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The accuracy of matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) in the identification of Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, and Kingella (HACEK) species was compared to that of phenotypic methods (Remel RapID and Vitek 2). Overall, Vitek MS correctly identified more isolates, incorrectly identified fewer isolates than both phenotypic methods.

The set of organisms used in this study was composed of American Type Culture Collection (ATCC) isolates, recent clinical isolates, and laboratory stock strains of Haemophilus, Aggregatibacter, Eikenella, and Kingella (Table 1). A total of 140 isolates representing 10 species were analyzed: 55 clinical isolates from the Cincinnati Children’s Hospital Medical Center, 2 isolates from the University of Cincinnati, 7 isolates from the Nationwide Children’s Hospital, 37 ATCC strains, and 39 isolates from bioMérieux.

Organisms were cultured on chocolate agar plates at 37°C in 5% CO₂ according to laboratory procedure. Remel RapID NH tests (Thermo Fischer Scientific, Lenexa, KS) were performed with each organism in accordance with the manufacturer’s instructions. For Vitek 2 NH card (bioMérieux, Durham, NC) analysis, isolates were tested according to standard laboratory policies and procedures for the Vitek2 XL instrument. Identification was considered definitive when a result was obtained with at least 85% confidence. Isolates were sent to the Genetic Variation and Gene Discovery Core Facility at the Cincinnati Children’s Hospital Medical Center, a Clinical Laboratory Improvement Amendments of 1988-approved laboratory, for identification by 16S rRNA sequencing. 16S RNA sequencing was performed for all isolates as the reference method.

MALDI-TOF analysis was performed with a bioMérieux Vitek MALDI-TOF mass spectrometer (bioMérieux, Durham, NC), and spectra were compared against Vitek MS SARAMIS research use only (RUO) database version 4.09 by using the SuperSpectra algorithm (referred to here as MALDI-TOF MS). Identification was considered definitive when the probability provided was greater than 70%. For MALDI-TOF MS analysis, one colony was applied to one spot of the test slide with a disposable inoculating loop, overlaid with 1 μl of matrix solution (α-cyano-4-hydroxycinnamic acid), and dried completely before analysis. MALDI-TOF MS analysis was performed in duplicate, with tests performed simultaneously on the same slide. If the duplicates gave different results, the analysis was repeated in triplicate. If methods contradicted one another, both were repeated.

As shown in Table 1, MALDI-TOF MS correctly identified the most isolates. Additionally, this method incorrectly identified the
To our knowledge, this is the first large-scale study of HACEK organisms or fastidious pediatric pathogens to be undertaken with MALDI-TOF MS. Additionally, it is the first to use MALDI-TOF MS correctly identified. Most commonly used methods for identifying members of the genus Haemophilus were correctly identified. However, previous studies of MALDI-TOF MS with the genera Moraxella, Actinobacillus, and Pasteurella have found that MALDI-TOF MS is not accurate for these genera.

### Table 2: Incorrect Identifications by each Method

<table>
<thead>
<tr>
<th>Method</th>
<th>Incorrect identifications</th>
<th>% incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitek 2 NH</td>
<td>20</td>
<td>7.5</td>
</tr>
<tr>
<td>Remel RapID NH</td>
<td>26</td>
<td>10.0</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>26</td>
<td>10.0</td>
</tr>
<tr>
<td>Vitek MALDI-TOF MS for HACEKs</td>
<td>26</td>
<td>10.0</td>
</tr>
</tbody>
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**Vitek MALDI-TOF MS for HACEKs**

The advent of MALDI-TOF MS for bacterial isolate identification allows a more accurate means of identification without the colorimetric change. IS-PCR sequencing, in contrast, is extremely accurate but typically is not done by clinical microbiology laboratories and is very costly.
SARAMIS RUO database version 4.09 correctly identified the most organisms of the three methods. As Vitek MS is more accurate than either of the commercially available methods tested here, it is appropriate for use with fastidious pediatric pathogens.

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REFERENCES