Identification of *Nocardia* species by MALDI-TOF mass spectrometry.

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Background

Routine MALDI-TOF mass spectrometry (MS) for identification of *Nocardia* species remains challenging. To improve identification, previous studies used modified sample preparation methods and\or considerably augmented the reference spectra database. We show that an approach using routine sample preparation, an up-to-date commercial database minimally augmented with custom spectra, and analysis at an early stage of growth is a promising approach for identification.

Methods

78 *Nocardia* isolates, representing 25 unique species that were identified to species level and 9 *Nocardia* isolates identified to complex level by partial 16S rRNA gene sequencing were tested by MALDI-TOF MS by a routine formic acid–acetonitrile sample preparation method. These spectra were analyzed using a commercial database (Bruker Biotyper, 5627 spectra) supplemented with 13 custom *Nocardia* species reference spectra. MALDI-TOF MS cutoff scores of ≥1.9 and ≥1.7 were used for species and genus level identifications.

Results

Among isolates identified to species by sequencing, MALDI-TOF MS identified 82% (62 of 78) and 94% (73 of 78) to species and genus level, respectively. Five of 78 (6.4%) of the isolates could not be identified (score <1.7), and there were no species or genus level misidentifications. Of the 5 isolates that scored <1.7, 4 were represented by only 1 reference spectrum. Of the isolates defined only to complex by sequencing, 89% (8 of 9) and 100% (9 of 9) were correctly identified to complex and genus level, respectively.

Conclusions

Recent updates in a commercial database coupled with routine extraction methods yielded improved identification of *Nocardia* spp. Supplementation of the database with custom spectra is still required for optimal performance.