

ISOLATION AND IDENTIFICATION OF *LISTERIA MONOCYTOGENES* BASED ON MORPHO-TINCTORIAL CHARACTERISTICS

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Abstract

Listeria monocytogenes is an opportunistic pathogenic bacterium responsible for listeriosis in humans and animals, a disease with major implications in the field of food safety. The aim of this study was the isolation and identification of *Listeria monocytogenes* based on morpho-tinctorial characteristics, using classical microbiological methods. The analyzed samples were subjected to a pre-enrichment and selective enrichment stage, followed by inoculation on specific differential media. The suspected colonies were subsequently examined microscopically, revealing the characteristic morphology of short, Gram-positive bacilli, arranged singly or in short chains. Gram staining and motility tests confirmed the presence of Gram-positive bacteria, motile at 25°C and non-motile at 37°C, suggesting their affiliation to the genus *Listeria*. The results obtained demonstrate the importance of morpho-tinctorial characteristics as an essential step in the preliminary identification of *Listeria monocytogenes*, providing a solid basis for subsequent confirmation through biochemical and molecular tests.

Keywords: *Listeria monocytogenes*: morphology, Gram staining, isolation, identification, Gram-positive bacteria.

INTRODUCTION

Listeria monocytogenes is a Gram-positive, facultatively anaerobic bacterium of major importance in medical and food microbiology, being the etiological agent of listeriosis. This severe zoonosis can affect both humans and animals, manifesting through septicemia, meningitis, spontaneous abortions, and neonatal infections. Due to its ability to multiply at low temperatures and resist unfavorable environmental conditions, *Listeria monocytogenes* represents a significant threat to the food industry and public health.

Accurate and rapid identification of this bacterium is essential for epidemiological control and the prevention of foodborne infections. The isolation process involves the use of selective and differential media that favor the growth of *Listeria* over other microorganisms. Subsequently, the analysis of morphotinctorial characteristics—such as cell shape, bacilli arrangement, and Gram staining reaction—constitutes a fundamental step in the preliminary recognition of the species.

Microscopic observation reveals specific features: short, slightly curved, Gram-positive bacilli, arranged singly or in short chains. These characteristics, correlated with its distinctive motility at 25°C and absence of motility at 37°C, facilitate the differentiation of *Listeria monocytogenes* from other morphologically similar bacteria. Thus, the study of

morphotinctorial characteristics represents an essential stage in the process of isolation and identification of this pathogenic agent, providing the basis for further confirmation through biochemical and molecular methods.

MATERIAL AND METHODS

We performed a prospective study, based on the microbiologic diagnosis registered in the bacteriologic register of the laboratory of medical analysis, S.C. Diaser, Oradea.

The period for which was extended the study is of 1 years, including the period 01.01.2024-31.12.2024.

For the performing of the study was used also the archive, registered in the specific program of the computer from the laboratory of S.C. Diaser, Oradea, the computerized data basis of the unit.

The necessary materials for the performing of the examination:

Type of samples: spontaneous urine samples collected from subjects included in the study.

• **Collection procedure:** samples were collected according to the medical unit's standard clinical procedures, with careful attention to minimizing external contamination.

• **Labeling and documentation:** each sample was uniquely identified (patient code, date, time, clinical observations) in the study register.

• **Transport and storage:** samples were

transported and handled in a manner that preserved their integrity for microbiological analysis, in accordance with clinical sample regulations.

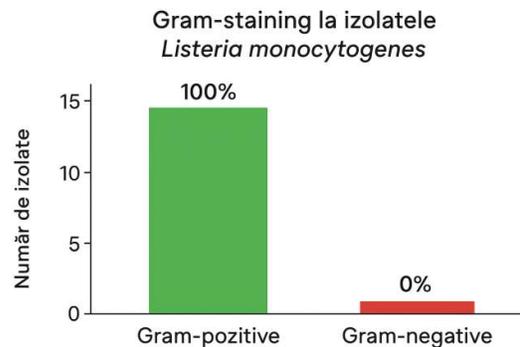
Materials and equipment (generic list)

- **Common sterile consumables:** tubes, pipettes, slides and cover slips, gloves, labels.
- **Staining reagents:** reagents for Gram staining and, where applicable, other complementary stains.
- **Selective/differential media and reagents** for screening and isolation (mentioned generically; applicable formulations and working parameters are in accordance with SOP/ISO standards).
- **Phenotypic systems/tests and commercial identification kits** used at a conceptual level.
- **Equipment:** optical microscope for morphological examination, appropriate laboratory equipment for microbial analyses (within authorized laboratories), and personal protective equipment.

RESULTS AND DISCUSSIONS

A total of 250 biological urine samples were analyzed in this study, collected aseptically from patients investigated for suspected bacterial urinary tract infection. The samples were inoculated on selective and differential media—Blood Agar, PALCAM, and Oxford—followed by evaluation of morphotinctorial and biochemical characteristics for the isolation and identification of *Listeria monocytogenes*.

Of the 250 samples examined, 15 samples (6.0%) were confirmed positive for *Listeria monocytogenes* based on morphological, staining, and biochemical criteria. The remaining 235 samples (94.0%) were negative or showed nonspecific polymicrobial growth, consisting mainly of other bacterial species, particularly Gram-negative bacilli.



Graf. 1. Distribution of *Listeria monocytogenes* cases on blood agar culture medium

On Gram-stained smears prepared from the isolated colonies, *Listeria monocytogenes* exhibited a coccobacillary or short bacillary morphology, Gram-positive, arranged singly, in pairs, or in short chains. All 15 isolates were Gram-positive (100%), confirming the typical characteristics of the genus *Listeria monocytogenes*.

In microscopic examination of wet mounts at 25 °C, most isolates displayed the characteristic tumbling motility, a slow oscillatory movement observed in 14 of the 15 isolates (93.3%). At 37 °C, motility was absent, consistent with classical descriptions for this species.

All positive isolates tested catalase-positive (100%), confirming typical enzymatic activity. On blood agar, 8 isolates (53.3%) showed clear β -hemolysis, while 7 were weakly

hemolytic or non-hemolytic, showing γ -hemolysis, a pattern compatible with the phenotypic variability of clinical *L. monocytogenes* strains.

On PALCAM agar, all isolates produced gray-green colonies with a black-green halo due to esculin hydrolysis and iron salt precipitation, 100% positive. The esculin hydrolysis test was positive in 12 out of 15 isolates (80%), confirming specific β -glucosidase activity.

In the study "*Listeria monocytogenes* isolation from urine: a series of 15 cases and review" by F. Danion et al., the authors conclude that urinary isolation is rare and occurs in three clinical patterns: primary urinary infection, a sign of systemic dissemination, or contamination/colonization.

Thus, regarding the results of the present study (15/250; 6%), the frequency is comparable with reported clinical series (Danion et al., 2017), which describe 15 cases and emphasize the need to differentiate between true urinary infection, systemic dissemination, and contamination.

In the paper titled "Pyelonephritis with bacteremia caused by *Listeria monocytogenes*: A case report" by S. Uno, R. Hase, A. Toguchi, Y. Otsuka, and N. Hosokawa, a clinical case is reported of a 90-year-old patient with obstructive pyelonephritis, who subsequently had *L. monocytogenes* detected in the urine.

CONCLUSIONS

1. *Listeria monocytogenes* was isolated in 6% of urine samples (15/250), a frequency comparable to reported clinical series.
2. All isolates were Gram-positive, catalase-positive, mostly motile at 25 °C,

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4. and 86.7% CAMP-positive; hemolysis was variable, confirming phenotypic variability.
5. Urinary isolations may reflect true urinary infection, a sign of systemic dissemination, or possible contamination; clinical data are necessary to stratify cases.
6. The combination of morphological, staining, biochemical, and culture tests on selective media allows correct identification of *L. monocytogenes*, with molecular confirmation or MALDI-TOF recommended to avoid false negatives.
7. The results highlight that urinary *Listeria*, although rare in infants, may be more frequent in adults or patients with risk factors for complicated infections, correlating with observations from the literature.
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