An Illustrative Example of the Need for Ongoing Clinical Microbiology Competency in the Era of MALDI-TOF MS Microorganism Identification: *Neisseria* spp.

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Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) has emerged as a rapid, accurate, and cost-effective methodology for organism identification in clinical microbiology laboratories. In many laboratories, this method has supplanted conventional phenotypic identification methods.

We report the case of a 2 year old boy with a superficial culture of a penile lesion containing a *Neisseria cinerea* misidentified as *Neisseria meningitidis* by MALDI-TOF MS. The isolate, initially analyzed using the Bruker MALDI Biotyper version 3.1 (Bruker, Billerica, MA) yielded a result of *N. meningitidis* as the top two identification matches with confidence scores of 1.722 and 1.721. Subsequent manual capture of spectra and data analysis on the same organism preparation also yielded *N. meningitidis* as the top two matches with confidence scores of 1.639 and 1.627. Importantly, none of these scores were high enough to be reported to a species level identification per manufacturers recommended cutoff of ≥2.000. However, the reproducibility of these results, the mid-level confidence values (1.700-2.000 corresponds to secure Genus level identification per manufacturer's recommendations), and the potential ramifications of an identification of *N. meningitidis*, prompted further workup of the isolate. A RapID^{TM} NH (Remel, Lenexa, KS) was performed and yielded an identification of *N. gonorrheae.* Recovery of this pathogen from a two year old patient could have major legal and ethical implications, obligating additional confirmatory testing. The isolate was eventually sent to the state public health laboratory for identification. Based on the consensus findings of a battery of phenotypic assays (including growth on nutrient agar and colistin disc susceptibility) the isolate was ultimately identified as *Neisseria cinerea*. In addition to the *Neisseria* spp., *Streptococcus*

pyogenes was also recovered from this clinical specimen, and was believed to be the causative agent of his penile lesion, with the *N. cinerea* representing a component of normal skin flora.

This experience prompted additional investigation into the accuracy of MALDI-TOF MS for the identification of *Neisseria* spp. Four well characterized isolates were used, including the patient isolated of *N. cinerea* described above, a laboratory isolate of *Neisseria mucosa* (confirmed by 16S rRNA gene sequencing), *Neisseria lactamica* (ATCC strain 23907), and *Neisseria sicca* (ATCC strain 9913). These isolates were grown on chocolate agar (Remel) at 35° C in 5% CO_2 and analyzed at 1, 2, and 5 days of growth by the Bruker MALDI Biotyper RUO library and the VITEK MS version 2.0 system (bioMérieux, Marcy l'Etoiile, France). Analysis was performed for both systems using the direct spotting technique and automated peak acquisition according to manufactures' specifications. Manual spectra acquisition and analysis was performed with samples analyzed on the Bruker platform if a score < 1.700 was achieved in automatic mode.

On Day 1, the Bruker Biotyper correctly identified three out of four isolates, with confidence scores between 1.700 and 2.000. On Day 2, the score for *N. lactamica* improved to 2.07, acceptable for species level identification. The isolate initially identified as *N. meningitidis* in the described case identified as *N. cinerea* on Day 1 with a score of 1.889. On Day 2 the highest match for this isolate reverted back to *N. meningitidis* with a score of 1.710. *N. meningitidis* was the first or second match for the three correctly identified organisms during at least one of the days tested. The VITEK MS correctly identified three out of four isolates each day tested. One isolate (*N. sicca* ATCC 9913) failed identification on both platforms, though on Day 5 was erroneously identified as *N. mucosa* (99.9% confidence) by the VITEK MS.

The identification of organisms within the genus *Neisseria* can be problematic due to the high degree of genetic similarity between species. Several studies have illustrated the efficacy of MALDI-TOF MS for the identification of *Neisseria* species, however, a handful of recent reports cite instances of MALDI-TOF MS misidentifying *Neisseria*(1, 2). Though rare, these instances of misidentifications are concerning given the clinical, legal, and public health implications of the identification and misidentification of *N. meningitidis* or *N. gonorrhoeae.* These previous

studies, as well the case described herein, suggest that MALDI-TOF MS may not be an accurate stand-alone identification methodology for *Neisseria* species.

References

1. **Cunningham SA, Mainella JM, Patel R.** 2014. Misidentification of Neisseria polysaccharea as Neisseria meningitidis with the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. Journal of clinical microbiology **52**:2270-2271.
2. **Vironneau P KR, Cambau E, Bercot B.** 2013. Neisseria polysacharea and Neisseria cinerea identified like Neisseria meningitidis by MALDI-TOF. **abstr P172. Abstr. 23rd Meet. Eur. Congr. Clin. Microbiol. Infect. Dis., Berlin, Germany**.