Short Communication

A case of primary meningococcal conjunctivitis in a young Japanese man rapidly identified as *Neisseria meningitidis* by MALDI-TOF MS

Noriko Mukai[1, 2]*, Yukiya Takeichi[2], Yamato Yoshikawa[3], Kensuke Tajiri[2], Toyofumi Nakanishi[4], Teruyo Kida[2]

[1] Department of Ophthalmology, Hirakata City Hospital, 2−14−1 Kinyahonmachi, Hirakata, Osaka 573−1013, Japan
[2] Department of Ophthalmology, Osaka Medical and Pharmaceutical University, 2−7 Daigaku-machi, Takatsuki, Osaka 569−8686, Japan
[3] Yoshikawa Eye Clinic, 1−5 Kizuekimae, Kizugawa, Kyoto 619−0214, Japan
[4] Department of Medical Technology, Faculty of Medical Science, Shubun University, 6 Nikko-cho, Ichinomiya, Aichi 491−0938, Japan

Abstract We report a rare case of keratoconjunctivitis in a young Japanese man rapidly identified as *Neisseria meningitidis* by MALDI-TOF MS (matrix-assisted laser desorption-ionization time-of-flight mass spectrometry). The patient was diagnosed with primary meningococcal conjunctivitis. A 26-year-old man presented to Osaka Medical and Pharmaceutical University with a 3-day history of sudden pain onset in both eyes, conjunctival redness, tearing, and purulent eye discharge. The bulbar and palpebral conjunctiva were hyperemic and thickened, with superficial punctate keratitis in both eyes, and an area of stromal infiltration in the left eye. No epithelial defects or corneal thinning were observed. He had a slight fever and fatigue. Initial Gram staining of the conjunctiva revealed gram-negative diplococci, and, he had a sexual encounter two weeks ago, therefore, we suspected gonococcal conjunctivitis. The patient was administered with 1 g of intravenous ceftriaxone every 12 h for 7 days, and topical cefmenoxime hydrochloride and topical gatifloxacin hydrate were administered eight times per day. His condition improved dramatically after treatment; however, on the third day after admission, Neisseria meningitidis was detected through conjunctival swab culture and biochemical testing using the VITEK® 2 NH card. We also rapidly identified *Neisseria meningitidis* through conjunctival swab mass spectrometry using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Later, the same bacterium was detected through genetic analyses. Following advice from our hospital's infectious disease control office, our patient was isolated in a private room for 1 week, and individuals who were in close contact with him were administered ciprofloxacin tablets. We strongly suspected gonococcal infection and initiated treatment, but *Neisseria meningitidis* was rapidly identified using conjunctival swab MALDI-TOF MS analysis. Therefore, appropriate treatment and infection prevention measures were possible.

Key words: *Neisseria meningitidis*, primary meningococcal conjunctivitis, matrix-assisted laser desorption-ionization time-of-flight mass spectrometry, gene sequence analysis

Introduction

Neisseria meningitidis is a gram-negative diplococcus that colonizes the nasopharyngeal mucosa and is transmitted via droplet infections from carriers or patients. In addition, it causes serious diseases, such as upper respiratory inflammation, pneumonia, urethritis, arthritis, pericarditis, bacteremia, and sepsis. Generally, meningococcal conjunctivitis can be classified as a primary or secondary systemic disease. Primary meningococcal conjunctivitis (PMC) is a rare cause of pediatric acute purulent bacterial conjunctivitis that is more frequently prevalent in children than in adults[1, 2]. Here, we report a rare case of PMC in a young Japanese adult who presented with clinical symptoms

***Corresponding author**
Noriko Mukai
Department of Ophthalmology, Hirakata City Hospital, 2−14−1 Kinyahonmachi, Hirakata, Osaka 573−1013, Japan
Tel: +81−72−847−2821, Fax: +81−72−847−2825
E-mail: mukanori@kcn.jp
Received: April 30, 2024. Accepted: December 24, 2024.
Epub March 10, 2025.
DOI: 10.24508/mms.2025.06.001

resembling gonococcal keratoconjunctivitis. We could rapidly identify PMC pathogenetic microorganism on conjunctival swabs from this patient by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Significant advances in microbiological methods have been through achieved innovation in mass spectrometry[3]. MALDI-TOF MS has become the standard for microbial identification, enabling microbiology laboratories to reliably, rapidly, and cost-effectively identify starting from colony material[4]. This is also the case in our hospital. In this case as well, we were able to make a definitive diagnosis and administer appropriate treatment for this patient by MALDI-TOF MS analysis and genetic analyses.

Case report

A 26-year-old man was referred to a specialized outpatient ophthalmology clinic at our hospital because of persistent conjunctival hyperemia with purulent eye discharge under the suspicion of gonococcal conjunctivitis following sexual intercourse with an unidentified woman 2 weeks prior. He had been treated with antibiotic eye drops for conjunctivitis at another eye clinic; however, his condition deteriorated. During the initial visit to our department, the best-corrected visual acuity and intraocular pressure were not measurable because he was unable to keep his eyes open due to severe pain in both eyes. Both eyes were injected with marked discharge from both eyelids (Fig. 1a, b). The bulbar and palpebral conjunctiva showed hyperemia and thickening (Fig. 1c, d), accompanied by superficial punctate keratitis in both eyes and an area of stromal infiltrate in the left eye. The posterior segments of both the eyes were unremarkable. In terms of systemic symptoms, he had a slight fever and fatigue without headache or a stiff neck. Blood tests revealed only mildly elevated C-reactive protein levels. He also had a history of bronchial asthma. Subsequently, a conjunctival swab smear test was quickly performed, and gram-negative diplococci were detected (Fig. 2). Based on his clinical findings and sexual history, gonococcal conjunctivitis was strongly suspected. Therefore, 1 g of intravenous ceftriaxone was started every 12 h for 7 days, and topical cefmenoxime hydrochloride and gatifloxacin hydrate were administered eight times per day at our hospital. Although the ocular findings improved, meningococcus was identified by culture and biochemical testing using the VITEK® 2 NH card three days after admission. Since adult case of PMC are rare in Japan and the results were unexpected, we additionally performed conjunctival swab MALDI-TOF MS. *Neisseria meningitidis* was rapidly identified. Furthermore, we conducted gene sequence analysis using conjunctival swabs to confirm the presence identify the same bacterium.

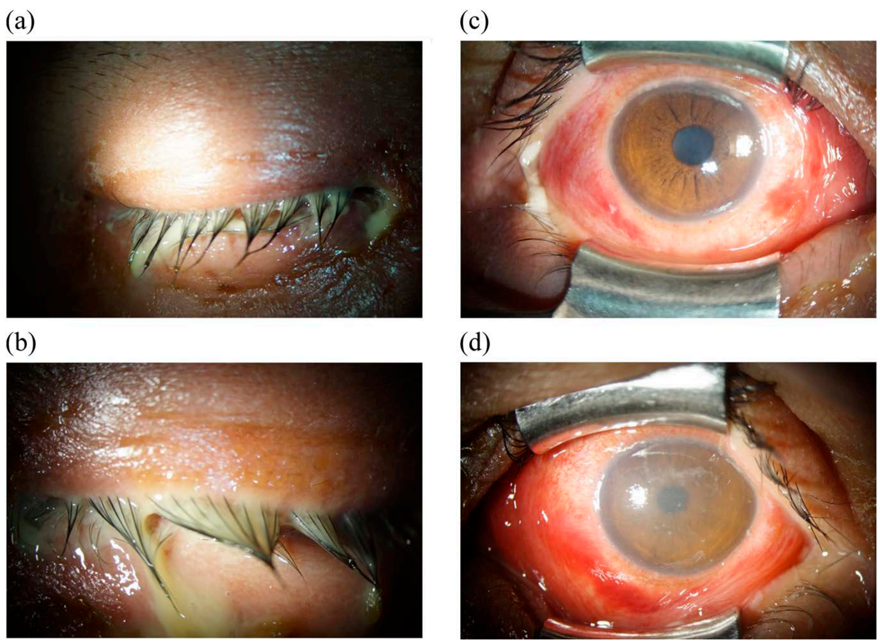


Fig. 1. Slit lamp photograph of the patient.
Slit lamp photograph shows a marked purulent discharge in the eyelids of right eye (a) and left eye (b). Slit lamp photograph shows the bulbar and palpebral conjunctiva presenting with hyperemia and thickening in right eye (c) and left eye (d).

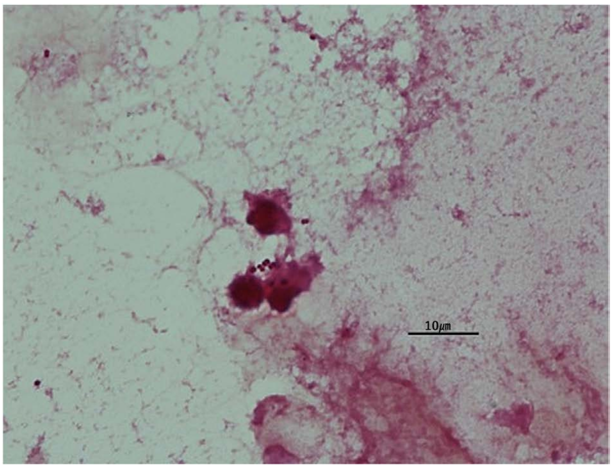


Fig. 2. Gram stain of the conjunctival swab characteristic of gram-negative diplococci polymorphs.

Materials and methods

Proteome analysis using MALDI-TOF MS was attempted to rapidly identify pathogenic microorganisms on conjunctival swabs from this patient. We applied matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a Linear mode, and identification was performed with the Biotyper software 3.0 program (Bruker Daltonics, Billerica, USA). The colonies were scraped off, directly placed on a MALDI plate, and then drying, 1 μL of pellets on the α-cyano-4-hydroxycinnamic acid (HCCA)-Matrix, we measured the MS. We checked the *m/z* calibration with the MBT calibrator (Bruker Daltonics KK., Tokyo) before MS analysis. The MS was carried out in the Autoflex-Speed using the Linear mode, and the results were analyzed using the Biotyper™ software 3.0 program (Bruker Daltonics, Billerica, USA).

All reagents except the HCCA were purchased from Nakalei tasque (Tokyo).

16S rRNA PCR amplification was performed for species identification according to the instruction manual from Nippon Gene Co., Ltd. PCR products were separated by electrophoresis on 1.5% (w/v) TAE agarose gel. DNA was purified using NucleoSpin® Gel and PCR Cleanup (TaKaRa Bio Inc., Japan), and its concentration and quality were assessed with a NanoDrop® system before DNA sequencing.

Results and discussion

Herein, we report a rare case of PMC in a young Japanese adult who presented with clinical symptoms resembling gonococcal keratoconjunctivitis. As shown in Fig. 1 (a), (b), these slit lamp photographs reveal a marked purulent discharge in the eyelids of right eye (a) and left eye (b). Additionally, slit lamp photographs show the bulbar and palpebral conjunctiva presenting with hyperemia and thickening in right eye (Fig. 1(c)) and left eye (Fig. 1(d)). Gram staining of the conjunctival swab identified the gram-negative diplococci, coffee bean appearance, as shown in Fig. 2. Furthermore, biochemical testing using the VITEK®2 NH card was performed, and the utilization capacity of glucose and maltose was confirmed to be positive, while that of sucrose and other sugars was confirmed to be negative.

Three colonies grew on blood agar, characterized by a grayish-white color, round shape, translucency, and glossy appearance. The colonies were centrifuged at 5,000 Xg at 4°C for five minutes, and the precipitate was placed on the pallet of the MALDI-TOF MS machine. As shown in Fig. 3a, the MALDI TOF raw mass spectra data was achieved using the autoflex speed™. We showed the obtained MALDI mass spectrum pattern (upper layer) and the standard pattern matching (lower layer) in Fig. 3b and c. The obtained the mass spectra pattern was compared and identified to the standard pattern matching with the Biotyper™ tool. As shown in Fig. 3, c, the score for *Neisseria meningitidis* is 2.165 by Biotyper™ database search (b), and that of *Neisseria gonorrhoeae* is 1.767 (c). Therfore, the pathogenic microorganism of this case was identified as *Neisseria meningitidis*, not *Neisseria gonorrhoeae.*

As shown in Fig. 4a, the PCR product was achieved approximately of 1600 bp in agarose gel electrophoresis. The 16S rRNA sequence for this PCR product compared with NCBI, and was showed the extremely high homology with a standard strain of Neisseria meningitidis, in Fig. 4b and 4c. The result of the direct DNA sequencing reconfirmed to support the identification with MALDI-TOF MS.

Our patient was 26 years old and had few systemic symptoms, with conjunctivitis and eye discharge as the main symptoms. PMC is extremely rare in adults. Barquet et al.[1] reviewed 84 cases of PMC globally from 1899 to 1988, and only 20 patients were adults (aged >18 years). Our literature search using PubMed yielded only a few additional case reports of adult PMC since 1990[5–11]. In Japan, there have been no reports of PMC in adults. In addition, our patient showed not only conjunctivitis but also stromal keratitis. We found several reported cases of keratitis, such as mild keratitis and perilimbal infiltrates, punctate epithelial

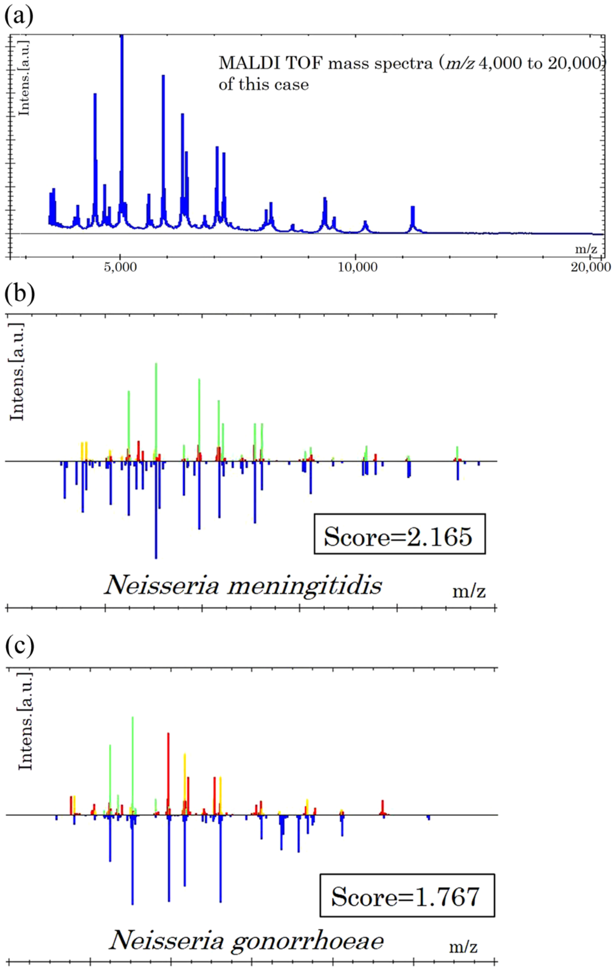
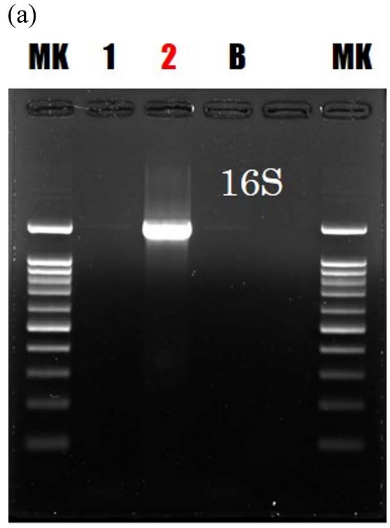


Fig. 3. MALDI-TOF mass spectra of this case and the score.
We analyze the standard pattern matching (a) and obtained MALDI mass spectrum pattern is compared to a database for identification.
The score for *Neisseria meningitidis* is 2.165 by Biotyper^{TM} database search (b).
The score for Neisseria gonorrhoeae is 1.767 (c).



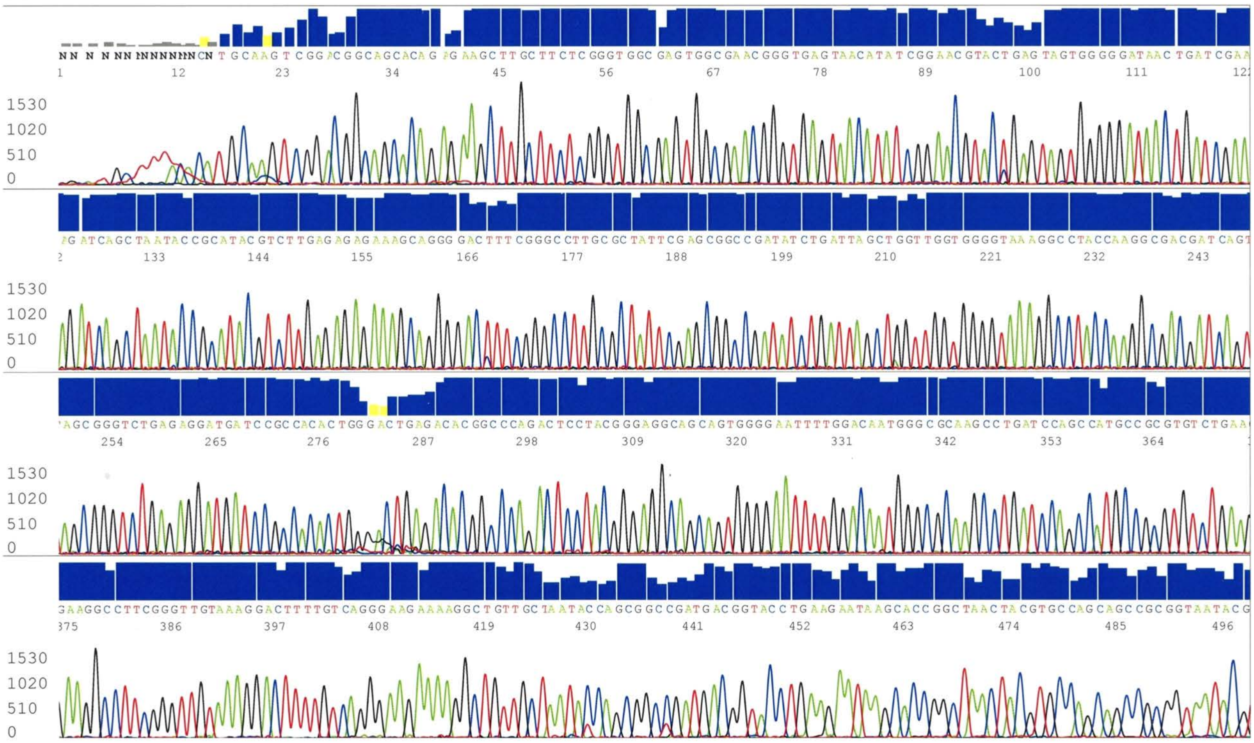
MK：marker, 1: other sample, 2: This case, B: dd-H2O

Fig. 4. The results of the image of the agarose electrophoresis and direct sequence of the PCR product of this case.

erosion of the cornea, subepithelial corneal infiltrates, peripheral stromal and limbal infiltrates, and corneal ulcers caused by *Neisseria meningitidis*[5, 7–11]. Unlike gonococcal conjunctival infections, no cases of corneal perforation have been observed in patients with PMC. PMC presents as an acute, purulent conjunctivitis and can mimic gonococcal conjunctivitis; however, differentiating between *Neisseria gonorrhoeae* and *Neisseria meningitidis* is important. PMC has the risk of corneal ulceration in 15.5% of cases, and after the onset of conjunctivitis, fatal systemic meningococcal infection (sepsis and/or meningitis) occurs in 10–29.9%, resulting in a high mortality rate of 13%[1, 12]. Moreover, Barquet et al.[1] reported that patients treated with only topical antibiotics develop systemic disease more frequently (approximately 32%) compared to those treated with both topical and systemic therapies (approximately 2%). Therefore, systemic therapy with adjunctive topical treatment is recommended to prevent invasive meningococcal infection in cases of hyperacute conjunctivitis caused by gram-negative diplococci[13–15].

In this case, gram-negative diplococci were confirmed, and gonococcal conjunctivitis was suspected; thus, systemic administration was promptly initiated. Fortunately, our patient did not develop a systemic meningococcal infection, and his ocular symptoms resolved. For the differentiation of between *Neisseria gonorrhoeae* and *Neisseria meningitidis*, both microbial and biochemical tests must be performed[16, 17]. The identification of *Neisseria* species. has always been considered a time-consuming task in routine laboratory work, and some publications have proposed tools to improve, accelerate, and simplify this process. MALDI-TOF MS is a new method for performing microbiology laboratory work that is capable of confirming the identity of several microorganisms known to be difficult to identify using conventional tests. Their method involves identifying a microorganism by searching a library for the ribosomal 16S protein. In our case, a gonococcal infection was suspected based on the clinical course, the patient's infection route episodes. However, using the MALDI-TOF MS method based on culture results, we were able to rapidly identify *Neisseria meningitidis* as the causative pathogen. As reported in reference[18], the MALDI-TOF MS

(b)



(c)

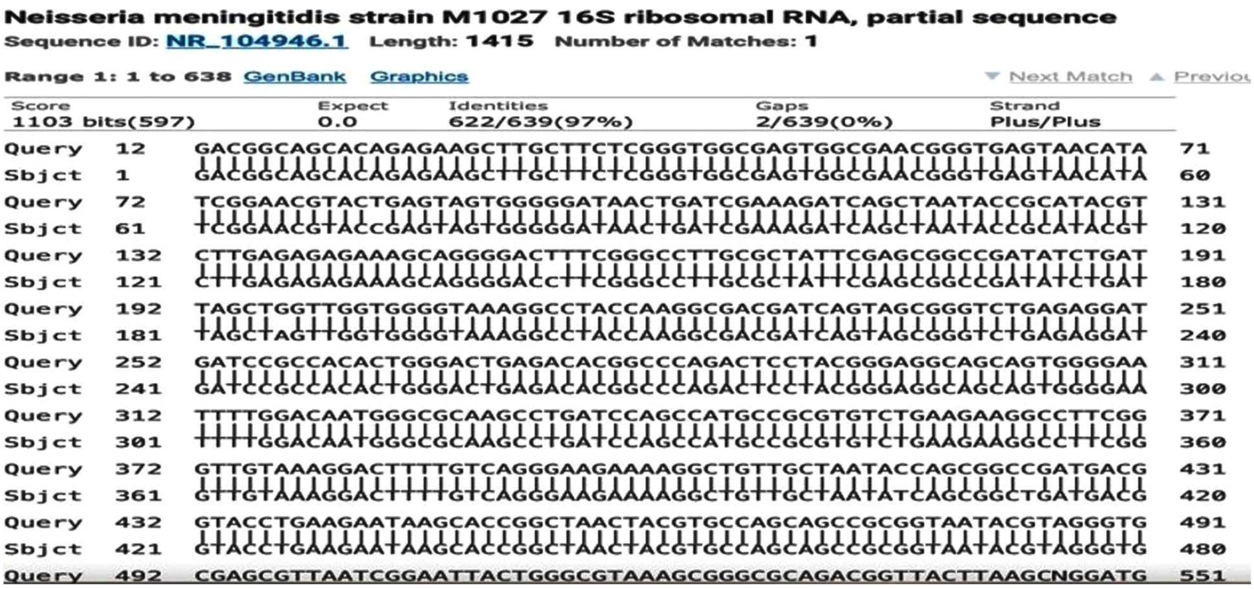


Fig. 4. Continued.

The agarose gel electrophoresis patterns of the PCR products of this case (a).
The result of the direct sequence of the r16S PCR product in this case (b).
The identification of Neisseria meningitidis by Database search (GeneBankTM) (c).

method is a powerful tool for more rapidly identifying pathogenic microorganisms compared to the 16S rRNA sequencing method. From the perspective of a mass spectrometry expert, microbial identification using MS is essentially a pattern recognition of the r16S protein, and does not involve indentifying or comparing individual proteins, Therefore, it is believed that the method may be usable even with crude samples. We believe that this allowed for the appropriate isolation of patients and prevention of infection. Fatalities after acquiring PMC from close contacts have been reported[1, 9, 12]. Therefore, individuals who were in close contact with patients should be administed with prophylactic antibiotics (such as ciprofloxacin), and intervention by infection control experts is required to prevent disease outbreaks. The risk of infection during a close slit-lamp examination is unknown; however, in this case, upon the advice of the hospital's infection control department, the patient was isolated in a private room for one week, and close contacts were administered ciprofloxacin tablets (500 mg).

In summary, we report a rare case of PMC in a young adult Japanese patient. Prompt and precise diagnosis from clinical eye findings and bacterial identification tests by ophthalmologists is important to initiate appropriate systemic antimicrobial therapy to prevent systemic disease and disease outbreaks. The MALDI-TOF MS analysis was a very useful for the rapid identification of *Neisseria meningitidis*.

Statement of ethics

This study complied with the guidelines for human studies and was conducted ethically, following the World Medical Association Declaration of Helsinki. Written informed consent was obtained from the patient for publication of this case report and any accompanying images. The Ethics Committee of our institute, Osaka Medical and Pharmaceutical University does not require an ethical approval for this study in accordance with local guidelines. The authors have no ethical conflicts to disclose.

Conflict of interest

The authors have no conflicts of interest to declare.

Funding sources

This study was not supported by any sponsor or funder.

Acknowledgements

I would like to express my appreciation for the test results and advice provided by the Microbiology Laboratory of the Central Testing Department at Osaka Medical and Pharmaceutical University Hospital.

References

1) Barquet N, Gasser I, Domingo P, Moraga FA, Macaya A, et al: Primary meningococcal conjunctivitis: report of 21 patients and review. *Rev Infect Dis* 12: 838–847, 1990.
2) Anderson J, Lind I: Characterization of Neisseria meningitidis isolates and clinical features of meningococcal conjunctivitis in ten patients. *Eur J Clin Microbiol Infect Dis* 13: 388–393, 1994.
3) Wieser A, Schneider L, Jung J, Schubert S: MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). *Appl Microbiol Biotechnol* 93: 965–974, 2012.
4) Seng P, Rolain JM, Fournier PE, LaScola B, Drancourt M, et al: MALDI-TOF-mass spectrometry applications in clinical microbiology. *Future Microbiol* 5: 1733–1754, 2010.
5) Andreoli CM, Wiley HE, Durand ML, Watkins LM: Primary meningococcal conjunctivitis in an adult. *Cornea* 23: 738–739, 2004.
6) Irani F, Ruddell T: Meningococcal conjunctivitis. *Aust N Z J Ophthalmol* 25: 167–168, 1997.
7) Stansfield RE, Masterton RG, Dale BA, Fallon RJ: Primary meningococcal conjunctivitis and the need for prophylaxis in close contacts. *J Infect* 29: 211–214, 1994.
8) Garcia-Lechuz JM, Alcala L, Gijon P, San Juan R, Bouza E: Primary meningococcal conjunctivitis in a human immunodeficiency virus-infected adult. *Clin Infect Dis* 27: 1556–1557, 1998.
9) Dryden AW, Rana M, Pandey P: Primary meningococcal conjunctivitis: an unusual case of transmission by saliva. *Digit J Ophthalmol* 22: 25–27, 2016.
10) Aranzasti C, Aguirreburualde R, Martinez R, Durán de la Colina JA, Gaztelurrutia L: Primary meningococcal keratoconjunctivitis in an extended contact lenses wearer. *Br J Ophthalmol* 78: 731, 1994.
11) Elias JA, Pandit RT: Primary Neisseria meningitidis Keratitis. *Cornea* 28: 714–715, 2009.
12) Parikh SR, Campbell H, Mandal S, Ramsay ME, Ladhani SN: Primary meningococcal conjunctivitis: Summary of evidence for the clinical and public health management of cases and close contacts. *J Infect* 79: 490–494, 2019.
13) Orden B, Martinez R, Millon R, Belloso M, Perez N: Primary meningococcal conjunctivitis. *Clin Microbiol Infect* 9: 1245–1247, 2003.
14) Andreoli CM, Wiley HE, Durand ML, Watkins LM: Primary meningococcal conjunctivitis in an adult. *Cornea* 23: 738–739, 2004.
15) Kanda P, Ioannidis S, Sim W, Weston B, Koaik M: Primary meningococcal conjunctivitis in an adult patient. *IDCases* 32: e01749, 2023.
16) Buchanan R, Ball D, Dolphin H, Dave J: Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry for the identification of Neisseria gonorrhoeae. *Clin*

Microbiol Infect 22: 815–815, 2016.

17) Ilina EN, Borovskaya AD, Malakhova MM, Vereshchagin VA, Kubanova AA, et al: Direct bacterial profiling by matrix-assisted laser desorption ionization time-of-flight mass spectrometry for identification of pathogenic neisseria. *J Mol Diagn* 11: 75–86, 2009.

18) Mellmann A, Cloud J, Maier T, Keckevoet U, Ramminger I, et al: Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria. *J Clin Microbiol* 46: 1946–1954, 2008.