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Study of Listeria Isolation from Milk on Nutrient Media with Tellurite

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Abstract: The wide distribution of listeria in nature, their significant resistance in the external environment, the difficulty of clinical diagnosis, and damage to humans place listeriosis in epidemiological terms among a number of dangerous diseases. The article provides data on the survival of Listeria in milk, the period of generation of Listeria in milk and dairy products is studied, and a comparative assessment of culture media for the accelerated isolation of Listeria from milk is carried out.

Keywords: Listeriosis, mononuclear leukocytosis, meat peptone agar, psychrophilicity, tellurite florimycin - chloride medium; Listeria agar cultures, optical turbidity standard.

Listeriosis is a little–known zoonotic infection that poses a danger to human health. The widespread occurrence of listeria in nature, their significant stability in the external environment, the difficulty of clinical diagnosis, and human damage place listeriosis in an epidemiologically dangerous range of diseases. In humans, the disease is caused by the species Listeria monocytogenes – spontaneous and experimental infection is accompanied by mononuclear leukocytosis. In humans, the disease occurs in most cases with damage to the nervous tissue or in the form of an anginous-septic form. From the body of a sick animal, the pathogen is excreted in urine, feces, nasal discharge, milk and amniotic fluid. The pathogen was isolated from humans by J. Dumont, L. Cotoni from the cerebrospinal fluid of a patient with meningitis. E. Jungherr isolated listeria in listeriosis encephalitis. P.P. Sakharov, E.I. Gudkova published a number of works on the isolation of listeria from the body of sick people.

Listeria (Listeria monocytogenes, L. monocytogenes) are bacteria that cause the infectious disease listeriosis in animals, including domestic, birds and humans. Thousands of cases of the disease are registered annually in the world, with a mortality rate of 20-30%, which is significantly higher than that from other foodborne toxic infections, including such as salmonellosis and botulism. Listeria is a great danger for pregnant women, newborns, the elderly and patients with severely reduced immunity. They affect mononuclear phagocytes, which is why they are called Listeria monocytogenes.

Listeria live in water, soil, plants and food. The ability of bacteria to multiply in food at low temperatures (home refrigerator conditions) can cause food poisoning in humans. The first symptoms of listeriosis appear only a few weeks after eating infected foods — raw milk, products made from raw milk, meat and meat products, greens, vegetables and fruits contaminated with soil, where the secretions of sick animals got.

Listeria enters the human body through the mucous membranes of the oral cavity and nose, conjunctiva, mucous membrane of the digestive tract, respiratory organs and damaged skin. The disease occurs in localized or generalized forms. With the mass reproduction of listeria monocotogenes in the blood of patients, sepsis develops.

The purpose of the work: to study the period of listeria generation in milk to clarify the role of milk and dairy products in the transmission of listeriosis infection, as well as to study in a comparative aspect the nutrient media for the isolation of listeria from milk.

The following nutrient media were tested: tellurite-florimycin-chloride medium; Martin agar; meat-peptone agar with potassium tellurite; meat-peptone liver agar with 1% glucose and 2% glycerin; meat-peptone agar; indicator medium with bromothymol blue, for the preparation of which 60 ml of

indicator is added to 1000 ml of MAPP 2% aqueous solution of bromothymol blue, the initial color of the medium is dark green, the causative agent of listeriosis changes it to yellow during growth; listeria colonies are smoky gray in the first 24 hours of growth, and after 36-48 hours. they turn golden yellow of characteristic morphology; indicator medium with bromocresol purple indicator (60 ml of 2% solution of bromocresol purple indicator is added to 1000 ml of MPPA); meat-peptone broth with the addition of 8.5% sodium chloride; MPPB with potassium rhodanide; BCH.

A suspension containing 1 billion mg was prepared from daily agar cultures of listeria strains 9-72, 9-127, 2795/ 5. microbial cells. in 1 ml according to the optical turbidity standard. After microscopy and investigation in the agglutination reaction, dilutions were made in sterile and fresh non-pasteurized milk from 1:100 to 1:100000. 0.1 ml of each dilution was applied to the surface of the medium in Petri dishes and incubated at 370. During cultivation on liquid nutrient media, they were subsequently transplanted to dense media and the presence of colonies, their number, morphology, etc. were determined.

Crops on dense media were viewed in transmitted light using an OI-31 type illuminator with a blue light filter. Listeria colonies in transmitted light, unlike colonies of other microorganisms, have a blue color with a greenish tinge and a fine-grained structure, have the smell of cottage cheese or whey due to the accumulation of carbohydrate metabolism products. In addition, smears were prepared and colored according to Gram. Pure listeria cultures were studied for control using the same tests.

The effectiveness of the medium was assessed by the time of listeria growth and the number of colonies, taking into account the dilution of the studied material.

The most effective for isolating listeria from artificially infected milk were: from dense ones - tellurite–florimycin-chloride medium, MPA with potassium tellurite; from liquid ones - MPPB with potassium rhodanide and MPB with 8.5% sodium chloride (see Table)

On tellurite–florimycin-chloride medium, an increase in the form of the smallest colonies, on the second day they turned black due to the reduction of potassium tellurite to metallic tellurium.

On liquid media – MPPB with potassium rhodanide and BCH with the addition of 8.5% sodium chloride – the growth manifested itself in the form of barely noticeable opalescence. When sown on these dilution media of 1:100,000, listeria grew in 100% of cases, when using other liquid nutrient media for this purpose, growth in 100% of cases occurred only when sowing a dilution of 1:100,000 or less.

Conclusion. The most effective for isolating listeria from milk were from dense nutrient media tellurite-florimycin-chloride medium, MPA with potassium tellurite; BCH with the addition of 8.5% sodium chloride.

The effectiveness of isolating listeria from pasteurized milk using a number of dense and liquid nutrient media.

Nutrient media	Colonies have grown during the sowing of different dilutions			
	1:100	1:1000	1:10000	1:100000
Tellurite-florimycin-chloride medium	740 ± 23,2	80 ± 5,2	12 ± 1,5	1
MPA with 0.5% glucose and 1% glycerin	420 ± 33,2	30 ± 12,9	3 ± 1,4	-
Agar Martin	640 ± 11,6	45 ± 1 5,8	7 ± 1,2	-
Medium with bromothymol ±blue	650 ± 17,4	30 ± 2,9	8 ± 1,2	-
Medium with	400 ± 40,6	25 ± 1,7	7 ± 1,2	-

bromocresol purple				
MPA with potassium tellurite	800 ± 62,8	70 ± 5,8	10 ± 2,6	1
MPPB with potassium rhodanide	500 ± 19,3	75 ± 2,9	10 ± 2,6	1
BCH with 8.5% sodium chloride	600 ± 11,6	60 ±2,9	7 ± 1,1	1
MPB	300 ± 11,6	40 ± 2,9	2 ± 5,8	-
MPA	410 ± 17,4	50 ± 1,7	3 ± 1,4	-

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