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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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Introduction

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.

Case Report

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 RapIDTM 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 MO 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.

Method

This experience prompted additional investigation into the accuracy of MALDI-TOF MS for the identification of *Neisseria* spp. Four 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% CO2 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, formic acid overlay (Bruker only), 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.

Results

- Results from the Bruker Biotyper are shown in Table 1 and results from the VITEK MS analysis are shown in Table 2.
- On Day 1, the Bruker Biotyper correctly identified three out of four isolates, with confidence scores between 1.700 and 2.000.
- The isolate initially identified as *N. meningitidis* by Bruker Biotyper 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. Obtained spectra from both days shown in Figure 1.
- *N. meningitidis* was the first or second match for the three correctly identified organisms during at least one day tested by the Bruker Biotyper.
- 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.

Figure 1. Similar obtained spectra from patient isolate on Day 1 (identified as *N. cinerea*) and Day 2 (identified as *N. meningitidis*)

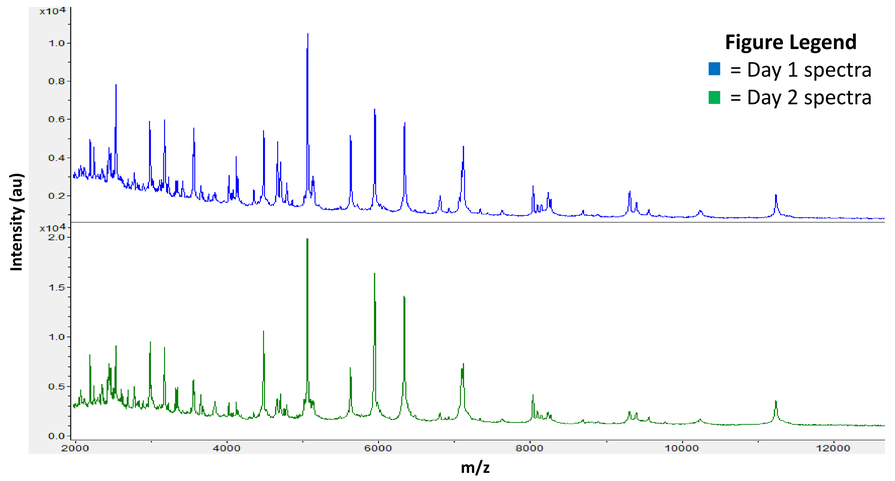

Table 1. Bruker MALDI Biotyper Data (top two matches shown)

Isolate	Bruker Day 1 ID	Bruker Day 1 score	Bruker Day 2 ID	Bruker Day 2 score	Bruker Day 5 ID	Bruker Day 5 score
Neisseria cinerea (patient isolate)	*Neisseria cinerea*	1.889	*Neisseria meningitidis*	1.710	*Neisseria cinerea*	1.700
	Neisseria meningitidis	1.610	*Neisseria meningitidis*	1.638	*Neisseria meningitidis*	1.652
Neisseria lactamica (ATCC 23970)	*Neisseria lactamica*	1.706	*Neisseria lactamica*	2.070	*Neisseria lactamica*	2.193
	Neisseria lactamica	1.678	*Neisseria lactamica*	1.870	*Neisseria meningitidis*	1.410
Neisseria sicca (ATCC 9913)	*Neisseria mucosa*	1.683	*Neisseria mucosa*	1.657	*Neisseria mucosa*	1.587
	Neisseria subflava	1.630	*Neisseria subflava*	1.692	*Neisseria flavescens*	1.483
Neisseria mucosa (confirmed by 16S rRNA)	*Neisseria mucosa*	1.914	*Neisseria mucosa*	1.855	*Neisseria mucosa*	1.748
	Neisseria cinerea	1.887	*Neisseria meningitidis*	1.518	*Neisseria meningitidis*	1.551

Table 2. VITEK MS Data

Isolate	Vitek Day 1 ID	Vitek Day 1 score	Vitek Day 2 ID	Vitek Day 2 score	Vitek Day 5 ID	Vitek Day 5 score
Neisseria cinerea (patient isolate)	*Neisseria cinerea*	99.9	*Neisseria cinerea*	99.9	*Neisseria cinerea*	97.8
Neisseria lactamica (ATCC 23970)	*Neisseria lactamica*	99.9	*Neisseria lactamica*	99.9	*Neisseria lactamica*	99.9
Neisseria sicca (ATCC 9913)	no identification	-	no identification	-	*Neisseria mucosa*	99.9
Neisseria mucosa (confirmed by 16S rRNA)	*Neisseria mucosa*	99.9	*Neisseria mucosa*	99.9	*Neisseria mucosa*	99.9

Conclusions

- The potential misidentification of *N. meningitidis* or *N. gonorrhoeae* is concerning given clinical, legal, and public health implications.
- The Bruker MALDI Biotyper will occasionally designate *N. meningitidis* as the first and/or second best match when analyzing non-meningitidis *Neisseria* isolates. Strict adherence to the manufacturer's recommended confidence value cutoffs and reliance on alternative testing can prevent misidentification.
- Based on the isolates tested the VITEK MS may have a higher identification rate to the species level for *Neisseria* sp., though occasionally misidentifies these organisms.
- MALDI-TOF MS may not be an accurate stand-alone identification methodology for *Neisseria* species.