sliced ham and turkey.
Because *L. monocytogenes* is an intracellular bacterium, some studies have used this bacterium as a vector to deliver genes *in vitro*. Current *transfection* efficiency remains poor. One example of the successful use of *L. monocytogenes* in *in vitro* transfer technologies is in the delivery of gene therapies for cystic fibrosis cases.

**Cancer vaccine**

A live attenuated *L. monocytogenes* cancer vaccine, ADXS11-001, is under development as a possible treatment for *cervical carcinoma*.