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Listeriosis: a Rare but Deadly Disease

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Abstract

Listeriosis is a serious foodborne illness that affects primarily those with weakened immune systems. Sporadic cases are uncommon, but incidence rates can be high in outbreak situations. Identification of the organism is important for early disease detection and treatment and also for epidemiologic surveillance and tracking. This article reviews the microbiology of the organism that causes listeriosis, *Listeria monocytogenes*, and the clinical aspects of the disease, as well as strategies for prevention.

Introduction

Infection with *Listeria monocytogenes* can lead to a rare but serious foodborne illness termed listeriosis. The disease is typically limited to those with weakened immune systems, including pregnant women and their fetuses, newborns, the elderly, and immunocompromised individuals. On occasion, otherwise healthy children and adults are also affected. The organism was first described in 1926 by Murray and colleagues (1) during an outbreak among laboratory guinea pigs and rabbits. It was first named *Bacterium monocytogenes* because of the increased number of circulating monocytes found in these animals. In 1927, Pirie (2) named the organism *Listerella hepatolytica* in honor of Joseph Lister, a British surgeon and pioneer of the antiseptic technique. Its current name, *L. monocytogenes*, came about in 1940 (3). Originally recognized as a cause of illness among animals, its pathogenic potential in humans was not recognized until 1949 during an outbreak in newborns. The bacteria were isolated from meconium, blood, and/or organs of 85 stillborn infants or newborns with severe illness and findings of granulomas in various organs (4).

Epidemiology

Ninety percent of listeriosis cases are diagnosed in people belonging to high-risk groups, including pregnant women, newborns, adults over 65 years of age, and immunocompromised individuals (5). The CDC estimates that around 1,600 illnesses and 260 deaths are caused each year by *Listeria* (6). Since 2000, listeriosis has been a nationally notifiable illness. The Foodborne Diseases Active Surveillance Network (FoodNet) conducts active population-based surveillance for listeriosis in 10 U.S. states. Data are published annually. The most recently published data, from 2013, reported an incidence of 0.26 per 100,000 people (7). Compared to 1996-to-1998 data, the incidence of listeriosis has declined by 42% (8). However, compared to the 2006-to-2008 data, the incidence of listeriosis has not significantly changed (7). The incidence of listeriosis is high among newborn infants. Past estimates of incidence in newborns ranged between 30% and 40% of all cases of listeriosis (9,10). However, more recent U.S. data demonstrated an incidence of 9% for infants (11). Of all pregnancy-associated cases, 29% resulted in fetal loss or death of the infant. Hispanic women comprise a much higher percentage of pregnancy-associated cases, likely due to dietary practices (11).

Classically, soft cheeses made from unpasteurized dairy, delicatessen meats, hot dogs, and fresh produce have been recognized as the most efficient vehicles for transmission of *Listeria*. On average, two outbreaks per year occur in the U.S. The largest outbreak occurred in 2011, involving 28 different states. During this outbreak, 33 deaths, 1 miscarriage, and 147 illnesses were associated with con-

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sumption of contaminated cantaloupes from a single farm in Colorado. Since that time, the majority of outbreaks have been caused by soft cheeses, such as Mexican-style cheese and ricotta. Interestingly, the majority were made with pasteurized milk and were likely contaminated during the cheese-making process. The most recent outbreak, which caused five illnesses and two deaths, was linked to consumption of contaminated mung bean sprouts (5). Coleslaw, alfalfa tablets, hummus, fish, and undercooked chicken have also been implicated as food sources. Although the disease is almost always foodborne, nosocomial transmission has also been documented. Nine infants in a hospital nursery in Costa Rica became infected with *L. monocytogenes* after being bathed in mineral oil from a multi-dose container that had previously been used to bathe an infected infant (12).

The Organism

The genus *Listeria* consists of several different species, *L. monocytogenes*, *Listeria seeligeri*, *Listeria innocua*, *Listeria grayi*, *Listeria welshimeri*, *Listeria ivanovii*, *Listeria fleishmannii*, *Listeria marthii*, *Listeria rocourtiae*, and *Listeria weihenstephanensis* (13). Recently, whole-genome sequencing helped to identify five new species, *Listeria aquatic*, *Listeria floridensis*, *Listeria cornellensis*, *Listeria grandensis*, and *Listeria riparia* (14). Of these, only *L. monocytogenes* is known to be a human pathogen. It is a gram-positive, facultatively anaerobic bacillus, which is non-sporulating, oxidase negative, and generally catalase positive. There have been rare reports of catalase-negative isolates, as well (15). The organism's characteristic tumbling motility under light microscopy, which occurs at room temperature, is due to the presence of polar flagellae. *Listeria* is tolerant of low temperatures (4 to 10°C), high pH, and high salt concentrations, which explains its ability to grow in a wide variety of environments, including soil, sewage, manure, and refrigerated foods.

Direct Gram stain of clinical material or Gram stain of colonies typically reveals short, gram-positive, intracellular and extracellular rods, but the organism may resemble diphtheroids, cocci, or diplococci. Overdecolorization may give a gram-negative or gram-variable appearance. Therefore, presumptive diagnosis based on the Gram stain may be confusing. Report of a "diphtheroid" or "coryneform" bacterium from blood or cerebrospinal fluid (CSF) in an ill patient with the right demography should alert medical practitioners to the possibility of *L. monocytogenes* as the causative organism.

Isolation of the organism

L. monocytogenes grows well on most nonselective media, including sheep's blood agar, chocolate agar, tryptic soy agar, brain heart infusion agar, and thioglycolate broth. Growth occurs at temperatures of 4°C to 37°C, with the most rapid growth occurring between 30°C and 37°C. Colonies are 0.2 mm to 1.5 mm in diameter, are blue-gray under normal light, and have a blue-green sheen under oblique light. A narrow zone of β-hemolysis is produced on blood agar, which may be noticed only after the colony has been moved aside. In contrast to *L. monocytogenes*, *L. ivanovii* produces a double or triple zone of hemolysis. Most other *Listeria* species are nonhemolytic. In addition to patterns of hemolysis, the Christie-Atikins-Munch-Peterson (CAMP) test, as well as patterns of sugar fermentation, can be helpful for species differentiation. The CAMP test detects a synergistic effect of hemolysins between *Listeria* spp. and *Staphylococcus aureus* or *Rhodococcus equi* and can be useful for differentiating between β-hemolytic species. Table 1 outlines the growth and biochemical characteristics of the different *Listeria* spp. More recently, molecular techniques, such as mass spectrometry, have proven useful for species identification.

In contrast to isolation from sterile specimens (e.g., CSF, blood, and amniotic fluid), isolation from contaminated specimens (e.g., stool and food) requires selective enrichment, followed by plating onto selective media. Because of its somewhat unique ability to grow in cooler temperatures, cold enrichment at 4°C in nonselective broth medium was used in the past to isolate the organism. However, it can take weeks for isolation of the organism using this method. In addition, cold enrichment is not as sensitive or specific as currently used selective media. Therefore, the cold-enrichment technique is no longer recommended. The most commonly used selective enrichment media include the Food and Drug Administration (FDA) broth, U.S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) broth, L-PALCAM broth (Oxoid Limited, Thermo Fisher Scientific, Carlsbad, CA) and nutrient broth that contains 3.75% potassium thiocyanate and nalidixic acid. McBride and LPM agars are two selective plating media for *Listeria*. Other differential media, such as Oxford, modified Oxford, and PALCAM (polymixin B, acriflavine, lithium chloride, ceftazadime, aesculin, and mannitol), allow presumptive identification based on the appearance of colony morphology or color. Therefore, differential media are often preferred. On Oxford agar, the organism appears as 1-mm black colonies

Table 1. Characteristics of different *Listeria* species[a]

		CAMP reaction		Type of acid production			
Species	**β-Hemolysis**	***S. aureus***	***R. equi***	**α-Methyl-D-mannoside**	**D-Xylose**	**D-Mannitol**	**L-Rhamnose**
L. monocytogenes	Narrow	+	+/-	+	-	-	+
L. seeligeri	Narrow	+/-	-	-	+	-	-
L. ivanovii	Wide or multiple zones	-	+	-	+	-	-
L. innocua	None	-	-	+	-	-	+/-
L. welshimeri	None	-	-	+	+	-	+/-
L. grayi	None	-	-	+	-	+	+/-

[a]Adapted from reference 30. +, positive; +/-, variable; -, negative.