Case Study:

Hemolytic organism makes headline news

Can You Identify This Organism?

An 89 year old man presented to a community hospital emergency room with weakness and inability to walk.

Among other tests, two sets of blood cultures were drawn to rule out sepsis, and submitted to the Microbiology laboratory where they were put into an automated blood culture instrument for incubation.

Within twenty four hours both aerobic bottles and one anaerobic bottle flagged as positive. Aerobic bottles were subcultured to Sheep Blood, Chocolate and MacConkey agar plates incubated at 35-37°C in 5-10% CO2, as well as an anaerobic Brucella agar plate incubated anaerobically. Anaerobic subculture included a BBE/LKV plate incubated anaerobically.

Direct and Day 1 gram stains showed small, non-spore forming gram positive rods.

Plates were examined on Day 1. A small, whitish, moist colony with a narrow zone of beta hemolysis around and under the colonies was noted on Blood agar plates. Growth was noted on Chocolate and anaerobic Brucella agar plate. A Bile Esclulin slant was inoculated and turned positive the next day.

A wet mount was done at the bench and the organism appeared very motile with a characteristic type of “tumbling” motility. The catalase test was positive. A CAMP test was performed; however, results were questionably negative.

An automated identification system identified the organism at the 99% level.

What is the organism?

Blood agar plate on left with reflected light. The same plate on right with transmitted light, demonstrating small zones of beta hemolysis.

Gram stain showing short Gram-positive rods.
ANSWER:

This organism identified with a 99% probability of being *Listeria monocytogenes*.

Correctly identifying this organism depends upon the microbiologist suspecting it as a possible etiologic agent when encountering beta hemolytic organisms, especially in blood, CSF, or genital and placental specimens. Although this patient’s history showed no evidence of possible *Listeria* exposure, the incubation period for *Listeriosis* can be four or more weeks, so for the patient to remember all that was previously consumed can be difficult. The information was given to Public Health for follow-up per reportable disease protocol.

The Colorado *Listeria* outbreak that began on July 31st of this year turned out to be the deadliest food-borne illness within the last 25 years. 123 persons became ill, and 25 died due to contaminated cantaloupe.

**Gram stains** and **catalase** should be performed to rule out *Listeria* from the Beta hemolytic Strep, which is also found in these specimen types. *Listeria* are facultatively anaerobic, non spore forming, short gram positive rods that may form short chains or diplococci when gram stained; careful staining and interpretation of any direct specimen smear must be done to avoid confusion with possible *Pneumococcus* or *Haemophilus* organisms.

*CAMP* tests must be interpreted with care as *Listeria* generally does not produce an arrowhead shaped zone of hemolysis but rather a rectangular zone. Robustness of the *Staphylococcus aureus* CAMP strain should also be ensured for proper hemolytic zone formation. Always perform positive controls.

![Scanning electron micrograph of *L. monocytogenes* showing flagella.](image1)

The organisms are ubiquitous in nature, found in fresh and salt water, sewage, soil, dust and decaying vegetable matter. They have been found in feces of both healthy and symptomatic humans and in over 40 species of animals and birds, including cattle, sheep, and wild and domestic fowl. *Listeria* can cause
meningoencephalitis in cattle and sheep and have caused stillbirths in other animals. Human acquisition of the organism is often due to consumption of raw foods of plant or animal origin which become contaminated with the organism during the growing or processing process.

Common foods include raw processed meat products including deli meats or hot dogs, unpasteurized milk products including cheese, and raw fruits and vegetables.

The current Listeria outbreak linked to contaminated cantaloupe grown in Colorado has sickened hundreds and killed over a dozen people as of this writing.

FDA investigators found the root cause of the latest cantaloupe Listeria outbreak in Colorado to be due to unsanitary processing conditions; pooled stagnant water, dirty tables, a contaminated produce washer, and a lack of a cooling system.

Human Listeriosis can range from asymptomatic, to a mild self limiting “flu-like illness”, to a severe sepsis or meningoencephalitis and death. Rare focal skin or ocular infections have been documented from occupational settings as well as rare nosocomial transmission. The elderly and immunosuppressed are more likely to suffer severe illness.

Pregnant women, if infected, may exhibit a range of disease from mild to severe, with fetal involvement or death a possibility.

According to the CDC, approximately 1,600 persons become seriously ill with *L. monocytogenes* every year, and about 260 will die from the illness. It is the third deadliest food borne illness in the U.S., after *Salmonella* (380 deaths annually) and *Toxoplasma gondii* (330 deaths per year). Listeria grows well at 4 deg. C, enhancing the virulence of the organism by allowing growth in refrigerated foods. CDC advises heating ready-to-eat foods to steaming hot, eating raw and perishable foods as soon as possible, avoiding unpasteurized milk, and washing raw fruits and vegetables thoroughly to reduce risk of infection with Listeria.

Diagram showing the infective intracellular nature of *Listeria monocytogenes*.

Listeria organisms are able to survive within cells of the monocyte-
macrophage system, as do *Mycobacterium*, *Brucella*, *Legionella*, and *Salmonella*.

It is this trait, in part, which allows the organism to travel from the GI tract through the body to blood, CSF, or placenta without being exposed to normal bacterial defense mechanisms such as PMN’s, antibodies or complement. Immunity to *Listeria* as with other intracellular pathogens is primarily cell mediated.

The genus *Listeria* contains species other than *L. monocytogenes*; other than *L. ivanovii*, formerly called *L. monocytogenes* serovar 4 or *L. bulgarica*, they are non pathogens. *L. ivanovii* also exhibits beta hemolysis on Sheep Blood Agar but its clinical significance has not been documented.

Although strains of *L. monocytogenes* can vary in their antimicrobial susceptibility, current recommendations for treatment of Listeriosis are Ampicillin or Penicillin in combination with gentamycin, or Trimethoprim/Sulfamethoxazole for Penicillin allergic patients. Treatment should be for at least three weeks or relapse may occur. There have been reports of some resistance to Trimethoprim which raises concern about the future of TMP/SXT for treatment.

In summary, *Listeria* is a serious pathogen which can be found in clinical specimens and needs to be considered when examining appropriate cultures growing beta hemolytic organisms. Development of chromogenic media to enhance growth of this organism can increase timeliness of identification and accuracy of patient management.

As a newspaper headline proclaimed this week, “*Lives Devastated by Listeria as Cantaloupe Outbreak Grows*”, we as microbiologists need to keep this organism in mind to ensure optimal patient diagnosis, treatment and care.

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Editor’s note: Do you have an interesting case history to share with your fellow MicroBytes readers? If so, please contact Newsletter@HardyDiagnostics.com.