

Misidentification of *Neisseria polysaccharea* as *Neisseria meningitidis* with the Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Scott A. Cunningham,^a Jill M. Mainella,^a Robin Patel^{a,b}

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,^a and Division of Infectious Diseases, Department of Medicine,^b Mayo Clinic, Rochester, Minnesota, USA

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) allows rapid and accurate identification of most bacterial isolates (1). This methodology may, however, impact safety when bacteria or fungi that can cause laboratory-acquired infection are unsafely manipulated (2). Laboratory workers who work with *Neisseria meningitidis* outside a biological safety cabinet are at risk for meningococcal disease; therefore, *N. meningitidis* must be safely, correctly, and rapidly identified. Moreover, prompt and accurate identification of *N. meningitidis* informs patient care and management of potentially exposed health care workers and other close contacts of infected patients (3, 4).

Traditional identification of *N. meningitidis* relied on the isolation of Gram-negative diplococci with positive catalase and oxidase tests, which produce acid from glucose and maltose in cysteine-rich media. In 1983, Riou et al. reported the isolation of a related organism, *Neisseria polysaccharea*, from the throats of healthy children during a meningococcal carriage study (5). Distinguishing this species from *N. meningitidis* is challenging since it also produces acid from glucose and maltose. It can be differentiated based on its acid production from sucrose and production of copious amounts of starch-like polysaccharide from sucrose, as well as by its lack of γ -glutamyltransferase activity. In 1986, investigators from the Spanish Meningococcal Reference Laboratory reported that 50 of 216 apparent nonencapsulated isolates of *N. meningitidis* were actually *N. polysaccharea* based on the characteristics described by Riou et al. and Boquete et al. (5–7). Their reports led to the practice of examining for polysaccharide production in the presence of 1% sucrose using a starch-free medium with iodine as the indicator, as well as inclusion of a test for γ -glutamyltransferase in subsequent *Neisseria* identification kits (8).

We encounter multiple isolates of *N. meningitidis* each year and have historically utilized the API Rapid NH kit and 16S rRNA gene sequencing for *Neisseria* identification. Since 2011, we have used MALDI-TOF MS and the Bruker MALDI Biotyper (Bruker, Billerica, MA) for bacterial identification. However, we have not been able to reliably identify all *Neisseria* species to the species level using the RUO MALDI Biotyper Reference Library (version 4.0.0.1; 4,627 main spectra [MSP] entries) with our user-supplemented library of 1,420 MSP entries, which includes 18 supplemented *Neisseria* entries, 4 of which are from *N. polysaccharea*.

Of particular concern are identification errors for all five isolates of *N. polysaccharea* that we have assayed with the Biotyper system, including *N. polysaccharea* ATCC 43768. Without library supplementation, all of the top scores (2.068 to 2.241) indicated an identification of *N. meningitidis*, and all of the top 10 matches were to *N. meningitidis*. Even with the library

supplementation described above, *N. meningitidis* remained the top match for all five *N. polysaccharea* isolates, with scores of 2.038 to 2.241, and all of the top 10 matches indicated an identification of *N. meningitidis* for three isolates, with *N. polysaccharea* and *N. meningitidis* identifications intermingled among the top 10 matches for the other two isolates. This is corroborated by data presented in abstract form by Vironneau et al., noting misidentification of two *N. polysaccharea* isolates as *N. meningitidis* (9).

Given this observation, we recommend that laboratories using the RUO Biotyper MALDI-TOF MS system consider verifying identities of isolates reported as *N. meningitidis* by an additional method. Testing may include assessment of γ -glutamyltransferase activity, production of starch-like polysaccharide from sucrose, and/or detection of acid production from sucrose. Growth of *N. polysaccharea* may be inhibited in the presence of 5% sucrose, so media assessing acid production from sucrose should contain no more than 1% sucrose (8). Alternatively, molecular methods such as 16S rRNA gene sequencing and amplification of *N. meningitidis*-specific genes (e.g., *sodC*, *porA*) may be considered (10, 11).

The current RUO Bruker MALDI-TOF MS library includes 45 entries covering 16 species of *Neisseria*, of which 40% represent *N. meningitidis* or *N. gonorrhoeae*; there is a single entry for *N. polysaccharea*. Our results suggest that *N. polysaccharea* may be misidentified as *N. meningitidis*, resulting in a cascade of unnecessary actions, including unnecessary public health reporting, management of patient care, and management of exposure for laboratory workers, as well as health care workers and other close contacts of the patient.

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Address correspondence to Robin Patel, patel.robin@mayo.edu.

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