Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mjafi

Case Report

Listerial meningitis: Atypical strain and misidentification by the automated identification system (Vitek2)

Maj Gurpreet Singh Bhalla ^a, Col Mahadevan Kumar, (Retd) ^{b,*},
Maj Pooja Mahajan ^c, Brig Kavita Sahai, vsm ^d

^a Graded Specialist (Microbiology), Department of Lab Sciences, Army Hospital (R&R), New Delhi, India

^b Senior Consultant & Head of Department (Microbiology), Medanta The Medicity, Gurugram 122001, India

^c Resident, Department of Lab Sciences, Army Hospital (R&R), New Delhi, India

^d DDG (Pension), Office of DGAFMS, Ministry of Defence, New Delhi, India

ARTICLE INFO

Article history:

Received 12 September 2017

Accepted 11 July 2018

Available online 15 October 2018

Keywords:

Listeria spp

Misidentification

Automated identification system

sewage.³ Closely related to *L. monocytogenes*, it is considered non-pathogenic. However, atypical and hemolytic strains have been described.^{3,4} *L. innocua* is considered non-pathogenic, and its isolation needs to be reconfirmed.

We report a case of meningitis in a known case of chronic lymphocytic leukemia (CLL) in which the isolation of a non-hemolytic isolate of *L. monocytogenes* was a diagnostic dilemma.

Case report

A 50-year-old male patient, a known case of CLL for 5 years, presented with complaints of moderate-grade fever associated with non-bilious, non-projectile vomiting (approx 10–12 episodes), headache, and alteration of behavior with irrelevant speech of 1 day duration. There was no history of any focal neurological deficit or involvement of any other system. The patient was having tachycardia, whereas other vital parameters were within normal limits. On examination, pupils were bilaterally symmetrical and responsive; neck rigidity was present, but Kernig's sign was negative. Motor examination was within normal limits; however, sensory examination could not be performed because the patient was disoriented. Systemic examination was unremarkable. The patient was admitted in the intensive care unit as a case of CLL with meningitis and started empirically on intravenous ceftriaxone (2 g/12h) and vancomycin (1 g/12h).

Introduction

Listeria monocytogenes is a pathogen known to affect the immune-compromised and other high-risk groups such as pregnant women, neonates, and the elderly.¹ It is a Gram-positive, non-sporulating, intracellular, facultative-anaerobic bacillus causing β -hemolysis on blood agar and being motile at 20°C–25°C but not at 37°C. Non-hemolytic mutants of *L. monocytogenes* have also been reported.²

Listeria innocua is a non-hemolytic saprophyte with wide environmental distribution, being able to survive in extreme conditions. It has been isolated from food, water, soil, and

* Corresponding author.

E-mail address: dr.mkumar011@gmail.com (M. Kumar).

<https://doi.org/10.1016/j.mjafi.2018.07.003>

0377-1237/© 2018, Armed Forces Medical Services (AFMS). All rights reserved.

Laboratory investigations revealed hemoglobin of 15 gm/dL and total leukocyte count of 59,200/ μ L with an absolute lymphocyte count of 47,800/ μ L. Cerebrospinal fluid (CSF) cytology revealed 500 WBwhite blood cells/ μ L with 65% lymphocytes. CSF glucose was 11 mg/dl (with a blood glucose level of 168 mg/dL); proteins were 249 mg/dL, ADA of 0.932 IU/L, and LDH of 50 U/L. On Gram staining of CSF, occasional Gram-positive bacilli were seen, and the findings were conveyed to the treating physician for the addition of ampicillin to the treatment regimen because *Listeria* is inherently not susceptible to third-generation cephalosporins and vancomycin has only limited activity.¹ Computer-aided tomographic (non-contrast) scan head revealed no focal lesions; however, a few parenchymal hyperdensities were seen. The condition of the patient worsened rapidly, and despite being placed on ionotropic support and other supportive measures, he continued to deteriorate and expired within a few hours.

The CSF was inoculated on 5% sheep blood agar and grew non-hemolytic, non-pigmented, grayish-white colonies that were 0.5 mm in size (Fig. 1). The isolate was catalase positive and motile at 25°C but non-motile at 37°C. Staining showed Gram-positive bacilli in coryneform arrangement. Christie–Atkins–Munch–Petersen (CAMP) test was negative. Initial bacterial identification performed using gram-positive identification (GP ID) card on an automated identification and AST system (Vitek2, bioMérieux, France) reported *L. innocua* with confidence of excellent identification. However, the analysis message displayed by the system mentioned the possibility of the isolate being *L. monocytogenes* and to confirm the same by β -hemolysis on blood agar.

Because *L. innocua* is not a known pathogen and only two cases have been reported as per our knowledge, a repeat test was put up.^{5,6} However, the same results and same analysis message were displayed as in the first instance, but this time with a confidence of low discrimination. So, it was sent for

identification by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI–TOF MS) because it is a rapid, accurate, and reproducible method of identification of bacterial species.^{7,8} The isolate was finally identified as *L. monocytogenes* by MALDI-TOF MS with 99.9% confidence value.

Discussion

Listeriosis, a severe food-borne disease, characterized by meningitis or meningoencephalitis, septicemia, gastroenteritis, abortion, and perinatal infections, is caused by *L. monocytogenes*. The organism is present in the environment all over the world and survives extreme conditions present in food processing, including high salt concentrations and wide-range pH and temperature. In fact, cold enrichment at 4°C is a method of isolation of the organism. *L. innocua*, which is considered a non-pathogenic species, possesses similar characteristics and is often associated with *L. monocytogenes* in environment and food. On consumption of the contaminated food, *Listeria* spreads from the intestine particularly to the central nervous system and the fetoplacental unit. Virulence factors include surface protein internalin (InlA) that helps the organism to cross the intestinal barrier. Other factors such as protein InlB promote invasion; proteins LLO and PlcA promote escape from the phagocytic vacuole; and the proteins ActA and PlcB favor intracellular actin-based motility and cell-to-cell spread. Clustered on a 10-kb virulence locus, these genes are absent from *L. innocua*.⁹

There is an increased risk of infection with *L. monocytogenes* among immune-compromised patients such as those with malignancies, hepatic or renal failure, or AIDS or those receiving drugs that suppress the immune system therapy. The organism has become an emerging cause of community-acquired meningitis owing to dietary changes and long life expectancy of immuno-compromised people.¹ The incidence of listeriosis among adult population in Europe and North America has been reported to range from 5 to 10% with high case fatality rate of up to 62% despite antimicrobial therapy.¹⁰ No such data were available for India.

Clinical features and biochemical findings of *L. monocytogenes* meningitis in CSF are not different from those of other bacterial meningitis, microscopy and culture of CSF being the main tools for diagnosis. In our case, lymphocytic pleocytosis with low CSF glucose concentration was highly suggestive of bacterial meningitis.

Clinical features coupled with culture characteristics, organism morphology, and biochemical tests of the isolate are adequate for diagnosis of meningitis due to *L. monocytogenes*. However, the present isolate was atypical as seen by the lack of hemolysis and a negative CAMP test. This posed difficulty in identification of the isolate. The Vitek2 ID AST system too misidentified the isolate on repeat testing albeit with low discrimination. Non-hemolytic mutants of *L. monocytogenes* have been reported, and these non-hemolytic strains are also reported to be phospholipase negative.^{2,4,9} Phospholipase is a substrate in the GP ID card, and the same being negative could be the reason for misidentification. Lappe et al also reported this limitation of Vitek2 which misidentified 4

Fig. 1 – Gray-colored non-hemolytic colonies seen on blood agar.

of the 288 isolates (1.4%) of *L. monocytogenes* as *L. innocua* and requires improvement in the algorithm for the identification of *Listeria* spp.¹¹ The microbiologist, therefore, needs to be careful while reporting atypical isolates that can be misidentified by automated systems, as in our case. Clinical features in the patient should be correlated while reporting the organism isolated.

Confirmation of all human isolates of *Listeria* spp. by molecular methods or MALDI-TOF MS at reference laboratories is recommended.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Zhang Y, Zang GQ, Tang ZH, Yu YS. *Listeria monocytogenes* meningitis in an immunocompetent adult: a case report. *Rev Soc Bras Med Trop*. 2012;45(3):410–411.
2. Kathariou S, Pine L, George V, Carlone GM, Holloway BP. Nonhemolytic *Listeria monocytogenes* mutants that are also noninvasive for mammalian cells in culture: evidence for coordinate regulation of virulence. *Infect Immun*. 1990;58(12):3988–3995.
3. Moreno LZ, Paixao R, Gobbi DD, et al. Characterization of atypical *Listeria innocua* isolated from swine slaughterhouses and meat markets. *Res Microbiol*. 2012;163(4):268–271.
4. Johnson J, Jinneman K, Stelma G, et al. Natural atypical *Listeria innocua* strains with *Listeria monocytogenes* pathogenicity island 1 genes. *Appl Environ Microbiol*. 2004;70(7):4256–4266.
5. Perrin M, Bemer M, Delamare C. Fatal case of *Listeria innocua* bacteremia. *J Clin Microbiol*. 2003;41(11):5308–5309.
6. Favaro M, Sarmati L, Sancesario G, Fontana C. First case of *Listeria innocua* meningitis in a patient on steroids and eternecept. *JMM Case Rep*. 2014;1(2).
7. Barbuddhe SB, Maier T, Schwarz G, et al. Rapid identification and typing of *Listeria* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl Environ Microbiol*. 2008;74(17):5402–5407.
8. Murray PR. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: usefulness for taxonomy and epidemiology. *Clin Microbiol Infect: Official Publ Eur Soc Clin Microbiol Infect Dis*. 2010;16(11):1626–1630.
9. Glaser P, Frangeul L, Buchrieser C, et al. Comparative genomics of *Listeria* species. *Science*. 2001;294(5543):849–852.
10. Mylonakis E, Hohmann EL, Calderwood SB. Central nervous system infection with *Listeria monocytogenes*. 33 years' experience at a general hospital and review of 776 episodes from the literature. *Medicine*. 1998;77(5):313–336.
11. De Lappe N, Lee C, O'Connor J, Cormican M. Misidentification of *Listeria monocytogenes* by the Vitek 2 system. *J Clin Microbiol*. 2014;52(9):3494–3495.