Misidentification of *Neisseria polysaccharea* as *Neisseria meningitidis* using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

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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 of 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 healthcare 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 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 acid production from sucrose, production of copious amounts of starch-like polysaccharide from sucrose, as well as lack of γ-glutamyl transferase activity. In 1986, investigators from the Spanish Meningococcal Reference Laboratory reported that 50 of 216 apparent non-encapsulated isolates of *N. meningitidis* were actually *N. polysaccharea* based on the characteristics described by Riou et al. (5-7). Their report lead to the practice of examining for polysaccharide production in the presence of 1% sucrose using a starch free media with iodine as the indicator as well as inclusion of a test for γ-glutamyl transferase 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 ribosomal RNA gene sequencing for *Neisseria* identification.
Since 2011, we have used MALDI-TOF MS using 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 3.3.1.2, 5627 entries) with our user-supplemented library of 1420 main spectra (MSP) entries which includes 18 supplemented Neisseria entries with four from N. polysaccharea. Of particular concern are identification errors for all five isolates of N. polysaccharea we have assayed with the Biotyper system, including N. polysaccharea ATCC 43768. Without library supplementation, the top scores (2.068-2.241) were all N. meningitidis and all top ten matches were N. meningitidis. Even with the library supplementation described above, N. meningitidis remained the top match for all five N. polysaccharea isolates with scores of 2.038-2.241 and all top ten matches being N. meningitidis for three isolates and N. polysaccharea and N. meningitidis intermingled among the top ten 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 Biotyper MALDI-TOF MS system consider verifying identities of isolates reported as N. meningitidis by an additional method. Testing may include assessment of γ-glutamyl transferase activity, production of starch-like polysaccharide from sucrose, and/or acid production from sucrose. Isolates 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 or amplification of N. meningitidis-specific genes (e.g., sodC, porA) may be considered (10, 11).
The current 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 of *N. polysaccharea*. Our results suggest that *N. polysaccharea* may be misidentified as *N. meningitidis* resulting in a cascade of unnecessary actions including exposure management of laboratory workers, healthcare workers and other close contacts of the patient, public health reporting and patient care.
References


sodC-based real-time PCR for detection of Neisseria meningitidis. PloS One 6:e19361.