P0291
Paper Poster Session
Non-culture techniques for challenging situations in diagnostics

Accurate identification of common pathogenic Nocardia species: evaluation of a multilocus sequence analysis platform and matrix-assisted laser desorption ionization-time of flight mass spectrometry

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Background: Species identification of *Nocardia* is not straightforward due to rapidly evolving taxonomy, insufficient discriminatory power of conventional phenotypic methods and limitations of single gene locus analysis including 16S rRNA gene sequencing.

Material/methods: Here we evaluated the ability of a 5-locus (16S rRNA, *gyrB*, *secA1*, *hsp65* and *rpoB*) multilocus sequence analysis (MLSA) approach as well as matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS; Bruker Biotyper database version 3.1) in comparison with 16S rRNA gene sequencing to provide identification of 25 clinical isolates of Nocardia.

Results: 16S rRNA gene sequence successfully assigned 24 of 25 clinical isolates (96%) to species level, namely *Nocardia cyriacigeorgica* (n=12, 48%), *N. farcinica* (n=9, 36%), *N. abscessus* (n=2, 8%) and *N. otitidiscaviarum* (n=1, 4%). The MLSA of clinical isolates showed concordance with 16S rRNA gene sequencing for the same 24 isolates. However, MLSA was able to identify the remaining isolate as *N. wallacei*, and clustered *N. cyriacigeorgica* into three subgroups. The Biotyper database correctly assigned none clinical isolates to species level. A small “in-house” spectral database was established by five clinical isolates representing five species identified in this study. After complementation with the “in-house” database, of the remaining 20 isolates, 19 (95%) were correctly identified to species level and one (an *N. abscessus* strain) to genus level.

Conclusions: In summary, MLSA showed superior discriminatory power compared with 16S rRNA gene sequencing for species identification of *Nocardia*. MALDI-TOF MS has good utility in rapid and accurate identification but relies on building up a robust mass spectra database.

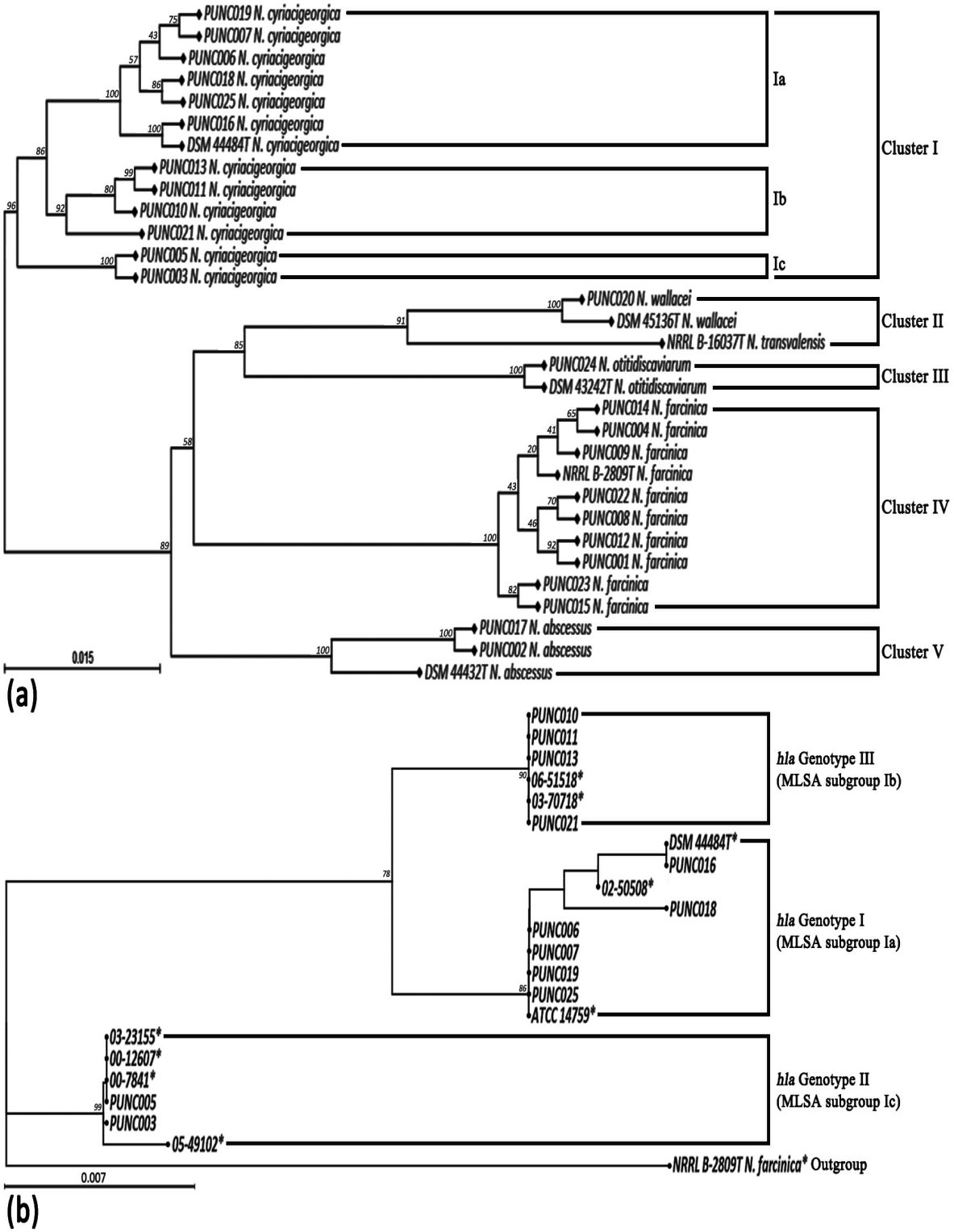

FIGURE. Phylogenetic trees are shown and were conducted using the N-J method. Fig. 1a. Phylogenetic tree based on the concatenated *gyrB*-16S-*secA1-hsp65-rpoB* sequences of six reference Nocardia strains and 25 clinical isolates. Fig. 1b. Phylogenetic tree based on the *hsp65* gene sequences of 12 *N. cyriacigeorgica* clinical isolates and nine *N. cyriacigeorgica* isolates whose genotypes have previously been determined using the sequence of a *Nocardia farcinica* strain as an outgroup.