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Misidentification of *Neisseria cinerea* as *Neisseria meningitidis* by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)

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Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) is used as a rapid and accurate microbial diagnostic method for species identification of pathogens. At our hospital, we use MALDI-TOF MS for rapid identification of microorganisms from pathogen-positive blood cultures. In this article, we report a case where *Neisseria meningitidis* was confirmed via blood culture using MALDI-TOF MS, but the microbe was ultimately confirmed to be *Neisseria cinerea*.

The 30-year-old patient received a diagnosis of Burkitt lymphoma, and chemotherapy (R-HyperCVAD/MA; rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, and cytarabine) was started on day 4 of hospitalization (Day 4). On Day 15, his body temperature rose to 39.2°C without symptoms of a common cold. Two sets of blood cultures were initiated on Day 15, and a Gram-negative coccus was detected in an aerobic bottle on Day 16. The acquisition and analysis of mass spectra were performed on a Microflex LT mass spectrometer (Bruker Daltonics, Bremen, Germany) and using the MALDI Biotyper software package (ver. 3.1) with the reference database ver. 3.1.2.0 (3,995 database entries) (Bruker Daltonics).

The cultivated bacterium was identified as *N. meningitidis* with Score Value 2.024 (best match) and Score Value 1.966 (second best match). The Score Value 2.024 of this pathogen was lower than that of a known strain, *N. meningitidis* (Score Value 2.437). Their MALDI-TOF MS spectra showed different wave patterns and peaks between the pathogen isolated from the patient (Fig. 1) and stocked *N. meningitidis* (Fig. 2).

Colony morphology and microscopic morphology were also different between these microbes. The blood culture sediment of the patient showed nonspecific agglutination in a latex agglutination assay (Pastorex Meningitis; Bio-Rad, Hercules, CA, USA). The strain from

patient's blood culture and the *N. meningitidis* strain were incubated on a chocolate agar plate (CAP) for 24 h with CO₂. The strain (from the patient's blood culture) growing on the CAP appeared more mucoid and smaller (~1 mm) than the *N. meningitidis* strain, which appeared as large, round, and smooth colonies. Two strains from CAP culture, one from the patient's blood culture and the other from *N. meningitidis* culture were subjected to carbohydrate utilization testing by means of a rapid diagnostic test kit (ID test HN-20 RAPID Nissui; Tokyo, Japan). Test results on the strain from the patient's blood culture were positive for glucose and maltose utilization, on the other hand, the *N. meningitidis* strain tested negative for glucose

Fig. 1. MALDI-TOF MS spectrum of the strain isolated from the patient's blood culture.

Fig. 2. MALDI-TOF MS spectrum of stocked *Neisseria meningitidis*.

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and maltose utilization. Judging by the results of the rapid diagnostic test, the strain from the patient's blood culture was identified as *N. cinerea*/*Neisseria flavescens* (60% probability), *Neisseria gonorrhoeae* (30%), or *Neisseria* 3 (7%). We conducted 16S rRNA gene sequencing analysis, and it revealed that the pathogen from our patients was actually *N. cinerea*.

In the present case, *N. cinerea*, a species of non-pathogenic *Neisseria*, from the blood culture of the Burkitt lymphoma patient was misidentified as *N. meningitidis* by the MALDI-TOF MS method. Although the MALDI-TOF MS Score Value was above 2.0 for the pathogen, we thought that the strain might not be *N. meningitidis* judging by the colony morphology and microscopic morphological examination. We conducted 16S rRNA sequence analysis for this pathogen, and this procedure revealed that the pathogen was *N. cinerea*.

We also doubted MALDI-TOF MS identification as *N. meningitidis* because of the antibiotic-sensitivity test results on the pathogen. The minimum inhibitory concentrations (MICs) of antimicrobial agents for the pathogen in the Etest, 1 µg/mL for benzylpenicillin, 1 µg/mL for ceftriaxone, 0.064 µg/mL for meropenem, and 0.5 µg/mL for ciprofloxacin, were higher than those of *N. meningitidis* reported in Japan (1) and those in Clinical and Laboratory Standards Institute guidelines (2,3).

As for the reason for misidentification, it was suspected that the number of non-pathogenic *Neisseria* spp. in the MALDI Biotyper library may not be sufficient. Only one strain of *N. cinerea* was registered in the MALDI Biotyper 3.1 software library, therefore, it could be misidentified because the differences from the *N. meningitidis* strain in the pattern could not be detected. The number of reference spectra for other non-pathogenic *Neisseria* spp. in the MALDI Biotyper

3.1 software library is also small: 11 strains for *N. meningitidis* and 7 strains for *N. gonorrhoeae*. The other species that can be misidentified as *N. meningitidis* by MALDI-TOF MS method is *Neisseria polysaccharea*. As Cunningham et al. reported, *N. polysaccharea* may be misidentified as *N. meningitidis* at the Score Value above 2.0 (4). For *N. polysaccharea*, like *N. cinerea*, there is only one entry in the MALDI Biotyper 3.1 software library.

Score Values ≥ 2.0 , < 2.0 but ≥ 1.7 , and < 1.7 are recommended for species, genus, and unreliable identification, respectively, in the Biotyper database. In clinical practice, these thresholds are generally adopted (5). Nonetheless, there have been reports that some isolates are identified correctly by means of low Score Values, whereas some others are misidentified via a high Score Value (5–7). Lower species cutoffs that may offer an optimal balance between an increased number of correct identifications and a limited number of misidentifications have been suggested in some reports (8–10). In our case, however, *N. cinerea* was misidentified as *N. meningitidis* with the Score Value above 2.0 (i.e., 2.024).

As Szabados et al. mentioned in their report (11), the MALDI-TOF MS-based species identification should be compared to previously obtained preliminary identification results, such as typical colony morphology, selective agar plate data, Gram staining, and catalase or oxidase activity, especially if a rare pathogen was identified. The differential characteristics of *N. meningitidis*, *N. cinerea*, and *N. polysaccharea* are presented in Table 1 (12–14).

In summary, we uncovered misidentification of *N. cinerea* as *N. meningitidis* by the MALDI-TOF MS method.

Table 1. Differential characteristics of *Neisseria meningitidis*, *Neisseria cinerea*, and *Neisseria polysaccharea*

	<i>Neisseria meningitidis</i>	<i>Neisseria cinerea</i>	<i>Neisseria polysaccharea</i>
Gram stain	Gram-negative	Gram-negative	Gram-negative
Cell morphology	diplococcus	diplococcus	diplococcus
Colony morphology	smooth, round, moist, uniform large grey/brown with a glistening surface and entire edges	small (1.0–1.5 mm in diameter), greyish white with entire edges and slightly granular	yellowish and 2 mm in diameter
Pigmentation	–	(yellowish pigment)	–
Oxidase test	+	+	+
Acid production	acid from glucose and maltose	weak acid from glucose in some acid detection tests	acid from glucose and maltose
Enzyme substrate test	GGT-positive	HAP-positive	PAP-positive
Nitrate reduction test	–	–	–
Polysaccharide from sucrose	–	–	+
Production of deoxyribonuclease	–	–	–
Superoxol test (reaction with 30% hydrogen peroxide)	weak (1+) to strong (4+)	weak (2+)	weak (1+) to strong (3+)
Catalase test (reaction with 3% hydrogen peroxide)	+	+	+
Colistin resistance	R	(R)	(R)

+, most strains positive; –, most strains negative; GGT, gamma-glutamyltransferase; HAP, hydroxyprolyl aminopeptidase; PAP, prolyl aminopeptidase; R, strains grow well on selective medium for *Neisseria gonorrhoeae* and/or show no inhibition around a colistin disk (10 µg); (R), most strains susceptible, some strains resistant.

Conflict of interest None to declare.

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