Listeriosis: A Foodborne Disease

Course # DL-009

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COURSE NAME: LISTERIOSIS – A FOOD BORNE DISEASE  COURSE # DL-009

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OUTLINE

A. Introduction
B. History of Foodborne Listeriosis
C. Transmission of *Listeria monocytogenes*
D. Illness/Clinical Symptoms of Listeriosis
E. Microbiology of *Listeria monocytogenes*
F. Pathogenic mechanisms of *Listeria monocytogenes*
G. Laboratory identification of *Listeria monocytogenes*
H. Treatment of Listeriosis
I. Prevention of *Listeria monocytogenes*
J. Conclusion
K. References

COURSE OBJECTIVES

At the conclusion of this course, the participant will be able to:

1. outline the history of foodborne listeriosis
2. discuss the incidence of foodborne listeriosis in the United States
3. explain the pathogenic mechanisms of *Listeria monocytogenes*
4. outline the clinical features of listeriosis
5. describe how the laboratory identifies *Listeria monocytogenes*
6. list various methods of preventing foodborne listeriosis

A. INTRODUCTION

Listeriosis is an infection caused by the bacterium *Listeria monocytogenes*, an organism that is widespread in the environment. *L. monocytogenes* can be found in water, soil, plants decaying vegetation, cattle, sheep, goats, poultry, animal sewage, manure, and slaughterhouse waste. *L. monocytogenes* received its current name in 1940 to honor Dr. Joseph Lister, a British surgeon and a pioneer of antiseptic surgery. There are currently 17 species of *Listeria*. *L. monocytogenes* is the predominant species that causes human disease and that is isolated from clinical specimens (1,3). Another species of *Listeria*, *L. ivanovi*, once considered to infect only ruminants, has been reported in extremely rare cases of listeriosis in humans and will not be discussed in this course (1,3).

Listeriosis is acquired by humans primarily through consumption of food or water contaminated with *L. monocytogenes*. Foods that are not pasteurized, foods that are not cooked,
and foods contaminated with animal feces or soil are the most frequent sources of infection (1,2,3). Cooking and pasteurization kill the organism. Therefore, cooking foods, treating or pasteurizing fluids, and avoiding food and fluids that may be contaminated with animal or human waste can prevent listeriosis. See Table 3. Major Foodborne Outbreaks of *Listeria monocytogenes*, 2011-2017, for list of other major foodborne outbreaks of *Listeria monocytogenes*.

*L. monocytogenes* is an important organism because it can cause widespread outbreaks of listeriosis in the general population, as happened in 2011 with contaminated cantaloupes. This caused one of the largest U.S. outbreaks involving 147 cases of listeriosis from 28 states (3).

*L. monocytogenes* infection can cause a spectrum of illness, ranging from mild flu-like symptoms and gastroenteritis to serious invasive illness, including sepsis and meningitis. Persons most at risk for acquiring listeriosis are in certain well-defined groups: pregnant women, newborns, the elderly with weakened immune systems, and immunocompromised patients, including persons with cancer, diabetes, kidney disease or AIDS, and those who take prescription steroid medications (1,3,4). However, rarely persons without these risk factors can also have serious disease due to *L. monocytogenes*. Generally, the prognosis for *L. monocytogenes* infection among healthy individuals is good; however, the prognosis rapidly declines in patients with risk factors if the disease is not quickly diagnosed and treated.

Human listeriosis is a disease with a low incidence but with a high mortality rate, i.e. relatively few people acquire listeriosis, but those with the disease are at greater risk of dying. *L. monocytogenes* is the most virulent of the food-borne pathogens and is the leading cause of death among foodborne bacterial pathogens, with fatality rates far exceeding even *Salmonella* (1, 3, 4, 5). See Table 5. Number of Cases, Hospitalizations, and Mortality Among the Most Common Foodborne Bacterial Pathogens in 2016 for comparison of fatality rates.

Accurate knowledge of the epidemiology and transmission of *L. monocytogenes* remains incomplete, since listeriosis can occur both as an epidemic disease and as sporadic cases. The Centers for Disease Control and Prevention (CDC) and its FoodNet program have an active surveillance program to monitor the incidence of disease, epidemiology, and transmission of *L. monocytogenes*. Because the organism can survive and multiply even in refrigerator and freezer temperatures, and in a wide range of pH, even a small amount of contamination may be significant. Therefore, food safety is a critical factor in the prevention of listeriosis.

**B. HISTORY OF FOODBORNE LISTERIOSIS**

*L. monocytogenes* was first isolated in 1926 by E.G.D. Murray, R.A.Webb, and M. B. Swann, microbiologists at the University of Cambridge, UK, from an epidemic among rabbits and guinea pigs. Dr. Murray and his colleagues described the organism and its biochemical characteristics, and named the organism *Bacterium monocytogenes*, because of the mononuclear leukocytosis observed in the animals. The organism was renamed *Listerella hepatolytica* by Dr. James Pirie in 1927 and then given its present name (*Listeria monocytogenes*) by him in 1940 (1). There are a few early reports of a Gram positive rod resembling *Listeria* detected from the spinal fluid of humans with meningitis in 1917 and again in 1920, but the cause of infection was unknown at that time (1). In 1952, *L. monocytogenes* was reported as a cause of neonatal sepsis and meningitis causing death, but it was believed then that these sporadic cases were due primarily to contact with diseased animals and not related to the consumption of contaminated food.
Until about 1960, *L. monocytogenes* was thought to be associated almost exclusively with infections in animals. In cattle, sheep, and goats it caused meningitis and encephalitis, making the animal move in circles; hence the common name of “circling disease.” Researchers also found that *L. monocytogenes* caused septic infections and intra-uterine infections resulting in abortion in sheep, goats, swine, rabbits, and cattle. The organism was rarely isolated from humans, and those cases where the organism was recovered often were from veterinarians or from others who handled animals.

It was not until 1981 that *L. monocytogenes* was identified as a cause of foodborne illness leading to manifestations of meningitis or septicemia and was called listeriosis. An early reported outbreak of *L. monocytogenes* in Halifax, Nova Scotia involved 111 patients and 18 deaths. Thirty-four of the infections occurred in pregnant women, among whom there were nine stillbirths and 23 infants born infected with the organism. Among 77 non-pregnant adults who developed overt disease mortality was nearly 30%. This outbreak was epidemiologically linked to the consumption of coleslaw made with cabbage from a local farmer who had been fertilizing his field with raw sheep manure from his flock. *L. monocytogenes* was also isolated from the contaminated raw sheep manure. During the outbreak investigation, it was learned that following the cabbage harvest each October, the crop was stored in a large cold-storage shed. As *Listeria monocytogenes* is able to grow at temperatures so low that other organisms either die or enter a stationary phase, the period of cold storage acted essentially as a period of selective enrichment for the organism.

Since this case in 1981 from Nova Scotia, *L. monocytogenes* has emerged as an important foodborne pathogen, and there have been many reported cases of foodborne outbreaks due to the organism. *L. monocytogenes* is now widely recognized as widely distributed in nature and is an important hazard in the food industry. See Table 1. Food Sources that are Typically Contaminated with *Listeria monocytogenes*. One large *L. monocytogenes* outbreak occurred in 1985 in California, where Mexican-style soft cheese was implicated as the source of the outbreak. In total, 142 people developed overt listeriosis with 34% mortality. Of the 142 cases, 93 cases were prenatal, and 49 cases were from non-pregnant individuals, 48 of whom were immunocompromised to some extent. Thirty newborn infants died and 22 adults died. The source of the *L. monocytogenes* was a particular brand of "pasteurized" soft cheese that apparently had become contaminated with unpasteurized (raw) milk during the manufacturing process (4,5).

The author has personal knowledge of two cases of listeriosis that also occurred in 1985 when he was a microbiologist at a San Jose, CA hospital. These two patients developed meningitis and septicemia with positive blood cultures due to *L. monocytogenes* after consuming ice cream made from unpasteurized milk. One of the two patients developed severe complications and died. The State of California Health Department later recovered *Listeria monocytogenes* from the ice cream food samples and from the manufacturing plant. The family sued the ice cream manufacturer and was threatening to sue the hospital and the primary care physician, but the hospital and physician were not part of the final litigation.

The World’s largest known *Listeria* outbreak to date began in March of 2017, which spread throughout South Africa. Upon updating this distance learning course in June of 2018, the disease outbreak is still ongoing for 15 months. So far, there have been 1,019 laboratory confirmed cases and 199 deaths. The number of hospitalizations is not known. The cause is related to a raw meat bologna product that is commonly consumed in South Africa.
There were a number of foodborne outbreaks of *L. monocytogenes* during the period of 2011 through 2017. See Table 3. Major Foodborne Outbreaks of *Listeria monocytogenes*, 2011-2017. One of the largest listeriosis outbreaks from multiple states in U.S. history according to the CDC (3,4) was in September 2011, involving the contamination of cantaloupe with *Listeria monocytogenes* from a single farm in Colorado. The Food and Drug Administration (FDA) found that the farm and processing environment contained *L. monocytogenes*. The identical strain of *L. monocytogenes* as the outbreak isolate determined by PFGE was found in pools of water and in the floor drain of the processing area. The farm had been fertilizing the cantaloupe field with animal manure. *L. monocytogenes*, which had the identical PFGE pattern, was recovered from the manure. The FDA considered the plant unsanitary and ordered the plant closed until changes were made and *L. monocytogenes* was no longer recovered from environmental samples. Overall, the contaminated cantaloupe caused 147 cases of listeriosis in 28 states; 143 people were hospitalized; 33 died; 7 cases were pregnant women; 3 cases were newborns, and one patient miscarried. The majority of cases were among individuals 65 years and older, pregnant women, and immunocompromised patients (3). Some excellent information about the outbreak and the CDC’s investigation is described in *Morbidity and Mortality Weekly Report* (MMWR) 2011:60(39):1357-1358.

An outbreak in September 2013 involved Crave Brothers Farmstead Cheeses contaminated with *L. monocytogenes*. A total of six infected persons were reported from five states (Illinois, Indiana, Minnesota, Ohio, and Texas). Major Foodborne Outbreaks of *Listeria monocytogenes*, 2011-2017. All six were hospitalized, one death was reported, and one illness in a pregnant woman resulted in a miscarriage. The *L. monocytogenes* contaminated cheeses were Les Frères, Petit Frère, and Petit Frère with Truffles, all manufactured by Crave Brothers Farmstead Cheese Company in Wisconsin. These cheeses were sold at high end grocery stores such as Whole Foods and others. The FDA closed the plant for several months while the manufacturer changed their procedures and cleaned the plant.

While the total incidence of listeriosis in the U.S. appears to have remained fairly stable since 2010, according to data collected by FoodNet (See Table 4. Number of Laboratory-Confirmed Bacterial Infections in the U.S. by Year and Pathogen, 2010-2016), there actually appears to be an increase worldwide, with the number of cases rising, especially in Europe (1,3). It is unclear whether this reflects a true increase in numbers or is due to better diagnosis and/or increased awareness of the disease. However, there is no doubt that the susceptible population is increasing, as are the number and types of foods in which *L. monocytogenes* is able to survive and grow. The public health importance of listeriosis is not always recognized, particularly because listeriosis is a relatively rare disease compared with other common foodborne illnesses. However, because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to foodborne illness. Since food is grown in close contact with animals, there is the possibility of a high exposure rate to the organism. Therefore, the implementation of preventive measures is vital to reduce the incidence of listeriosis.

Recent investigations by the CDC have shown that most foodborne outbreaks of listeriosis involve a small number of closely related genetic isolates, known as epidemic clones. An epidemic clone is a group of isolates that are genetically related and presumably from a common ancestor but are implicated in different or geographically unrelated outbreaks (1, 2, 3, 6). Determining genetic similarities or differences helps identify the source of the organism in outbreaks as well as in determining routes of infection and how the organism is transmitted in foods. Consequently, rapid subtyping assays such as PFGE and other methods capable of
discerning closely related epidemic clones are extremely important tools for public health laboratories to use in foodborne outbreaks.

**C. TRANSMISSION OF *LISTERIA MONOCYTOGENES***

Contaminated food and water are the primary vehicles of transmission for *Listeria monocytogenes*. Humans typically are infected by consuming raw vegetables that have been contaminated from the soil or from manure used as fertilizer; fresh, frozen, and processed meat and poultry that have been improperly prepared; milk or dairy products made from unpasteurized milk, including ice cream and yogurt; cheeses (particularly soft-ripened varieties); cold smoked salmon; fermented raw-meat sausages; hot dogs that have not been cooked; deli meats that have been contaminated during or after processing; and ready to eat foods (3). See Table 1, Food Sources that Can Be Contaminated with *Listeria monocytogenes*.

*L. monocytogenes* is widely distributed in the environment and has many opportunities to enter food production and processing environments, contaminating food during and after processing. The organism can survive on environmental surfaces that have not been cleaned, easily contaminating the next batch of food. *Listeria* has been found in many types of raw food (even seafood), but especially in meats, deli meats, vegetables, and cheeses. Some deli meats, for example, may become contaminated with *L. monocytogenes* from environmental surfaces, and because many deli meats are fermented rather than cooked, the organism can survive in the food. Contamination of beef, pork, lamb, and poultry is widespread. Some studies show contamination rates ranging from 12% to 50% of poultry (1,2,3).

*L. monocytogenes* is frequently associated with meningitis and disease in newborns, therefore pregnant mothers are advised not to eat soft cheeses such as Brie, Camembert, feta, and queso blanco fresco, which are more frequently contaminated with *L. monocytogenes* than hard cheeses. If the mother ingests *L. monocytogenes*, the organism can infect the placenta via the bloodstream and subsequently infect the fetus. Newborn infants can also be exposed to *L. monocytogenes* while traversing the vagina if the mother has been infected with the organisms. *L. monocytogenes* is the third-most-common cause of meningitis in newborns.

A particular property of *L. monocytogenes* that affects its food-borne transmission is its ability to multiply at a wide range of temperatures (1). Unlike most bacteria, *Listeria* can survive refrigeration and even freezing. Its ability to survive at temperatures as low as 0°C allows potential contamination of food and transmission of the organism when the food is thawed (1). Since *L. monocytogenes* grows in contaminated food stored in the refrigerator it is not surprising that listeriosis is frequently associated with products that have been refrigerated for a long period of time. It has been shown that *Listeria monocytogenes* can survive in certain foods at freezing temperatures (-20°C), and at refrigeration temperatures (4°C) over a 12 week period (1,2,3). *Listeria monocytogenes* is killed by cooking and pasteurization; however, in some ready-to-eat foods, such as hot dogs and deli meats, contamination may occur after factory cooking but before packaging. When *Listeria* gets into a food processing factory, it can live in the processing plant on environmental surfaces for years, contaminating food products.

**D. ILLNESS/CLINICAL SYMPTOMS OF LISTERIOSIS**

*Listeria monocytogenes* can cause a spectrum of illness in patients from mild flu-like symptoms with diarrhea to those illnesses where the organism has invaded past the
gastrointestinal tract lining causing symptoms such as septicemia and meningitis. Usually, mild cases of *Listeria* infection are not diagnosed or reported as “listeriosis.”

Healthy adults and children can get infected with foodborne *L. monocytogenes*, but they rarely become seriously ill. These symptoms can mimic other foodborne illnesses. When symptoms are present, they usually consist of mild gastroenteritis with nausea, vomiting, or diarrhea, and sometimes with fever and muscle aches, generally considered as “flu-like symptoms” (6). The onset time for flu-like and gastrointestinal symptoms to appear after ingestion of contaminated food or water is not certain, but probably exceeds 12 hours. Most healthy people spontaneously clear the infection within a week and require no treatment.

Host immunity is very important in the extent of disease and in prevention of disease. A decrease in T-cell mediated immunity (a decrease in phagocytes and cytotoxic lymphocytes called T-cells) and a decrease in the activation of macrophages, as seen in immunocompromised individuals, the elderly, or among pregnant women, can make the patient more susceptible to listeriosis.

If a patient develops listeriosis in which *L. monocytogenes* has invaded past the intestinal mucosa (See Section F., Pathogenic Mechanisms of *Listeria monocytogenes* for more details), the initial clinical symptoms can include fever, muscle aches, and nausea and/or diarrhea (flu-like symptoms) leading to septicemia or meningitis, which are the most typical clinical presentations in patients with listeriosis. The usual symptoms of sepsis are high fever, shaking chills, rapid breathing, rapid pulse, and low blood pressure. The patient can go into shock and die if not treated promptly. There are reports of sepsis due to *L. monocytogenes* leading to endocarditis in some patients (1). If the infection spreads to the meninges or to the brain, signs and symptoms may include: severe headache, stiff neck, fever, seizures, sensitivity to light, confusion or changes in alertness, loss of balance, fever, vomiting, convulsions, and death. There are rare reports of its causing brain abscess or encephalitis in patients with meningitis (1). There also have been very rare reports of *Listeria monocytogenes* causing lymphadenitis, cellulitis, pneumonia, osteomyelitis, and septic arthritis in immunologically compromised patients after septicemia or meningitis (1,2,3). The onset time of listeriosis with symptoms of septicemia and meningitis is unknown but may range from 3 to 70 days depending on the dose of organisms ingested and the host immune system (3,4,5).

Listeriosis primarily affects older adults >65 years, pregnant women, newborns, and adults with weakened immune systems, such as those with cancer, HIV infection, renal disease, diabetes, and patients on high steroid use. At least 90% of people who get listeriosis are in these high risk groups (1). See Table 2. Risk Factors for Acquiring Foodborne Listeriosis.

According to the CDC, the overall mortality rate of listeriosis is 20-30%. In those patients with risk factors the mortality rate is 70-90% in untreated patients, and 30-50% even when treated (3,4,5,6). *Salmonella*, in comparison, has a case mortality rate estimated at less than 1% (4,5,6). The mortality rates of meningitis due to *L. monocytogenes* may reach 70%, from septicemia --50%, and from *Listeria* endocarditis -- 50% (3,4). Patients who survive septicemia or meningitis often have lifetime neurologic sequelae (1,2,3). People with risk factors for listeriosis need to be treated quickly with IV antibiotics to reduce mortality. The CDC estimates that nationwide 1,600 persons become seriously ill with listeriosis each year, of these about 92 percent are hospitalized with 450 deaths, and there are 100 still-born births annually in the United States (4,5,6).

FoodNet, a real-time surveillance program of the CDC, collects data on actual laboratory confirmed foodborne cases from 10 states and on 5 foodborne pathogens. For example, FoodNet
published laboratory confirmed foodborne data for the years 2005 through 2016. The most recent FoodNet data from 2016 showed 116 cases of listeriosis from the 10 states. See Table 4. Number of Laboratory Confirmed Bacterial Infections in the U. S. by Year and Pathogen, 2010-2016. FoodNet also collects data on incidence, number of hospitalizations, the number of deaths due to each foodborne pathogen, and the case fatality ratio. See Table 5. Number of Cases, Hospitalization and Mortality among the Most Common Foodborne Bacterial Pathogens in 2016. Table 5 shows that \textit{L. monocytogenes} has a higher percentage of hospitalizations and a higher case-fatality ratio compared to other foodborne pathogens. For example, 95.7\% of patients with Listeria infection were hospitalized, and almost 13\% of the patients died in 2016.

More than half (58\%) of \textit{Listeria} infections occur among adults 65 and older. They are about 4 times more likely than the general population to get \textit{Listeria} infection (2,3). As adults age it is normal for there to be changes in their immunity, making them more susceptible to contracting a foodborne illness such as listeriosis. Pregnant women and neonates represent 14\% of \textit{Listeria} infections, and individuals with a weakened immune system (cancer, diabetes, AIDS, etc.) represent about 12\% of all \textit{Listeria} infections (2,3,4). Data from the CDC showed that 29\% of listeriosis patients had septicemic infections, 24\% had meningitis or another type of central nervous infection, and the remaining percentage was from patients with atypical forms of listeriosis (3).

Listeria sepsis or meningitis occurs more commonly in adults > 65 years and persons with impaired immune systems. In these patients, gastrointestinal symptoms and flu-like symptoms may precede more serious forms of listeriosis. In persons with meningitis, the cerebrospinal fluid (CSF) is purulent but the Gram stain is negative in over 60\% of patients. Cultures of blood and CSF are positive in 60\% to 75\% of patients and remain the most reliable means of diagnosis. The case fatality rate is estimated to be about 35\% (1). Approximately 20\% of meningitis cases in patients older than 65 years of age is due to \textit{L. monocytogenes}, making it the 5\textsuperscript{th} most common pathogen, yet it has the highest mortality. The mortality rate is estimated to be about 35\% (1).

Pregnant women are particularly susceptible to \textit{L. monocytogenes} infection and are approximately 20 times more likely than other healthy adults to get listeriosis (1). About 14\% of all listeriosis infections reported in the U.S. occurs in pregnant females (2,3,4). Pregnant Hispanic women are about 24 times more likely than the general population to acquire \textit{L. monocytogenes} infection (1,2,3). Illness may occur at any time during pregnancy, but usually in the third trimester, probably related to the major decline in cell-mediated immunity normally seen in women at 26-30 weeks of gestation (third trimester of pregnancy). 22\% of prenatal \textit{L. monocytogenes} infection result in stillbirth, spontaneous abortion, or neonatal death (1). In infections during pregnancy, the mother usually survives, but the infant mortality during the perinatal/neonatal period may be greater than 80\% (1).

If listeriosis occurs during pregnancy, the pregnant woman may initially experience a mild flu-like illness sometimes with diarrhea, abdominal cramps, lower-back pain, or with a mild fever and other non-specific symptoms such as fatigue and ache, which may or may not lead to further disease. If untreated, the initial flu-like symptoms in the pregnant woman may precede more serious forms of listeriosis where the mother may develop septicemia or meningitis and serious illness to the unborn baby. An early diagnosis can often be made in some cases by detecting \textit{L. monocytogenes} in maternal blood cultures or from her spinal fluid. Septicemia in the mother often manifests clinically as an acute febrile illness often accompanied by headache and backache, fever, myalgia, shaking chills, rapid breathing, rapid pulse, and low blood pressure.
The progression of *L. monocytogenes* infection in the mother can infect the placenta causing amnionitis and infection of the fetus, resulting in miscarriage, stillbirth, and premature birth.

The pregnant woman may go to her doctor with decreased fetal movements or early labor, unaware that she has or had listeriosis. The fetus may be stillborn or die within hours. It is believed that the frequency of spontaneous abortion or miscarriage due to listeriosis is under-reported and is often not diagnosed (1). The case fatality rate ranges from 30% to 50% with prenatal listeriosis (4,5). Exogenous transmission of *L. monocytogenes* can also rarely occur from the infected female genital tract at the time of delivery producing disease of the fetus or newborn resulting in an acutely ill infant or sometimes death.

There are two clinical forms of neonatal listeriosis, early- and late-onset forms. The mean time of incubation in early onset is 1.5 days and presumably occurs in infants infected *in-utero*. The newborn may develop a life-threatening infection within the first few days after birth or develop life-long health problems as a consequence of listeriosis. Fetal infection may also cause bacteremia and/or meningitis in the fetus. The consequences for the baby may be devastating. The organism is widely disseminated in the body of the newborn, with lesions being found most typically in the liver. The poorest prognosis appears to occur in the early-onset group. In late-onset neonatal listeriosis, the mean onset is 14.3 days, with meningitis as the predominant form of the disease. The source of the *L. monocytogenes* in these late-onset cases is unclear, although it is believed that the infection is acquired from the mother’s genital tract during birth.

Manifestations of neonatal listeriosis include respiratory distress syndrome, rash, pneumonia, vomiting, shortness of breath, shock, and hematologic abnormalities. The signs and symptoms of listeriosis in a newborn can be subtle, but may include: little interest in feeding, irritability, fever, and vomiting. At birth, the diagnosis is made by detecting the organism in CSF, blood, amniotic fluid, respiratory secretions, placental or cutaneous swabs, gastric aspirates, or meconium of the neonate (1, 2, 3). Direct microscopic examination of these specimens to detect small Gram-positive rods can be invaluable for early diagnosis and treatment. Additional focal infections may rarely occur after an episode of bacteremia in the infant, such as cutaneous lesions, endocarditis, arthritis, osteomyelitis, intra-abdominal abscesses, and pleuropulmonary infections (1). Surviving neonates of maternal listeriosis may suffer granulomatosis infantiseptica (skin abscesses or granulomas distributed over the whole body), and/or may suffer from physical or mental retardation.

**E. MICROBIOLOGY OF *LISTERIA MONOCYTOGENES***

Microscopically, *L. monocytogenes* appear as small, non-spore-forming Gram-positive rods, with rounded ends sometimes showing palisade formation (bacterial cells lying adjacent to each other in a picket fence arrangement). The organism can sometimes appear coccoid or be arranged in short chains and can thus be mistaken for *Streptococcus* sp. on direct smears. Occasionally, longer cells that may resemble *Corynebacteria* sp. or diphtheroids can be observed on direct smear. These morphological variations can at times be confusing and cause the microbiologist to incorrectly identify the organism. Generally, the cells of *L. monocytogenes* are approximately 0.5 µ micron in width by 1 to 2 µ microns in length (*E. coli* by comparison is 0.6 x 3 µ).

*L. monocytogenes* are facultatively anaerobic. The organism is capable of growing between - 0.4°C and 50°C (1). They are catalase positive, oxidase negative and esculin hydrolysis positive. *L. monocytogenes* grows well on most simple media, although growth is improved markedly by the addition of blood. Colonies are small (1 to 2 mm after 1 or 2 days of incubation
at 37°C). *L. monocytogenes* produces a narrow zone of clearing in the medium due to beta hemolytic activity on blood agar; this is often used as a marker to distinguish *L. monocytogenes* from other organisms. More on this topic is discussed in Section G. Laboratory Identification of *Listeria monocytogenes*.

Young broth cultures of *L. monocytogenes* show a characteristic tumbling end-over-end motility when viewed on a wet-mount with light microscopy. Although *L. monocytogenes* is actively motile by means of peritrichous flagella (flagella uniformly distributed all over the exterior of the organism) at room temperature (20–25°C), the organism does not synthesize flagella at body temperatures (37°C). More on this topic in Section G. Laboratory Identification of *Listeria monocytogenes*.

*L. monocytogenes* is resistant to many environmental and physical agents and can multiply and survive for years in the environment. It is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. For example, the organism has been shown to survive 5 minutes at 70°C and can survive for weeks at 4°C (1). *L. monocytogenes* is capable of surviving on environmental surfaces, but usually when occurring in niches or biofilms (where organisms stick together to form a matrix) where moisture and a carbohydrate are present. Because of the unusual physical characteristics of *L. monocytogenes*, the heat during pasteurization needs to be carefully controlled or the organism will not be killed during this process. According to the United States Department of Agriculture (USDA), pasteurization is the process in which milk is flash heated to 72°C for 15 seconds (3). The virulence of *Listeria monocytogenes* may also be affected by its growth temperature. For example, it has been shown that when grown at a reduced temperature (4°C) *L. monocytogenes* is able to increase its virulence, thus increasing its disease producing qualities in refrigerated foods (1).

**F. PATHOGENIC MECHANISMS OF *LISTERIA MONOCYTOGENES***

When food or water contaminated with *L. monocytogenes* is ingested the organisms pass into the gastrointestinal tract, causing some patients to develop a mild gastroenteritis with diarrhea and flu-like symptoms. These patients may not even know they have a mild listeria gastrointestinal tract infection. The development of an invasive infection with *L. monocytogenes*, however, depends on several factors including host immunity, gastric acidity, inoculum size, growth temperature, and various virulence factors of the organism.

For *L. monocytogenes* to produce listeriosis, the organism must be capable of penetrating the epithelial mucosal barrier of the intestinal tract. The gastrointestinal tract is the initial site of the bacterial replication that allows invasion, intracellular growth, and cell-to-cell spread. The incubation period for listeriosis is believed to be in the range of 3 to 70 days (1). The infective dose of *L. monocytogenes* varies with the strain and with the susceptibility of the patient, but it is believed that fewer than 1,000 organisms may cause disease.

*L. monocytogenes* has mechanisms that enable it to initially combat the effect of various acidic environments to which it may be exposed, such as that of the gastrointestinal tract and various acidic foods. *L. monocytogenes* is capable of neutralizing an acid environment because it is able to initiate the glutamate decarboxylase system. When *L. monocytogenes* is exposed to various environmental conditions such as the acid from the stomach, or acidic foods such as fruit juices, yogurt, salad dressings, or mayonnaise, *L. monocytogenes* switches on a specific gene called an ArcA gene, which permits acid tolerance and survival (1,2).
The most important pathogenic mechanisms of *L. monocytogenes* center on its ability to survive, evade, and multiply in phagocytic host cells. Attachment of *L. monocytogenes* to host gastrointestinal cells, monocytes and lymph nodes (Peyer’s patches) is the first step in the development of disease. This step is accomplished through D-galactose residues on the bacterial cell surface attaching to D-galactose receptors on host cells of the intestinal mucosa lining. Once attachment occurs, the second step involves a bacterial protein named internalin, which attaches to a protein called cadherin on the host intestinal cell membrane (1). The protein cadherin is an adhesion molecule that forms a barrier and binds cells and tissues together. This adhesion molecule is found in very high amounts in two other barriers in humans — the blood brain barrier and the fetal–placental barrier. The unique adhesion molecules from these two sites may explain the apparent affinity *Listeria* has for causing meningitis in adults and infecting the fetus *in-utero*.

After attaching to host cells, a third pathogenic step occurs when *L. monocytogenes* produces a protein called invasin that induces phagocytosis of *Listeria* into host phagocytes and mononuclear cells. Following induced phagocytosis and entry into the host cell, *Listeria monocytogenes* needs to escape from the phagosome (a product of phagocytosis containing the organism formed in the host cell) that is formed before it can enter into the cytoplasm of the host cell and proliferate. The phagosome membrane undergoes cytolysis, mediated by three main virulence factors of *L. monocytogenes* that allow it to escape into the host cytoplasm. They are:

1. a listeriolysin O (a hemolysin which causes lysis of cells)
2. a phospholipase (an enzyme that hydrolyzes phospholipids a component of cell membranes)
3. a lipopolysaccharide (an enzyme that hydrolyzes lipids) (1).

Each factor disrupts the phagosome membrane and permits the bacterium to escape into the host cell cytoplasm and then to multiply. Many investigators feel that listeriolysin O is the most important virulence factor for permitting cytolysis of the phagosome (1). Before lysis of the host phagosome occurs, *L. monocytogenes* is able to survive within the toxic environment of the phagosome by producing catalase and superoxide dismutase, which neutralize the various enzymes that are normally produced within the phagosome.

Once in the cytoplasm of the host cell, *L. monocytogenes* utilizes the host’s actin (a protein that is associated with the cellular membrane) to form filaments. Within the host cell cytoplasm and surrounded by sheets of actin filaments, *L. monocytogenes* can reside and multiply (1). Actin filaments are able to coat *L. monocytogenes* within 2 hours of entry in the host cytoplasm. A gene in *L. monocytogenes* turns on and promotes the polymerization of actin to form protrusions which propel the organism into and through the host cell membrane until *Listeria* finally reaches the host cell surface. At the cell surface, the actin-propelled *Listeria* pushes against the cell's membrane to form protrusions called filopods or "actin-rockets." The protrusions reach the cell's outer edge to put it in contact with adjacent cells. The adjacent cells then engulf the “actin-rocket” containing *Listeria* and the process is repeated, perpetuating the infection. The actin protrusions or filaments are essential to the pathogenesis by driving *Listeria* into the internal structure of an adjacent cell, causing direct cell-to-cell spread without an extracellular stage, thereby bypassing the immune system of the host (1).

This direct cell to cell spread mechanism is known as paracytophagy (1). Paracytophagy is the process whereby a cell engulfs a protrusion containing an organism from a neighboring cell. The protrusion contains material and organisms which are actively transferred between cells. *L. monocytogenes* is a classic example of an intracellular pathogen that can move directly
from cell to cell. Once phagocytosis occurs, the bacterium is never again extracellular and is never required to leave the intracellular environment. *L. monocytogenes* is then capable of causing tissue damage while it is within a host cell. Another example of an intracellular parasite is *Shigella flexneri* (1).

Once direct cell-to-cell spread is initiated, *Listeria* are taken up by phagocytes and spread to the regional lymph nodes, where the organisms multiply. *L. monocytogenes* can then spread to the host hepatic and splenic macrophages to produce multiple abscesses and granulomas. From these sites, *Listeria* can enter the circulatory system to produce septicemia often accompanied by maculopapular lesions of the legs and trunk due to lysis of the monocytes and macrophages. Once in the bloodstream, hematogenous dissemination may occur to any site, although *L. monocytogenes* has a particular predilection for the central nervous system and the placenta. Dissemination from the circulatory system to the meninges produces meningitis, the most commonly recognized form of listeriosis. *Listeria* is the leading cause of bacterial meningitis among adult cancer and renal transplant patients.

Organisms gain access to the fetal or newborn circulation through the placenta of the mother, resulting in disease. Pathogenesis of fetal and newborn listeriosis is initiated by organisms that gain access to the fetal or newborn circulation through the mother and are taken up by monocytes and macrophages, where they multiply and produce a septicemia in the neonate accompanied by maculopapular lesions of the legs and trunk.

Normally, the immune system eliminates the infection before it spreads. Normal monocytes and macrophages are capable of effectively eliminating and controlling *Listeria monocytogenes* before it fully initiates disease. However, if the immune system is compromised, systemic disease may develop. A failure to control *L. monocytogenes* by means of cell mediated immunity (activation of phagocytes, T-lymphocytes and cytokines) allows the bacteria to spread systemically.

**G. LABORATORY IDENTIFICATION OF *LISTERIA MONOCYTOGENES***

The initial diagnosis of listeriosis by the physician is usually based on the patient's history and physical exam, especially after the patient gives a history of a likely exposure to a contaminated food during a *L. monocytogenes* outbreak. Without this information, the diagnosis is difficult to differentiate from many other diseases. This situation may result in a delay of treatment, as the physician may order other tests to rule out other diseases such as salmonellosis, shigellosis, and *E. coli* O157 infection. Definitive diagnosis of listeriosis depends on isolation and identification of *L. monocytogenes* from the patient's blood, cerebrospinal fluid, placenta, lochia, amniotic fluid, or from another source.

As was mentioned in Section E, Microbiology of *Listeria monocytogenes*, the organism appears as a non-spore-forming, short, Gram-positive bacillus on Gram stain. The cells may be coccobacillary, or they may occur in short chains or in pairs suggestive of *Streptococcus pneumonia* or another species of streptococci. The organism may also appear as longer rods on a Gram stain smear suggesting *Corynebacterium* sp., which may lead to an inaccurate identification. A useful piece of advice is to question whether the organism may actually be *Listeria* when you see “diphtheroids” on Gram stain from a CSF specimen. The author has seen this error in the laboratory, so be aware of the various Gram stain morphological variations of *Listeria monocytogenes* that can cause misidentification of the organism. See Figure 1. *Gram Stain Photographs of Listeria monocytogenes* from Blood and Amniotic Fluid.
*L. monocytogenes* grows well on most laboratory media, but grows better on media containing blood, such as tryptic soy agar containing 5% sheep blood. Incubation under aerobic conditions at 37°C is optimal for isolation of the organism. Although *L. monocytogenes* grows readily from CSF, blood, and most other specimens, it can be difficult to isolate from placental and other fetal tissue. Placing the specimen at 4°C for several weeks and subculturing at frequent intervals may serve to enhance recovery. This procedure is called “cold enrichment” and is particularly useful for epidemiological studies when specimens are contaminated with other flora. Although not practical time-wise in most laboratory situations, it can be used by public health laboratories to improve recovery of *L. monocytogenes*.

Some laboratories may use specialized media for the differential and selective isolation of *L. monocytogenes* from clinical specimens—media such as McBride agar, Oxford agar, or Brilliance Listeria agar (BD Biosciences, Hardy Diagnostics, Oxoid, Remel, and others) (1,6). However, most likely the presence of *L. monocytogenes* from a clinical specimen will be unknown before the specimen is plated onto media, so these media are often not used or considered. Generally, these selective and differential agars are used by the public health laboratory or by the food industry. Likewise, other specialized media, such as chromagar (BD Biosciences, Hardy Diagnostics, Oxoid, Remel), are often used by public health laboratories or the food industry for faster isolation and identification. Occasionally the specialized media may be used by clinical laboratories during a known foodborne outbreak of *L. monocytogenes*. Occasionally the specialized media may be used by clinical laboratories during a known foodborne outbreak of *L. monocytogenes* where direct specimen plating is used on the chromagar, or suspected colonies are plated on chromagar for rapid confirmation.

The colonies of *L. monocytogenes* on 5% sheep blood agar after 24 hours of incubation at 37°C are small (1-2 mm in diameter), smooth, translucent gray colonies with a characteristic narrow zone of beta hemolysis. Although rarely isolated, *L. ivanovii* produces a very wide zone of beta hemolysis or sometimes a double zone of hemolysis (1,3). The colony morphology of *L. monocytogenes* closely resembles that of group B beta-hemolytic *Streptococci*, with which it is often confused. Sometimes the beta hemolysis is directly under the colony, or is very narrow, so that hemolysis can only be detected by moving the colony out of the way and directly observing the medium from where the colony was growing for indications of clearing indicating beta hemolysis. Care must be taken that colonies on blood agar are not confused with hemolytic streptococcus or mistakenly discarded as diphtheroids. Generally, motility at room temperature and beta hemolysis are useful characteristics that help differentiate *L. monocytogenes* from diphtheroids or *Corynebacterium* sp.

Another method useful for the identification of *L. monocytogenes* is the CAMP factor test. In this test, the beta hemolysin of *Listeria monocytogenes* acts synergistically with the beta hemolysin of *Staphylococcus aureus* on sheep blood agar, producing the effect known as the CAMP factor—an acronym for Christie, Atkins, and Munch-Petersen, three researchers who first described the phenomenon from Group B *Streptococci* (1). In this technique, *L. monocytogenes* is streaked at right angles, but not touching, the streaked *S. aureus*. If the CAMP factor test is positive, the hemolysis pattern of *L. monocytogenes* is enhanced in the vicinity of *S. aureus* forming an arrow shaped pattern within the medium.

Further biochemical characterization may be necessary to distinguish between *Listeria monocytogenes* and other Gram-positive bacteria with which they may be confused. *Streptococcus* spp. may be differentiated from *L. monocytogenes* on the basis of Gram stain morphology, motility, and catalase activity. *L. monocytogenes* may be distinguished from Group
Streptococcus by several laboratory tests; however, the initial beta hemolytic characteristic on blood agar, the coccobacillary appearance on Gram stain, or the tendency of *L. monocytogenes* to grow in short chains can often result in an erroneous identification of Group B *Streptococcus*. *Listeria monocytogenes* is positive for catalase, and hydrolyze esculin, while Group B *Streptococcus* is negative for both. Another Gram positive rod, *Erysipelothrix* spp., can be differentiated from *Listeria* by motility, catalase reaction, and ability to grow at 4°C. (*Erysipelothrix* spp. do not grow at that temperature.) *Kurthia* spp., another Gram-positive rod which might be confused with *L. monocytogenes*, are strictly aerobic and esculin negative. See Table 6. Basic Biochemical Tests Used to Differentiate *L. monocytogenes* from Other Bacteria It Resembles. Remember that the key biochemical characteristics to identify *Listeria monocytogenes* are catalase, esculin, motility, and a small zone of beta-hemolysis on blood agar media.

*L. monocytogenes* has a characteristic type of motility that can be helpful in its identification. *L. monocytogenes* is actively motile at room temperature (20-25°C); however, at body temperature (37°C) the organism does not generally synthesize flagella. Broth cultures of *Listeria monocytogenes* incubated at room temperature show a characteristic tumbling end-over-end motility when viewed on a wet mount with light microscopy, but very little tumbling motility, if at all, when the broth is incubated at body temperature (35°C). This characteristic is due to the lack of flagellin production at 37°C, but when the organism is grown between 20-25°C, flagellin is produced at the cell surface to allow the synthesis of flagella.

Another useful characteristic for identification is the unique “umbrella” shape motility pattern when *L. monocytogenes* is stabbed (inoculated) into semi-solid tubed media and incubated at room temperature (20-25°C) for 24 hr. After 24 hr of incubation at room temperature, an umbrella (parasol) pattern appears in the medium showing diffuse motility of the organism. The classic umbrella-shaped appearance generally does not occur when *Listeria monocytogenes* is incubated at 37°C. The umbrella-shaped pattern develops at the top of the semi-solid agar (where it was initially inoculated) and curves downward at the tube edges toward the bottom of the tube after overnight incubation at room temperature. The umbrella-shaped pattern not only illustrates the unique type of motility, but also illustrates the requirement of the organism for oxygen. The further the organism moves away from the top of the tube and oxygen, the less motile *L. monocytogenes* becomes. This requirement changes the motility pattern of the organism resulting in the umbrella-shaped diffusion pattern in the medium. See Figure 2. Photograph of *L. monocytogenes* with Umbrella-Shaped Motility Pattern.

Various commercial biochemical identification systems may also be used to help confirm the identification of *L. monocytogenes*, such as API Listeria (bioMérieux, www.bioMerieux.com). Various Gram positive automated panel systems are available such as MicroScan Siemens Healthcare (www.healthcare.siemens.com), Vitek bioMerieux (www.biomerieux.com), and BD Biosciences (wwwbdbiosciences.com) to name just a few of the automated systems. Another approach is to use the Listeria Latex Test on isolated colonies, or Listeria O Antisera on isolated colonies; both are available from Hardy Diagnostics (www.hardydiagnostics.com), Oxoid (www.Oxoid.com), or Remel (www.remel.com) to name just a few sources. The Listeria Latex Test and Listeria O Antisera are serological tests that produce clumping or agglutination of the reagent if *L. monocytogenes* is present.

One rapid identification method of *L. monocytogenes* for the clinical laboratory is a DNA probe assay manufactured by AccuProbe (Gen-Probe, San Diego, CA) for the confirmation of *L. monocytogenes* from colonies on solid media or from broth. This assay is highly specific (99%)
for detecting *L. monocytogenes* nucleic acid RNA sequences by chemiluminescence in 30 minutes. The AccuProbe method is approved for use in clinical laboratories as well as for use in the food industry.

Public health laboratories may use various molecular and other DNA sequencing techniques to rapidly identify and confirm the presence of *L. monocytogenes*. Public health laboratories use PFGE to determine genetic similarities or differences in a *Listeria monocytogenes* outbreak situation and for epidemiological purposes. Public health laboratories may also use various PCR or molecular methods, such as AccuProbe (Gen-Probe, San Diego, CA) for rapid identification of *L. monocytogenes*.

There are a variety of PCR, DNA-probe, and molecular methods available to the food industry to use for the rapid identification and confirmation of *L. monocytogenes*. These identification systems are available for the rapid detection of *L. monocytogenes* from food samples; however, these kits are not approved for use for the detection of *Listeria monocytogenes* from clinical specimens.

In the past few years, the number of subtyping assays has increased due to the development of DNA-based methods and other molecular techniques to accurately identify organisms and to identify their sources. The use of DNA-sequence-based molecular subtyping tests and procedures, such as PFGE, are essential to correctly determine the clonal relationship among different isolates to accurately recognize outbreaks and epidemics and to identify their routes of transmission and sources.

**H. TREATMENT OF LISTERIOSIS**

Most minor foodborne cases of *L. monocytogenes* in which the patient has only symptoms of gastroenteritis are usually not treated. In the majority of cases, the patient is unaware their gastroenteritis is due to *L. monocytogenes*. The majority of people with *Listeria monocytogenes* gastroenteritis spontaneously clear the infection in about seven days.

However, when *L. monocytogenes* has disseminated beyond the gastrointestinal tract and causes symptoms typical of listeriosis, e.g., high fever, chills, severe headache, and confusion due to septicemia or meningitis, then antibiotic treatment is initiated. In patients at increased risk, especially pregnant women, immediate IV antibiotic treatment to prevent, slow, or halt the development of more severe disease is essential. Early effective antibiotic treatment of pregnant females may be lifesaving for the fetus.

*L. monocytogenes* is susceptible to a wide variety of antimicrobial agents, including penicillin, ampicillin, tetracyclines, erythromycin, trimethoprim-sulfamethoxazole (TMP-SMX), and the aminoglycosides. Most authorities suggest ampicillin plus gentamicin for treatment of bacteremia, meningitis, and endocarditis (1,3,6). In patients who have an allergic response to penicillin-like antibiotics, the combination of TMP-SMX plus an aminoglycoside is recommended (1,3,6). Trimethoprim-sulfamethoxazole in combination with an aminoglycoside is bactericidal (kills bacteria rather than inhibits bacteria), which may be necessary in some clinical situations. No currently available cephalosporin, including the newer generations, should be used until further study is conducted (1). Further study is also required for the use of fluoroquinolones, such as ciprofloxacin, to determine their place in the treatment of *Listeria monocytogenes* infections (1).

In general, the length of antibiotic treatment increases with the severity of the infection. Septicemia due to *L. monocytogenes* is usually treated by IV for two weeks; meningitis is treated for three weeks; brain abscesses are treated for six weeks. However, each patient's treatment
should be individualized for optimal results. Many clinicians recommend an infectious-disease consultant be involved, and if the patient is pregnant, her obstetrician and a pediatric specialist should help manage the treatment plan because of the high mortality rate of \textit{L. monocytogenes}.

If diagnosed early enough in high risk patients, antibiotic treatment can prevent serious consequences of the disease. However, early diagnosis is the exception rather than the rule in pregnant women, since the first signs of a case or an outbreak are reports of stillbirth or serious infections of sepsis or meningitis. Even with prompt treatment, some cases of listeriosis result in death. This is particularly likely in older adults and in persons with other serious underlying medical problems.

**I. PREVENTION OF \textit{LISTERIA MONOCYTOGENES}**

Governmental agencies take various preventive measures to reduce the impact of foodborne infections in general and specifically for \textit{Listeria monocytogenes} infection throughout the United States. For example, the USDA implements various food safety requirements and procedures in the food industry and performs periodic inspections to monitor and reduce foodborne illness. The FDA protects the food supply and reduces contamination of food with periodic inspections, food safety requirements, recall notices, and plant closure if necessary. The FDA performs periodic inspections of food manufacturers, testing food samples and the environment of the food processing center looking for various pathogens using rapid detection molecular methods to permit “real-time” recall of food. If a food is found to be contaminated, food monitoring and plant inspection are intensified by the FDA to prevent cases of listeriosis and to ensure the safety of the food. Early detection and reporting of outbreaks of listeriosis to local and state health departments can help identify sources of infection, recall food, and prevent more cases of the foodborne illness.

In addition, the FDA publishes a list of product recalls on the internet to allow manufacturers, distributors, and individuals to check on specific products and to avoid them. For example, See Table 7. \textit{A Partial List of FDA Recalled Contaminated Food with \textit{Listeria monocytogenes} in 2017}.

The CDC protects overall health, controls disease, and implements various guidelines and procedures to reduce food contamination. In addition, the CDC has various surveillance programs to look at trends and potential outbreaks to monitor the incidence of disease. Surveillance of manufacturers and data collection and analysis conducted by the CDC and FoodNet does provide vital information but does not capture every illness. Even so, the CDC needs to have information about the potential total burden of illness in order to set public health goals and allocate adequate resources to prevent further foodborne illness. Each year, FoodNet reports on the number of people in the United States sickened with foodborne infections that have been confirmed by laboratory tests. See Table 4. \textit{Number of Laboratory-Confirmed Bacterial Infections in the U. S. by Year and Pathogen, 2005-2016}.

The CDC provides various recommendations and publications for issues such as food safety, handling of produce, dangers of raw milk, and special handling requirements for ready-to-eat foods (3). The CDC also publishes guidelines for the prevention of \textit{L. monocytogenes} foodborne infection in general and recommendations for persons at a higher risk. See Table 8. \textit{Prevention of \textit{Listeria monocytogenes} Foodborne Infections}. Further, because of the large outbreak of \textit{L. monocytogenes} from melons, the CDC has published guidelines specific to the safe handling and consumption of melons. See Table 9. \textit{Safety Tips for Eating Melons}. 

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Important information about safely consuming melons and further information about this outbreak is in *Morbidity and Mortality Weekly Report*, 2011; 60(39): 1357-1358.

Consumers must be proactive to prevent listeriosis by following the recommendations from the CDC to prevent foodborne infection. It is important to adhere to the food safety guidelines published by the CDC and the FDA to prevent *Listeria monocytogenes* infection (2,3,4). These guidelines include the following:

- keep kitchen clean;
- wash hands, utensils and cutting boards after use with uncooked food;
- keep ready-to-eat food (salads, deli meats, etc.) cold;
- cook meat and poultry thoroughly (read the label and use a food thermometer, usually meat should be cooked until 160°C and poultry until 165°C);
- thoroughly wash raw vegetables;
- avoid unpasteurized (raw) milk and dairy products made from unpasteurized milk;
- keep foods in the refrigerator until used;
- keep uncooked meats separate from vegetables and from cooked and ready-to-eat foods.

Various foods have a higher risk of contamination with *Listeria monocytogenes* and should be avoided, particularly unpasteurized milk and milk product – fresh cheeses (especially imported, and soft), yogurt, and ice cream. Other high risk foods are raw vegetables, raw sausages, raw/cooked poultry, raw meats, smoked fish, deli meats and hot dogs. Avoid soft cheeses (Mexican-style cheeses such quesillo blanco and quesillo fresco, feta, Brie, Camembert, and blue cheese), unless it’s clear from the packaging that the product was made using pasteurized milk. Leftover foods and ready-to-eat foods should be served only steaming hot. Avoid food from delicatessen counters or cook it thoroughly before consumption. Avoid meat spreads or pâté unless they are canned and previously cooked. Avoid cold smoked seafood such as salmon, trout, whitefish, cod, tuna, or mackerel that is most often labeled as "nova-style," "lox," "kippered," "smoked," or "jerky." Hot smoked seafood is not an issue.

**J. CONCLUSION**

Listeriosis is a rare foodborne disease caused by the organism *Listeria monocytogenes*. For most individuals the foodborne disease does not cause major problems other than a mild gastroenteritis which abates usually within a week. However, it can be devastating for individuals within certain high-risk groups such as pregnant women and newborns and others. Listeriosis can result in septicemia, meningitis, miscarriage, premature delivery, and serious infection of the newborn. Rarely, persons without risk factors can also be affected. The mortality rate can be very high for high-risk individuals. Early symptoms of listeriosis are often general and can mimic other foodborne illnesses.

The bacteria *L. monocytogenes* can be found in soil, water, plant material, and in animals that may contaminate our food. Generally, foods such as unpasteurized milk, improperly cooked meat, deli meats, or ready-to-eat foods are the prime sources of the organism. The organism can also live in food processing plants and contaminate a wide variety of foods. *L. monocytogenes* has the ability to survive under a wide range of environmental and temperature conditions, which contributes to the spread of the organism.

Although the total number of infections in the United States is not as high as other foodborne organisms, the mortality can be very high according to the CDC. For example, in
2011 there was a large outbreak from contaminated cantaloupe that involved 147 cases and 33 deaths from 28 states (3).

In high risk individuals, \textit{L. monocytogenes} invades past the intestinal mucosa to spread to the blood stream and to the meninges. The organism has a variety of pathogenic mechanisms to permit invasion past the intestinal mucosa and to facilitate the spread of the organism throughout the body. One unique pathogenic mechanism is the ability of \textit{L. monocytogenes} to have direct cell-to-cell spread.

\textit{L. monocytogenes} can be isolated and identified in the clinical laboratory using standard methods and techniques. It is a non-spore-forming Gram-positive small bacillus, but other Gram stain morphological variations often occur and can cause misidentification. It grows readily on media containing blood, although its morphology on blood agar may resemble other organisms with which it may be confused. The organism has a unique type motility that can be helpful in its identification. Other biochemical tests as well as thorough rapid molecular techniques can be useful in its identification. The public health department and the food industry may use other molecular techniques and other techniques such as PFGE that allow isolate differentiation for epidemiological purposes. Many of these techniques may not be approved for use in the clinical laboratory.

\textit{Listeria monocytogenes} is susceptible to a variety of antibiotics, but the usual treatment is ampicillin and gentamicin. Early treatment in its clinical course is helpful to reduce mortality.

Individuals can help prevent or reduce the risk of listeriosis by following the CDC recommendations for safe food preparation, consumption, and storage and by avoiding certain high-risk foods. High-risk individuals should avoid certain foods such as unpasteurized milk in which \textit{L. monocytogenes} may be present.

K. REFERENCES
Table 1. Food Sources That Can Be Contaminated with *Listeria monocytogenes*

1. Unpasteurized milk and unpasteurized dairy products, including yogurt and ice cream
2. Soft cheese made with unpasteurized milk, such as queso fresco, feta, Brie, or Camembert
3. Produce and raw sprouts
4. Improperly prepared meat
5. Ready-to-eat deli meats and hot dogs and other ready-to-eat foods
6. Refrigerated pâtés or meat spreads
7. Refrigerated smoked seafood

*Adapted from Centers for Disease Control and Prevention. Reference No. 3.

Table 2. Risk Factors for Acquiring Foodborne Listeriosis*

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1. Pregnancy
2. People who are over 65 with weakened immunity
3. Have AIDS
4. Are undergoing chemotherapy
5. People with certain diseases such as cancer, renal disease, liver disease, alcoholism, and diabetes
6. Take high-dose prednisone or certain rheumatoid arthritis drugs
7. Take medications to block rejection of transplanted organ
8. People who have a decreased immunity

* Adapted from Centers for Disease Control and Prevention, Reference No. 3.

Table 3. Major Foodborne Outbreaks of *Listeria monocytogenes*, 2011-2013*

<table>
<thead>
<tr>
<th>Date</th>
<th>Source</th>
<th>Outbreak Details</th>
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<tbody>
<tr>
<td>September 2011</td>
<td>Fresh cantaloupe from Jensen Farms, Colorado</td>
<td>147 persons infected from 28 states. 143 of the cases were hospitalized. Majority of cases were in persons 60 years and older. 33 persons died. 7 cases among pregnant women; one woman had miscarriage. Contamination within farm, equipment, and processing environments with animal waste. This was the largest single outbreak of <em>L. monocytogenes</em> in the U.S. The two owners were arrested on misdemeanor charges of introducing adulterated food into interstate</td>
</tr>
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commerce due to careless farming practices.

August 2012  Frescolina Marte Brand Ricotta Salata Cheese from Italy  
22 persons infected from 13 states. 20 of the cases were hospitalized. Infected persons ranged in age from 30 to 87. 4 deaths. 9 cases among pregnant women, resulting in 3 cases in newborns and 1 newborn death. The cheese was recalled, and FDA banned distribution of product.

July-September 2013  Cave Brothers Farmstead Cheeses from Wisconsin  
Three types of cheeses: Les Frères, Petit Frère, and Petit Frère with truffles  
6 persons infected from five states. All six were hospitalized. One death. Once case in a pregnant woman resulted in a miscarriage. Infected persons ranged in age from 30 to 67. FDA closed plant and investigated outbreak and firm’s corrective actions before plant resumed operations.

*Adapted from Centers for Disease Control and Prevention, References No. 3 and 4.

Table 4. Number of Laboratory-Confirmed Bacterial Infections in the U.S. by Year and Pathogen, 2005-2012a.

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<td>Campylobacter</td>
<td>5,692</td>
<td>5,770</td>
<td>5,871</td>
<td>5,854</td>
<td>6,058</td>
<td>6,372</td>
<td>6,785</td>
<td>6,365</td>
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<tr>
<td>Listeria</td>
<td>131</td>
<td>129</td>
<td>118</td>
<td>121</td>
<td>149</td>
<td>125</td>
<td>135</td>
<td>125</td>
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<tr>
<td>Salmonella</td>
<td>6,504</td>
<td>6,689</td>
<td>6,828</td>
<td>7,456</td>
<td>7,023</td>
<td>8,273</td>
<td>7,813</td>
<td>8,256</td>
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<tr>
<td>Shigella</td>
<td>2,095</td>
<td>2,765</td>
<td>2,869</td>
<td>3,043</td>
<td>1,854</td>
<td>1,779</td>
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<tr>
<td>STEC O157b</td>
<td>473</td>
<td>590</td>
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</tbody>
</table>

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Adapted from Centers for Disease Control and Prevention, References No. 3 and 4.

Shigella toxin-producing E. coli

Table 5. Number of Cases, Hospitalization, and Mortality Among the Most Common Foodborne Bacterial Pathogens in 2012*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No. Cases</th>
<th>Incidence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. Hospitalizations</th>
<th>Percentage&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. Deaths</th>
<th>Case Fatality Ratio&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>6,365</td>
<td>37.5</td>
<td>928</td>
<td>14.6</td>
<td>8</td>
<td>0.1</td>
</tr>
<tr>
<td>Listeria</td>
<td>125</td>
<td>0.73</td>
<td>112</td>
<td>89.6</td>
<td>16</td>
<td>12.8</td>
</tr>
<tr>
<td>Salmonella</td>
<td>8,256</td>
<td>48.6</td>
<td>2,290</td>
<td>27.7</td>
<td>29</td>
<td>0.4</td>
</tr>
<tr>
<td>Shigella</td>
<td>1,780</td>
<td>10.4</td>
<td>333</td>
<td>18.7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>STEC O157&lt;sup&gt;d&lt;/sup&gt;</td>
<td>442</td>
<td>2.6</td>
<td>184</td>
<td>41.6</td>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Total cases 16,968 3,847 55

*Adapted from Centers for Disease Control and Prevention, References No. 3 and 4. The year 2012 had the most recent data. Key = <sup>a</sup> incidence is the percentage of the total number of 16,968 cases, <sup>b</sup> percentage shows the percent of patients ill with that particular bacteria who are hospitalized, <sup>c</sup> case fatality ratio is the number of deaths among the total number of cases of that particular bacteria, <sup>d</sup> STEC is Shigella toxin producing E. coli.

Table 6. Biochemical Tests Used to Differentiate L. monocytogenes from Other Bacteria It Resembles*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Catalase</th>
<th>Esculin</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. monocytogenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>+</td>
<td>-</td>
<td>-/+</td>
</tr>
<tr>
<td>Erysipelothrix sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactobacillus sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. agalactiae (grp B)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enterococcus sp.</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ 90% of strains positive, - >90% of strains negative, -/+ variable (most strains negative)


<sup>1</sup> Test for the production of hydrogen sulfide from triple sugar iron agar medium.
Table 7. A Partial List of FDA-Recalled Contaminated Food with *L. monocytogenes* in 2017*

<table>
<thead>
<tr>
<th>Date</th>
<th>Contaminated Food</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>Raw Dog and Cat Food</td>
<td>Distributed nationwide by Blue Ridge Beef. Frozen beef was a risk to humans.</td>
</tr>
<tr>
<td>January</td>
<td>Frozen vegetable mix</td>
<td>Distributed in Florida</td>
</tr>
<tr>
<td>January</td>
<td>Sourellette cheese by Fromi</td>
<td>Distributed in California, New York and New Jersey</td>
</tr>
<tr>
<td>February</td>
<td>Pimento spread by Ruth’s</td>
<td>A variety of Ruth’s spread cheese mixtures. Distributed in S.E. United States</td>
</tr>
<tr>
<td>February</td>
<td>Various cheese by Sargento</td>
<td>Jack cheese, cheddar, nacho and Colby cheese. Nation-wide distribution.</td>
</tr>
</tbody>
</table>
March
Vulto Creamery cheeses

March
Smoked fish and salmon
Smokehouse brand of New York. Distributed nation-wide

May
Aunt Jemina and Hungry Man pancakes, waffles and toast
Major recall of products that are distributed nation-wide

June
Wildway and Trader Joe’s grain free granola products
Various grain free and granola products that are distributed nation-wide

July
Hummus and hummus products from Fresh Foods Market
Various products that are distributed nation-wide.

September
Fajita blend stir-fry vegetables
Diced peppers and other vegetables that are distributed primarily in the Southern U. S.

October
Various vegetable products by Mann Packing Co.
Major recall of packaged vegetables from Salinas, Ca. Broccoli, Brussel sprouts that are distributed nation-wide.

October
Fresh vegetable trays and cups
Distributed by Albertson’s Safeway, Vonns, and Pack and Save. Distributed nation-wide by Mann Packing Co.

November
Seafood meal bags
Distributed nation-wide by Albertson’s Market

November
Hummus, tahini, yogurt dips, and tabouli salad
Distributed nation-wide by Trader Joe’s

December
Smoked fish products
Distributed nation-wide by Nova Salmon Products found in Safeway, Albertson’s Whole Foods and other markets.

* Adapted from FDA Recall Products 2017. https://www.fda.gov/Safety/Recalls/Archives Recalls 2017
Table 8. Prevention of *Listeria monocytogenes* Foodborne Infections*

- Do not drink raw (unpasteurized) milk, and do not eat foods that have unpasteurized milk in them.
- Wash hands, knives, countertops, and cutting boards after handling and preparing uncooked foods.
- Rinse raw produce thoroughly under running tap water before eating.
- Keep uncooked meats, poultry, and seafood separate from vegetables, fruits, cooked foods, and ready-to-eat foods.
- Thoroughly cook raw food from animal sources, such as meat, poultry, or seafood, to a safe internal temperature.
- Consume perishable and ready-to-eat foods as soon as possible.

**Recommendations for people at high risk, such as pregnant women and people with weakened immune systems, in addition to the recommendations listed above:**

- Do not eat refrigerated smoked seafood, unless it is contained in a cooked dish.
- Do not eat refrigerated pâtés or meat spreads.
- Do not eat hot dogs, luncheon meats, or deli meats unless they are reheated until steaming hot.
- Do not eat soft cheeses such as feta, Brie, and Camembert, blue-veined cheeses, or Mexican style cheeses such as queso blanco, queso fresco, and Panela, unless they have
labels that state they are made from pasteurized milk.

* Adapted from Centers for Disease Control and Prevents, Reference No. 3.

Table 9. Safety Tips for Eating Melons*

- Wash hands thoroughly after handling and cutting melon.
- Scrub the melon surface under running water and dry it before cutting. Be sure that your scrub brush is sanitized after each use to avoid transferring bacteria between melons.
- Promptly consume cut melons or refrigerate promptly. Keep cut melon refrigerated for no more than 7 days.
- Discard cut melons left at room temperature for more than 4 hours.

*Adapted from Centers for Disease Control and Prevention, Reference No. 3.

REVIEW QUESTIONS

Course #DL-009

Choose the **one** best answer

1. Which set of symptoms is most typical of listeriosis?
   a. flu-like symptoms, chronic arthritis, pneumonia
   b. flu-like symptoms, diarrhea, chronic arthritis
   c. flu-like symptoms, sepsis, meningitis
   d. flu-like symptoms, endocarditis, pneumonia

2. Which of the following statements about listeriosis is correct?
   a. a disease with a high incidence and a high mortality rate
   b. a disease with a low incidence and a low mortality rate
   c. a disease with a high incidence but with a low mortality rate
   d. a disease with a low incidence but with a high mortality rate

3. According to the CDC, the highest incidence for listeriosis is among:
   a. individuals who are immunologically compromised
   b. > 65 years old
   c. pregnant women
   d. neonates

4. The CDC reports that 90% of the cases of listeriosis are among:
a. cancer patients, teenagers and >50 year old men  
b. patients >65, pregnant women and immunologically compromised patients  
c. patients >50, AIDS patients and pre-teenage patients  
d. male patients >65, pregnant women, and adolescent patients  

5. The incubation period of listeriosis after ingestion of contaminated food is:  
   a. 7 days to 3 weeks  
   b. less than 12 hours to 3 weeks  
   c. 3 days to 70 days  
   d. a few days to one week  

6. The number of *L. monocytogenes* organisms required to initiate symptoms of listeriosis is:  
   a. greater than 10,000 organisms  
   b. fewer than 1,000 organisms  
   c. fewer than 100 organisms  
   d. greater than 100,000 organisms  

7. Which virulence factor is associated with listeriosis?  
   a. hemotoxin  
   b. cytotoxin  
   c. invasin  
   d. leukocidin  

8. The correct biochemical profile of *Listeria monocytogenes* is:  
   a. catalase positive, esculin hydrolysis negative, oxidase positive  
   b. catalase negative, esculin hydrolysis positive, oxidase negative  
   c. catalase positive, esculin hydrolysis positive, oxidase negative  
   d. catalase negative, esculin hydrolysis negative, oxidase positive  

9. Which of the following three steps are recommended to prevent listeriosis?  
   a. wash hands before and after handling food, avoid meat, avoid raw produce  
   b. wash hands before and after handling food, avoid poultry, consume smoked seafood  
   c. wash hands before and after handling food, rinse produce, avoid meat  
   d. wash hands before and after handling food, rinse produce, thoroughly cook meat  

10. Which of the following is most likely not to be a source of *L. monocytogenes* infection:  
    a. cooked meat and vegetables  
    b. raw milk  
    c. refrigerated smoked seafood  

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d. refrigerated meat spreads

11. Which set of features best describes the appearance of *L. monocytogenes* on TSA with 5% sheep blood:
   a. small size colony, rough, white, with a wide zone of beta hemolysis
   b. medium size colony, smooth, white, with a narrow zone of beta hemolysis
   c. small size colony, smooth, gray translucent, with a narrow zone of beta hemolysis
   d. medium size colony, rough, gray translucent, with a narrow zone of beta hemolysis

12. Which pathogenic mechanism is **not** associated with the virulence of *L. monocytogenes*:
   a. D-galactose residues
   b. internalin
   c. cadherin
   d. fibronectin

13. Which are the correct Gram stain morphological features of *Listeria monocytogenes*:
   a. Gram positive coccus in chains, occasional long chains
   b. Gram positive bacillus, occasional spore, palisade formation
   c. Gram positive non-sporeforming bacillus, palisade, small cells
   d. Gram positive non-sporeforming bacillus, large cells, usually in pairs

14. The first step in the attachment of *L. monocytogenes* to host cells is initiated by:
   a. the protein invasin
   b. D-galactose residues
   c. the protein internalin
   d. the ArcA gene

15. What is the function of actin in the pathogenesis of *L. monocytogenes*:
   a. produces phagosome enzymes
   b. forms protrusions to propel organism into an adjacent cell
   c. promotes attachment to host cells
   d. causes cytolysis of host cells

16. Direct cell to cell spread is called:
   a. peristalsis
   b. paracytolysis
   c. paracytophagy
   d. paraphilia

17. Why is *L. monocytogenes* able to produce tumbling motility at 22-25°C, but not at 35°C?
a. due to the production of flagellin at 22-25°C  
b. due to the production of motility enzymes at 22-25°C  
c. due to the inhibition of growth of *L. monocytogenes* at 35°C  
d. due to the increase in growth rate at 22-25°C  

18. What is the recommended antibiotic therapy for listeriosis?  
   a. ampicillin plus a cephalosporin  
   b. TMP-SMX plus ampicillin  
   c. cephalosporin plus TMP-SMX  
   d. ampicillin plus gentamicin  

19. The most common manifestations of severe listeriosis include:  
   a. high fever, pneumonia  
   b. shock, gastroenteritis, high fever  
   c. meningitis, septicemia  
   d. encephalitis, brain abscess  

20. Which characteristic of a pregnant woman increases the possibility of getting listeriosis?  
   a. being black  
   b. carrying multiple infants  
   c. being Hispanic  
   d. living in an urban area  

21. Pasteurization needs to be carefully controlled to prevent listeriosis because:  
   a. *L. monocytogenes* is able to survive 10 minutes at 4°C  
   b. *L. monocytogenes* is able to survive weeks at 4°C  
   c. *L. monocytogenes* is able to survive 10 minutes at 95°C  
   d. *L. monocytogenes* is able to survive 5 minutes at 70°C  

22. The role of invasin in the pathogenesis of *L. monocytogenes* is:  
   a. induces formation of filaments in the host cell  
   b. induces phagocytosis of the organism  
   c. promotes cytolysis of the phagosome  
   d. induces the production of catalase and superoxide dismutase  

23. *Listeria monocytogenes* is capable of neutralizing an acid environment by:  
   a. initiating the internalin and cadherin system  
   b. initiating superoxide dismutase  
   c. initiating actin polymerization  
   d. initiating the glutamate decarboxylase system
24. One trick to improve the observation of beta-hemolysis in *L. monocytogenes* is:
   a. to move the colony out of the way
   b. to look at underside of the agar plate
   c. to observe the agar plate with indirect lighting
   d. to observe the agar plate with fluorescent lighting

25. Which physiological characteristic aids in the pathogenesis of *L. monocytogenes*:
   a. able to survive for a month at -30°C
   b. able to survive for 12 weeks at 4°C
   c. able to survive for 10 minutes at 85°C
   d. able to survive for 10 weeks at 0°C

26. Which is not a recommendation from the CDC about melon safety:
   a. keep cut melon in refrigerator for no more than 7 days
   b. discard cut melons left at room temperature for more than 4 hours
   c. do not leave seeds in melon after cutting
   d. scrub the surface of melon and dry before cutting

27. The CDC estimates that the incidence of listeriosis in the U.S is:
   a. 5,000 cases nationwide with 50% hospitalized
   b. 1,600 cases nationwide with 92% hospitalized
   c. 125 cases nationwide with 50-75% hospitalized
   d. 3,800 cases nationwide with 85% hospitalized

28. The function of actin in the pathogenesis of *L. monocytogenes* is:
   a. to form protrusions to propel the organism into adjacent cell
   b. to promote attachment of *L. monocytogenes* to intestinal cells
   c. to neutralize enzymes produced within the phagosome
   d. to disrupt the phagosome membrane

29. Meningitis is a common syndrome in listeriosis because of the predilection of *L. monocytogenes* for:
   a. the phagosome membrane
   b. internalin molecules
   c. cadherin molecules
   d. actin molecules

30. Compromised patients and pregnant women should avoid this food to prevent listeriosis:
   a. cooked smoked seafood
   b. pasteurized milk and its products
   c. cooked meat
   d. soft cheeses