CASE OF *STREPTOCOCCUS MASSILIENSIS* ISOLATION FROM BLOOD OF PATIENT WITH MENINGOENCEPHALITIS

Lyang OV[1,2] ✉, Pakhilova-Popova AV[1], Kabaeva AR[1], Boyko OV[1], Shamalov NA[1]

[1] Federal Center of Brain Research and Neurotechnologies of the Federal Medical Biological Agency, Moscow, Russia

[2] Peoples' Friendship University of Russia, Moscow, Russia

To date, *Streptococcus massiliensis*, the representative of human oral normobiota, was detected in the patients' blood only twice (which was confirmed by the reports published in 2004 and 2015). The patient with the demyelinating disease of the nervous system was routinely admitted to the neurological department for further examination and treatment. The diagnosis of meningoencephalitis was established based on the laboratory and instrumental tests. Meningoencephalitis was later complicated by bilateral multilobar pneumonia, systemic inflammatory response syndrome, and multisystem organ failure. After sharp deterioration of patient's health in the form of the decreased level of consciousness and brainstem symptoms, cerebrospinal fluid was tested by PCR for markers of viral and bacterial infections, and blood was cultured. Bacterial growth was detected in blood culture, and then *Streptococcus massiliensis*, susceptible to benzylpenicillin, vancomycin, levofloxacin, linezolid, sulfamethoxazole/trimethoprim, and erythromycin, was identified by time-of-flight mass spectrometry. Thus, it seems necessary to focus attention on this case of the *Str. massiliensis* isolation from blood due to the near total lack of data on the *Str. massiliensis* biological role and the more frequent isolation of bacteria of the genus *Streptococcus* from sterile human body fluids.

Keywords: meningoencephalitis, *Streptococcus massiliensis*, MALDI-TOF mass spectrometry

Author contribution: Lyang OV — literature review, manuscript writing; Pakhilova-Popova AV — bacteriological testing, manuscript writing; Kabaeva AR — writing clinical parts of the manuscript; Boyko OV — writing clinical parts of the manuscript; Shamalov NA — concept, manuscript editing.

✉ **Correspondence should be addressed:** Olga V. Lyang
Ostrovitianov, 1, str. 10, 117997, Moscow, Russia; lyang@fccps.ru

Received: 15.07.2022 **Accepted:** 07.08.2022 **Published online:** 08.09.2022

DOI: 10.47183/mes.2022.030

СЛУЧАЙ ВЫДЕЛЕНИЯ *STREPTOCOCCUS MASSILIENSIS* ИЗ КРОВИ У ПАЦИЕНТА С МЕНИНГОЭНЦЕФАЛИТОМ

О. В. Лянг[1,2] ✉, А. В. Пахилова-Попова[1], А. Р. Кабаева[1], О. В. Бойко[1], Н. А. Шамалов[1]

[1] Федеральный центр мозга и нейротехнологий Федерального медико-биологического агентства, Москва, Россия

[2] Российский университет дружбы народов, Москва, Россия

На сегодняшний день *Str. massiliensis*, представитель нормобиоты полости рта человека, был обнаружен в крови пациентов всего дважды (что подтверждают публикации 2004 и 2015 гг.). В неврологическое отделение для проведения дообследования и лечения в плановом порядке поступил пациент с демиелинизирующим заболеванием нервной системы. По результатам лабораторных и инструментальных исследований был выставлен диагноз «менингоэнцефалит», позднее осложненный двусторонней полисегментарной пневмонией, синдромом системной воспалительной реакции и полиорганной недостаточностью. После резкого ухудшения состояния пациента в виде угнетения сознания, нарастания стволовой симптоматики было проведено исследование ликвора на маркеры вирусных и бактериальных инфекций методом ПЦР и выполнен микробиологический посев крови. В крови обнаружен рост и затем методом времяпролетной масс-спектрометрии идентифицирован *Streptococcus massiliensis*, чувствительный к бензилпенициллину, ванкомицину, левофлоксацину, линезолиду, сульфаметоксазолу / триметоприму, эритромицину. Таким образом, ввиду практически полного отсутствия данных о биологической роли *Str. massiliensis*, а также в связи с повышением частоты выделения бактерий рода *Streprococcus* из стерильных жидкостей человека представляется необходимым заострить внимание на данном случае выделения *Str. massiliensis* из крови.

Ключевые слова: менингоэнцефалит, *Streptococcus massiliensis*, MALDI-TOF масс-спектрометрия

Вклад авторов: О. В. Лянг — анализ литературы, написание текста статьи; А. В. Пахилова-Попова — выполнение бактериологических исследований, написание текста статьи; А. Р. Кабаева — написание клинической части текста статьи; О. В. Бойко — написание клинической части текста статьи; Н. А. Шамалов — идея написания и редактирование статьи.

✉ **Для корреспонденции:** Ольга Викторовна Лянг
ул. Островитянова, д. 1, стр. 10, 117997, Москва, Россия; lyang@fccps.ru

Статья получена: 15.07.2022 **Статья принята к печати:** 07.08.2022 **Опубликована онлайн:** 08.09.2022

DOI: 10.47183/mes.2022.030

Bacteria belonging to the genus *Streptococcus* are one of the most widespread groups of microorganisms that colonize various areas of the human body. Besides the well-known and well-studied species *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus mutans*, attention has been increasingly paid to other representatives of the genus in the last decade. The emergence of modern and high-tech methods for identification of microorganisms, such as matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, had a critical impact. All these methods have greatly facilitated the task of identifying bacteria. However, further challenges, faced by both medical microbiologists and doctors of other specialties, have arisen when performing the microbiological study data interpretation and assessing the clinical significance of various bacterial pathogens. The clinical case reported is dedicated to one of such pathogens, *Streptococcus massiliensis*. Because of the small number of publications on the topic, attention should be paid to this pathogen, especially in case of isolation from the normally sterile sites.

Clinical case

Patient D., 41 years old, diagnosed with the demyelinating disease of the central nervous system, was routinely admitted to the Federal Center of Brain Research and Neurotechnologies

of FMBA on 8 July 2021 for further examination and treatment. It was known from the case history that he had an acute disorder, severe headache arose on 19 May 2021. Hyperthermia (up to 39 °C) developed within a week, however, the patient tested negative for COVID-19 (PCR), and his chest CT was normal. The patient started to notice the decline in short-term memory. He was admitted to hospital with a diagnosis of acute ischemic cerebrovascular accident on 8 June 2021 due to severe speech disorder. Ten days after the improvement the patient was discharged. However, weakness in his arms and legs increased, cerebral symptoms and disorientation developed and increased within two weeks of staying at home. After readmission the diagnosis of hemorrhagic necrotizing encephalitis was established based on the MRI scan, the patient was discharged again and subsequently routinely admitted to the neurological department of the Federal Center of Brain Research and Neurotechnologies of FMBA of Russia. Clarification of past medical history showed that the patient had no chronic infections, including those involving paranasal sinuses, oropharynx, and nasopharynx.

On admission, the patient's condition was assessed as of medium severity. The patient was in a state of clear consciousness, he had severe cognitive, speech, and bulbar impairment. The results of routine laboratory tests (complete blood count, urinalysis, biochemical profile, coagulation profile) showed no significant deviations. Assessment of cerebrospinal fluid revealed cytosis (up to 19 cells/μL), moderate increase in the levels of protein (up to 0.95 g/L) and glucose (up to 4.1 μmol/L). MRI scan was performed. MRI features were typical for encephalitis involving bilateral mediobasal temporal lobe with posthemorrhagic component and areas of postinflammatory encephalomalacia on the left. The diagnosis of meningoencephalitis was established.

Worsening of the condition occurred on 12 July 2021: meningeal signs and oculomotor dysfunction emerged. On the next day, 13 July 2021, the loss of consciousness to the level of sopor occurred, and brainstem symptoms increased. No bacterial growth was detected in the blood and cerebrospinal fluid samples collected on 13 July 2021 for bacteriological testing. The patient was transferred to the department of anesthesiology and intensive care, where the loss of consciousness to the level of coma 1 occurred, oxygen saturation decreased, and bradycardia developed two days later. The patient underwent intubation and was put on a ventilator. Laboratory tests revealed hyperglycemia (up to 9.8 μmol/L), uric acid levels up to 10.5 mmol/L, leukocytosis (up to 13.4 × 10^9/L with further increase up to 20.4 × 10^9/L), aPTT reduced to 19 s, D-dimer levels increased up to 2,925 ng/mL, and the C-reactive protein levels up to 56 mg/L. Regardless on the increasing levels of inflammatory markers, the patient's body temperature was within the range of 36.1–36.8 °C. PCR tests for herpes virus types 1, 2 and 6, *Mycobacterium tuberculosis*, Epstein–Barr virus, and cytomegalovirus, performed on 15 July 2021, were negative; no growth was also detected. Such complications of the underlying disease, as bilateral multilobar pneumonia (*Acinetobacter baumannii*, *Corynebacterium striatum*, *Klebsiella pneumoniae* were found in bronchoalveolar lavage fluid), systemic inflammatory response syndrome, and multisystem organ failure were revealed, complex antimicrobial therapy was prescribed.

Because of the patient's serious condition and persistent loss of consciousness, on 19 July 2021 blood and cerebrospinal fluid were sampled for culture for the second time in the BactAlert Aerobic and BactAlert Anaerobic bottles, 10 mL of blood and 2 mL of cerebrospinal fluid per bottle. The bottles were transferred to the bacteriology laboratory within an hour. The BactAlert 3D automated microbial detection system was used to incubate the bottles. Growth was observed in blood contained in the BactAlert Anaerobic bottle in 72 h.

Primary isolation from blood samples was performed on the ready-to-use solid culture media (Biomedia; Russia): Columbia agar with sheep blood, chocolate agar with growth factors, chromogenic agar medium for detection and enumeration of uropathogens, MacConkey agar, Sabouraud agar. Incubation was carried out under anaerobic conditions. Primary growth was observed after 48 h of incubation at a temperature of 37 °C on the Columbia blood agar and chocolate agar. Tiny gray matte colonies were found.

Microorganisms were identified with the Microflex MALDI-TOF mass spectrometer (Bruker; Germany) using the method of the direct application of pure culture onto the target [1]. *Streptococcus massiliensis* was identified by this method. *S. massiliensis* was isolated from biomaterial obtained from the patient admitted to the Federal Center of Brain Research and Neurotechnologies of FMBA of Russia for the first time in 2.5 years.

Clinical case discussion

Streptococci are a group of the spherical-shaped aerobic Gram-positive microorganisms that include numerous facultative anaerobes. Streptococci inhabit diverse environments in the human body not only as pathogens, but also as a part of normal microbiome. Based on the ability to induce hemolysis when grown on blood agar, streptococci are divided into alpha-hemolytic, beta-hemolytic and gamma-hemolytic. Alpha-hemolytic streptococci (generally referred to as the viridans streptococci) produce a greenish zone due to partial hemolysis [2]. The viridans streptococci constitute part of normal microflora of the human oral cavity, respiratory, reproductive and digestive tracts [3].

S. massiliensis, that is curerently considered to belong to the viridans group, was first isolated from blood of the patient admitted to the La Timone hospital (Marseille) with a gunshot wound to the head in June 2004. In 2006, the researchers, who had identified the new microorganism, published the paper reporting the details of the identification process [3]. The strain was named massiliensis in honor of Massilia (Ancient Greek and Roman name of Marseille, the city, where the strain was isolated). The colonies grown on blood agar were described as surface colonies, round-shaped, white to grayish, glossy, raised, with a diameter of 1–2 mm within 48 h of incubation at 37 °C in the CO_2-enriched atmosphere.

The second currently available scientific report that described *S. massiliensis* was issued in 2015. The group of researchers performed phylogenetic analysis of 17 *Streptococcus species*, including *S. massiliensis*, in order to define their habitats. It was shown that *S. massiliensis* found in human blood was a common inhabitant of human oral cavity [4].

S. massiliensis was assessed with the Vitek 2 Compact microbial detection system (bioMerieux SA; France) using the Vitek 2 AST-ST03 card in order to define antibiotic susceptibility. AST cards are used to determine minimum inhibitory concentration (MIC) of antibiotic by the turbidimetric technique, and the results are analyzed using the specialized AES software. Data interpretation was performed in accordance with the criteria of EUCAST-2021. Susceptibility to the following antibacterial drugs was revealed: benzylpenicillin, vancomycin, levofloxacin, linezolid, sulfamethoxazole/trimethoprim, and erythromycin. Ethiological role of this microorganism is

uncertain, and the risk of possible contamination is minimized by using the standard procedure, developed based on the Russian Practice Guidelines for the Preanalytical Phase of Microbiology Testing [5], when collecting venous blood for culture.

The control blood culture and cerebrospinal fluid culture tests, performed on 28 July 2021 and 2 August 2021, were negative. The patient in fair condition was transferred to the neurology department in 16 August 2021, then transferred to the department of medical rehabilitation, and discharged on 9 September 2021 due to significant improvement of neurological symptoms.

CONCLUSION

In recent years the reports of the *Streprococcus* species isolation from human blood and other sterile body fluids have become more frequent in domestic and foreign literature. This is probably due to the growing number of immunocompromised patients with primary and secondary immunodeficiencies. There are also cases of streptococci isolation from blood of patients with various neurological and cardiovascular disorders [6–8]. That is why the cases of isolating rare *Streprococcus* species require close attention of both medical microbiologists and the doctors of other specialties.

References

1. Chebotar IV, Polikarpova SV, Bocharova YuA, Mayanskij NA. Ispol'zovanie vremyaproletnoj mass-spektrometrii s matrichno-aktivirovannoj lazernoj desorbciej/ionizaciej (MALDI-TOF MS) dlya identifikacii bakterial'nyx i gribkovyx vozbuditelej III–IV grupp patogennosti. Laboratornaya sluzhba. 2018; 7 (2): 78–86. Russian.
2. Bush LM, Vazquez-Pertejo MT. MSD Manual for the Professional. Streptococcal Infections. 2021. Available from: https://www.msdmanuals.com/professional/infectious-diseases/gram-positive-cocci/streptococcal-infections.
3. Glazunova OO, Raoult D, Roux V. Streptococcus massiliensis sp. nov., isolated from a patient blood culture. Int J Syst Evol Microbiol. 2006; 56 (Pt 5): 1127–31.
4. Póntigo F, Silva C, Moraga M, Flores SV. Streptococcus massiliensis in the human mouth: a phylogenetic approach for the inference of bacterial habitats Genetics and Molecular Research. 2015; 14 (4): 19184–90.
5. Alieva EV, Kaftyreva LA, Makarova MA, Tartakovskij IS. Prakticheskie rekomendacii po preanaliticheskomu ehtapu mikrobiologicheskix issledovanij. Chast' I. Bakteriologicheskie issledovaniya. Laboratornaya sluzhba. 2020; 9 (2): 45–66. Russian.
6. Hachiya Y, Miyata R, Tanuma N, Hongou K, Tanaka K, Shimoda K, et al. Autoimmune neurological disorders associated with group-A beta-hemolytic streptococcal infection. Brain Dev. 2013; 35 (7): 670–4. DOI: 10.1016/j.braindev.2012.10.003. Epub 2012 Nov 9. PMID: 23142103.
7. Madathil S, Matsumoto S, Mathews KD, Glykys J. Central Nervous System Infections Due to Streptococcus anginosus Group: A Single-Center Case Series. J Child Neurol. 2022; 37 (3): 210–7. DOI: 10.1177/08830738211052132. Epub 2021 Dec 1. PMID: 34851209.
8. Bhatia NS, Farrell JJ, Sampath R, Ranken R, Rounds MA, Ecker DJ, et al. Identification of Streptococcus intermedius central nervous system infection by use of PCR and electrospray ionization mass spectrometry. J Clin Microbiol. 2012; 50 (12): 4160–2. DOI: 10.1128/JCM.01296-12. Epub 2012 Oct 3. PMID: 23035188; PMCID: PMC3502992.

Литература

1. Чеботарь И. В., Поликарпова С. В., Бочарова Ю. А., Маянский Н. А. Использование времяпролетной масс-спектрометрии с матрично-активированной лазерной десорбцией/ионизацией (MALDI-TOF MS) для идентификации бактериальных и грибковых возбудителей III–IV групп патогенности. Лабораторная служба. 2018; 7 (2): 78–86.
2. Bush LM, Vazquez-Pertejo MT. MSD Manual for the Professional. Streptococcal Infections. 2021. Available from: https://www.msdmanuals.com/professional/infectious-diseases/gram-positive-cocci/streptococcal-infections.
3. Glazunova OO, Raoult D, Roux V. Streptococcus massiliensis sp. nov., isolated from a patient blood culture. Int J Syst Evol Microbiol. 2006; 56 (Pt 5): 1127–31.
4. Póntigo F, Silva C, Moraga M, Flores SV. Streptococcus massiliensis in the human mouth: a phylogenetic approach for the inference of bacterial habitats Genetics and Molecular Research. 2015; 14 (4): 19184–90.
5. Алиева Е. В., Кафтырева Л. А., Макарова М. А., Тартаковский И. С. Практические рекомендации по преаналитическому этапу микробиологических исследований. Часть I. Бактериологические исследования. Лабораторная служба. 2020; 9 (2): 45–66.
6. Hachiya Y, Miyata R, Tanuma N, Hongou K, Tanaka K, Shimoda K, et al. Autoimmune neurological disorders associated with group-A beta-hemolytic streptococcal infection. Brain Dev. 2013; 35 (7): 670–4. DOI: 10.1016/j.braindev.2012.10.003. Epub 2012 Nov 9. PMID: 23142103.
7. Madathil S, Matsumoto S, Mathews KD, Glykys J. Central Nervous System Infections Due to Streptococcus anginosus Group: A Single-Center Case Series. J Child Neurol. 2022; 37 (3): 210–7. DOI: 10.1177/08830738211052132. Epub 2021 Dec 1. PMID: 34851209.
8. Bhatia NS, Farrell JJ, Sampath R, Ranken R, Rounds MA, Ecker DJ, et al. Identification of Streptococcus intermedius central nervous system infection by use of PCR and electrospray ionization mass spectrometry. J Clin Microbiol. 2012; 50 (12): 4160–2. DOI: 10.1128/JCM.01296-12. Epub 2012 Oct 3. PMID: 23035188; PMCID: PMC3502992.