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Short Communication

Characteristics of Meningitis Due to *Methylobacterium mesophilicum*: A Rare Case

Alexander Zinchuk[1]*, Olena Zubach[1], Andrij Zadorozhnyj[1], Yulia Chudina[2], and Valentyna Bilavka[2]

[1]*Department of Infectious Diseases. Danylo Halytsky Lviv National Medical University, Lviv; and*
[2]*Department of Diagnostics, Lviv Regional Infectious Hospital, Lviv, Ukraine*

SUMMARY: *Methylobacterium mesophilicum* — is a gram-negative bacillus most often isolated in medical establishments. In humans, *M. mesophilicum* is considered a conditionally pathogenic flora. Infections in humans generally occur in immunodeficient individuals. This article describes a rare case of *M. mesophilicum* infection that developed into acute meningitis in a 26-year-old patient. Decreased neutrophil bactericidal activity was also detected. To our knowledge, there have been no previous reports of meningitis due to *M. mesophilicum*.

There have been few recent reports of *Methylobacterium mesophilicum* infection in humans (1,2). These gram-negative bacilli are commonly referred to as conditionally pathogenic flora (3). Sewage and soil are considered the most common transmission routes to humans. It has also been isolated from the surface of plant leaves (4). *M. mesophilicum* infection has been described in significantly immunosuppressed patients (1).

We present our own clinical observation of a human infected with *M. mesophilicum* and the subsequent development of meningitis.

On October 28, 2013, a 26-year-old man presented to our hospital complaining of general weakness and slight headache. The next day, his headache had worsened, and eye pain, body ache, and chills appeared. After 3 days, his headache became intolerable; his weakness increased and repeated vomiting developed. On November 1, the patient visited Lviv Regional Infectious Hospital. On examination, his general condition was of moderate severity: he was conscious, adequate, and adynamic. His face was symmetric, and his tongue was in the midline. Weak convergence (S > D), horizontal nystagmus, moderate neck stiffness, and Kernig's signs on both sides were observed. His tendon reflexes were normal, without pathological reflexes or disorders of the peripheral nervous system.

In addition, laboratory testing revealed a hemoglobin concentration of 13.6 g/dL, white blood cell (WBC) count of $7.4 \times 10^3/\mu$L (2% eosinophils, 12% band neutrophils, 48% segmented neutrophils, 28% lymphocytes, 10% monocytes), and an erythrocyte sedimentation rate (ESR) of 2 mm per hour. His level of blood glucose was 91.9 mg/dL, urea was 58.3 mg/dL, and creatinine was 1.0 mg/dL. The cerebrospinal fluid (CSF) was colorless, slightly cloudy, with a protein concentration of 165 mg/dL, WBC count of 287/μL (lymphocytes 75%, neutrophils 25%), and glucose level of 37.8 mg/dL.

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Corresponding author: Mailing Address: Department of Infectious Diseases. Danylo Halytsky Lviv National Medical University, Pekarska str. 54. Lviv, Ukraine. Tel/Fax: +380322755406, E-mail: infectio@ukr.net

The patient was administered ceftriaxone 2 g twice daily, dexamethasone 8 mg 3 times a day, in addition to detoxification and anti-inflammatory therapy. His condition did not improve during treatment: his headache persisted and his fever remained at 37.8°C (Fig. 1). On the 10th day of treatment, his condition deteriorated: his headache worsened, nausea and repeated vomiting appeared, and the meningeal symptoms persisted. On November 11, lumbar puncture was performed. Transparent, colorless CSF was obtained, with a protein concentration of 49.5 mg/dL, WBC count of 240/μL (93% lymphocytes, 7% neutrophils), and a glucose level of 61.3 mg/dL.

Bacteriological blood analysis was negative. CSF inoculation was performed on serum, blood, and chocolate agar. Tiny pale grey colonies grew on all media. Bacterioscopy revealed gram-negative bacilli. The culture was non-fermentative and was identified as *M. mesophilicum*. The accuracy of these bacteriological investigations was confirmed in Lviv regional reference laboratory using a standardized system for automatic identification of gram-negative rods (ID 32 GN, BioMerieux, Etoile, France), which uses 32 miniaturized assimilation tests and a database. The results are shown in Table 1.

Table 1. Biochemical characteristics of strain *M. mesophilicum* in the patient with meningitis

Substrate	Result	Substrate	Result
L-Rhamnose	−	D-Mannitol	−
N-Acetyl-Glucosamine	−	D-Glucose	−
D-Ribose	−	Salicin	−
Inositol	−	D-Melibiose	−
D-Saccharose (sucrose)	−	L-Fucose	−
D-Maltose	−	D-Sorbitol	−
Itaconic acid	−	L-Arabinose	±
Suberic acid	−	Propionic acid	−
Sodium malonate	−	Capric acid	−
Sodium acetate	−	Valeric acid	−
Lactic acid	+	Trisodium citrate	−
L-Alanine	−	L-Histidine	−
Potassium 5-ketogluconate	−	Potassium 2-ketogluconate	−
Glycogen	−	3-Hydroxybutyric acid	+
3-Hydroxybenzoic acid	−	4-Hydroxybenzoic acid	−
L-Serine	−	L-Proline	−

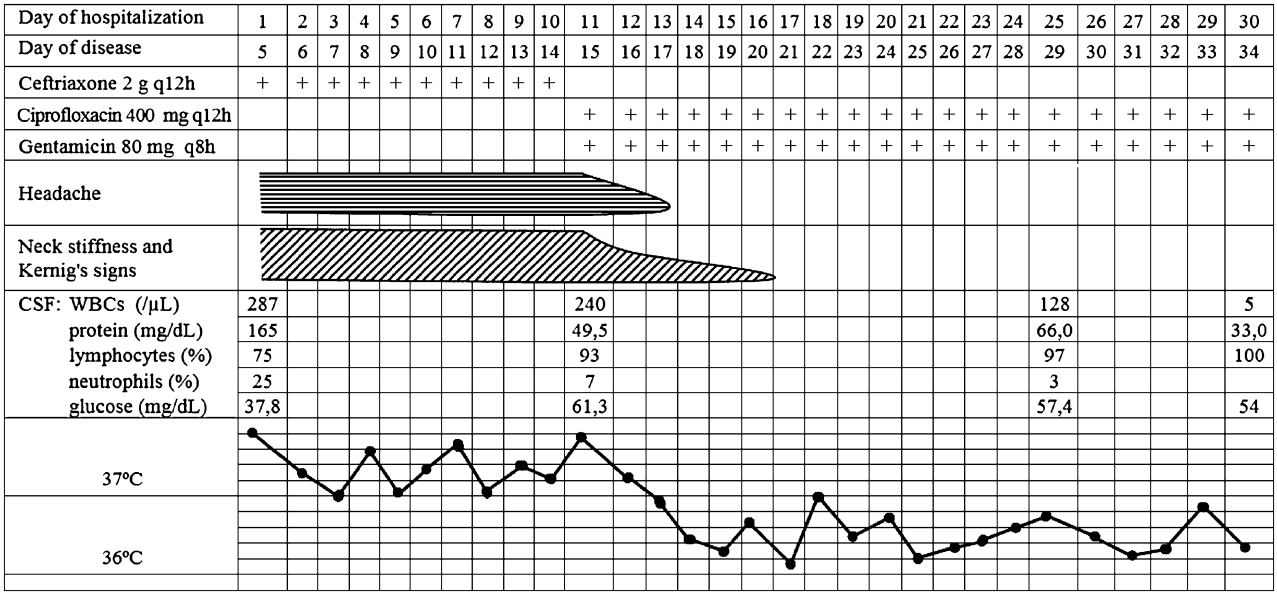

Fig. 1. Treatment, clinical and laboratory data of the patient with meningitis, caused by *M. mesophilicum*.

Table 2. Distribution of antibiotic resistance among *M. mesophilicum* in the patient with meningitis

Antibiotic	Content of antibiotic in disk (μg)	Evaluation scale according to diameter of growth inhibiting zone (mm)			Diameter of growth inhibiting zone (mm)	Result
		Resistant	Moderately sensitive	Sensitive		
Cefoperazone	75	<14	14–17	>17	34	Sensitive
Ceftazidime	30	<15	15–17	>17	17	Moderately sensitive
Cefepime	30	<15	15–17	>17	22	Sensitive
Imipenem	10	<14	14–15	>15	27	Sensitive
Meropenem	10	<14	14–15	>15	28	Sensitive
Gentamicin	10	<13	13–14	>14	29	Sensitive
Ciprofloxacin	5	<16	16–20	>20	30	Sensitive

Antimicrobial sensitivity was tested using the disk diffusion method and according to standard technique on Mueller-Hinton agar. The following antibacterial agents were tested: cefoperazone, ceftazidime, cefepime, imipenem, meropenem, gentamicin, and ciprofloxacin (Table 2).

CSF in PCR for herpes viruses types 1, 2, and 6; *M. tuberculosis*; and M and G antibodies against *Borrelia burgdorferi* were performed to exclude other etiological factors of meningitis.

A thorough immunological investigation, particularly of cell-mediated immunity, was performed to detect possible immunodeficiency conditions. There were no significant findings in the relative and absolute amount of lymphocyte subpopulations ($CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD22^+$, $CD25^+$, $CD71^+$, $CD95^+$, $CDHLA\text{-}DR^+$). The immunoregularory index ($CD4^+/CD8^+$) was 1.4. However, despite normal phagocyte activity, there was a significant disturbance in the neutrophil bactericidal activity, with an index of 2% on the spontaneous nitroblue tetrazole test (normal range 5–15%). The content of general complement and circulating immune complexes in the blood serum was within normal limits.

Based on bacteriological investigation, ciprofloxacin (400 mg) and gentamicin (80 mg) were also administrated intravenously twice and 3 times daily, respectively. The patient's condition gradually improved: his headache weakened, the rigidity of his occipital muscles disappeared, and his body temperature normalized. After treatment and CSF normalization, the patient was discharged from the hospital in satisfactory condition on December 12.

M. mesophilicum is a member of the *Methylobacterium* family. They are gram-negative bacilli with single polar flagella and they form pink colonies on media with methanol (3). They are characterized by slow growth; tap water, soil, and plant leaves are their main transmission routes to humans (4). *Methylobacterium* are most frequently isolated from sewage in medical establishments (5). *Methylobacterium* are also characterized by their resistance to chlorination (6). Organisms with characteristics similar to *M. mesophilicum* include *Pseudomonas mesophilica* (4), *Beijerinckia* spp. (7), *Chromobacterium* spp. (8,9), *Protaminobacter* spp. (9), *Mycoplana rubra* (10), and *Vibrio extorquens* (4). Bacteria in the *Methylobacterium* family may have potential use in the development of biotechnologies, particularly the manufacture of ectoine and phytohormones. (11).

In humans, *M. mesophilicum* is considered a conditionally pathogenic flora. Cases of human infection, causing different forms of disease, have been reported in immunocompromised individuals (3,12,13). These

patients had AIDS or oncological pathologies, had undergone organ transplantation, were receiving chronic hemodialysis, or had been diagnosed with alcoholism (14–17). *M. mesophilicum* infections were previously reported only in hospitalized patients and the disease was described as a nosocomial infection. At present, despite a number of cases of *M. mesophilicum* infection reported worldwide, most describe immunocompromised patients treated on an outpatient basis. The incidence and the general number of these cases have been increasing (14).

According to Sanders' data, 29 cases of *M. mesophilicum* infection in humans were reported before 2000 (14). The patients had signs of immunodeficiency caused by AIDS (3 patients), alcoholism (1 patient), malignant tumors (5 patients), T-cellular lymphoma (1 patient), multiple sclerosis (1 patient), bone marrow transplant (5 patients), hematologic pathology (3 patients), chronic renal diseases on decompensation stage (3 patients), tuberculosis (2 patients), abscess of the pancreas (1 patient), diabetes mellitus (1 patient), dumping syndrome with prolonged artificial nutrition (1 patient), and after topical application of steroids in ophthalmology (1 patient). The most common symptoms were fever, lung disorders (pneumonia, presence of infiltrates, empyema), septic processes (septic damage of the joints, catheter-associated infection), uveitis, keratitis, and chronic skin ulcers (14).

In the last 20 years, only single cases of *Methylobacterium* infection in humans have been reported. In 2001, Australian scientists described a clinical case of periodic bacteremia caused by *M. mesophilicum* in a 51-year-old patient on chronic hemodialysis. A catheter for intravenous infusions, which had been in the patient for a prolonged period, was considered the infection entry point (18).

Italian scientists also reported similar findings (19). According to 2006–2007 research data from the Department of Nephrology and Hemodialysis, several patients with acute and chronic renal insufficiency who were on hemodialysis and who had catheters for intravenous infusions developed periodic fevers. *Methylobacterium* were isolated from the blood or venous catheter cultures in 37 patients. After thorough examination, *M. radiotolerans* was confirmed as the infectious agent. *M. mesophilicum* and *M. radiotolerans* belong to the same family and have similar properties and characteristics. These patients were negative for the bacteria after removal and replacement of the catheter and antibacterial therapy modification.

Finally, researchers from Taiwan reported human infection by a member of the *Methylobacterium* family in 2011 (15). The investigation was conducted at the Taiwan National Medical University during 2000–2010. During this period, *Methylobacterium* bacteremia was observed in 6 patients. The infections were considered hospital infections. The researchers showed that the infection was associated with the presence of cubital catheters in 5 patients; the infection was therefore classified as catheter-associated.

In 1 patient, the *Methylobacterium* infection was classified as primary bacteremia. *Methylobacterium* were isolated from the blood all patients in this study. In addition, all patients were also immunocompromised (3 patients with terminal renal insufficiency, 2 with leukemia, and 1 with chronic obstructive pulmonary disease).

The patients were administered antibacterial agents alone or in combination, including meropenem, levofloxacin, combinations of vancomycin and imipenem or piperacillin-tazobactam and ciprofloxacin in combination with cefepime or gentamicin. All patients recovered.

Fluorquinolones and aminoglycosides are the treatments of choice for *M. mesophilicum* infections. Patients with *Methylobacterium* infections are generally have good prognosis with adequate antibacterial treatment (14).

In conclusion, we report a case of acute meningitis due to *M. mesophilicum*. *M. mesophilicum* rarely causes human infections, although it has been reported in immunodeficient patients. We have reported and described what we believe to be the first case of meningitis due to *M. mesophilicum*. Immunological investigation revealed a significant decrease in the bactericidal activity of neutrophils in this patient.

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Conflict of Interest None to declare.

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