

Serotyping of pneumococci using FT-IR spectroscopy

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Introduction and Purpose

Pneumococcal vaccines are powerful tools to protect patients from severe diseases. Until today more than 90 different serotypes of this capsule have been described, but only 23 serotypes are included in the available vaccines. Therefore serotyping of pneumococci is mandatory to monitor distribution of different serotypes. The traditional method of typing is the “Neufeldsche Quellungs” reaction, which is expensive, difficult to perform and therefore limited to a few reference laboratories.

Fourier Transformation-Infrared spectroscopy (FT-IR) is a technique with the potential to differentiate closely related species. We used FT-IR to investigate its typing capabilities for the 23 serotypes included in the 23-valent vaccine.

Materials and Methods

We studied 112 isolates covering the 23 serotypes included in the 23-valent polysaccharide vaccine. Isolates were cultivated overnight on blood agar and homogenous suspensions of bacteria were dried on silicon plates as thin films. Infrared spectra were recorded with an FT-IR spectrometer (Tensor27 with HTS-XT module). In total a minimum of three independent measurements per isolate with aspired 5 technical replicates summed up to 2060 infrared spectra. Acquisition of spectra and cluster analysis were performed with OPUS software (Bruker Optics) using specific algorithms.

- The results for FTIR “serotyping” were available within one day, i.e. the same day the pure culture was available.
- All isolates generated good spectra and the method was capable of distinguishing between serotypes/serogroups with an accuracy of >99%.
- To reliably distinguish between members of serogroup 6 more isolates have to be studied.

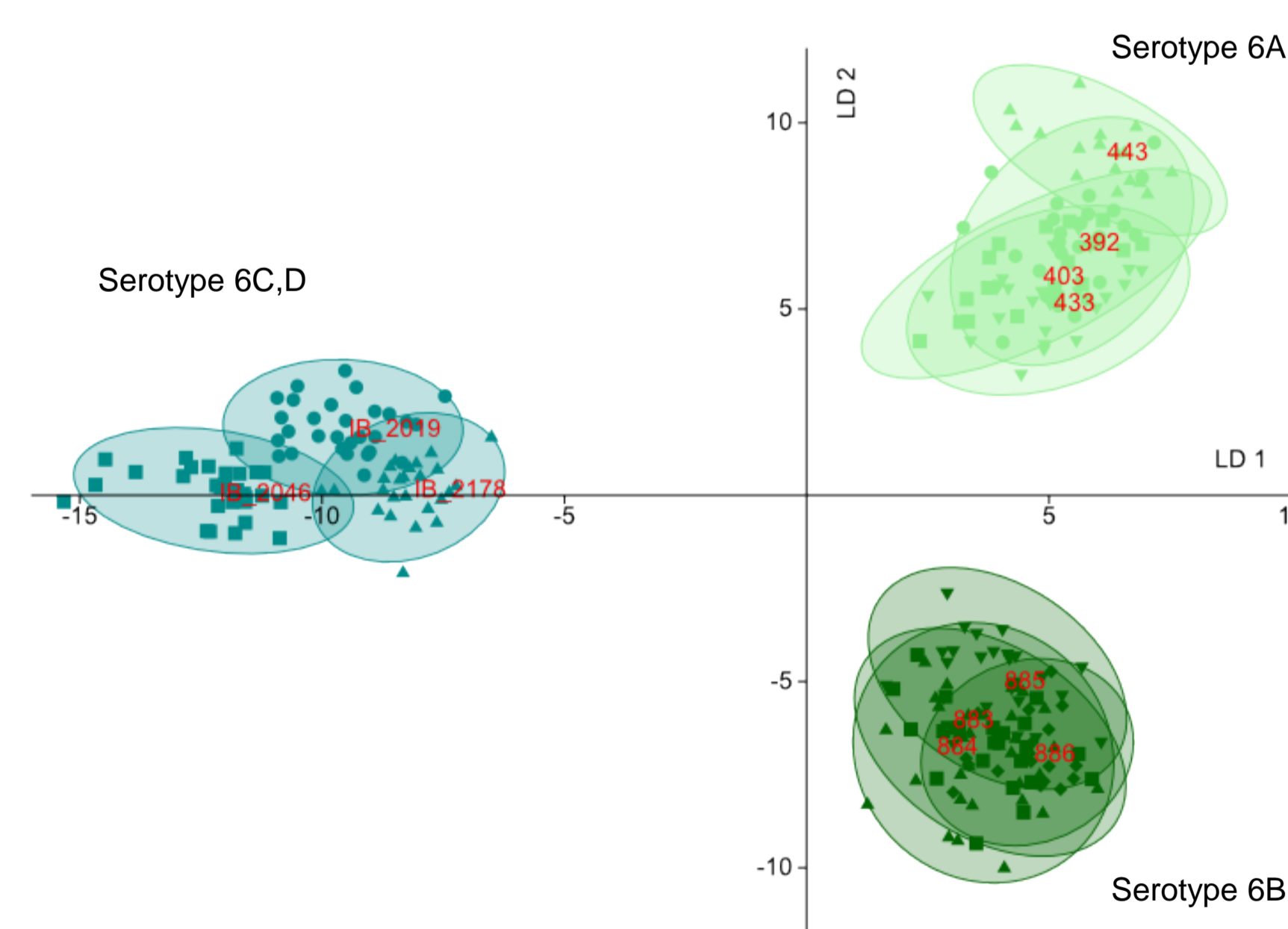


Figure 2: 2D scatter plot infrared spectra of serogroup 6 isolates. Dimensions are the first two linear discriminants of an LDA grouping isolates (not serotypes!). This LDA bases on the first 38 principal components of the IR spectra (2nd derivative, wavenumbers 800-1300 /cm, vector-normalized), that contain at least 95% of the spectral variance.

Results

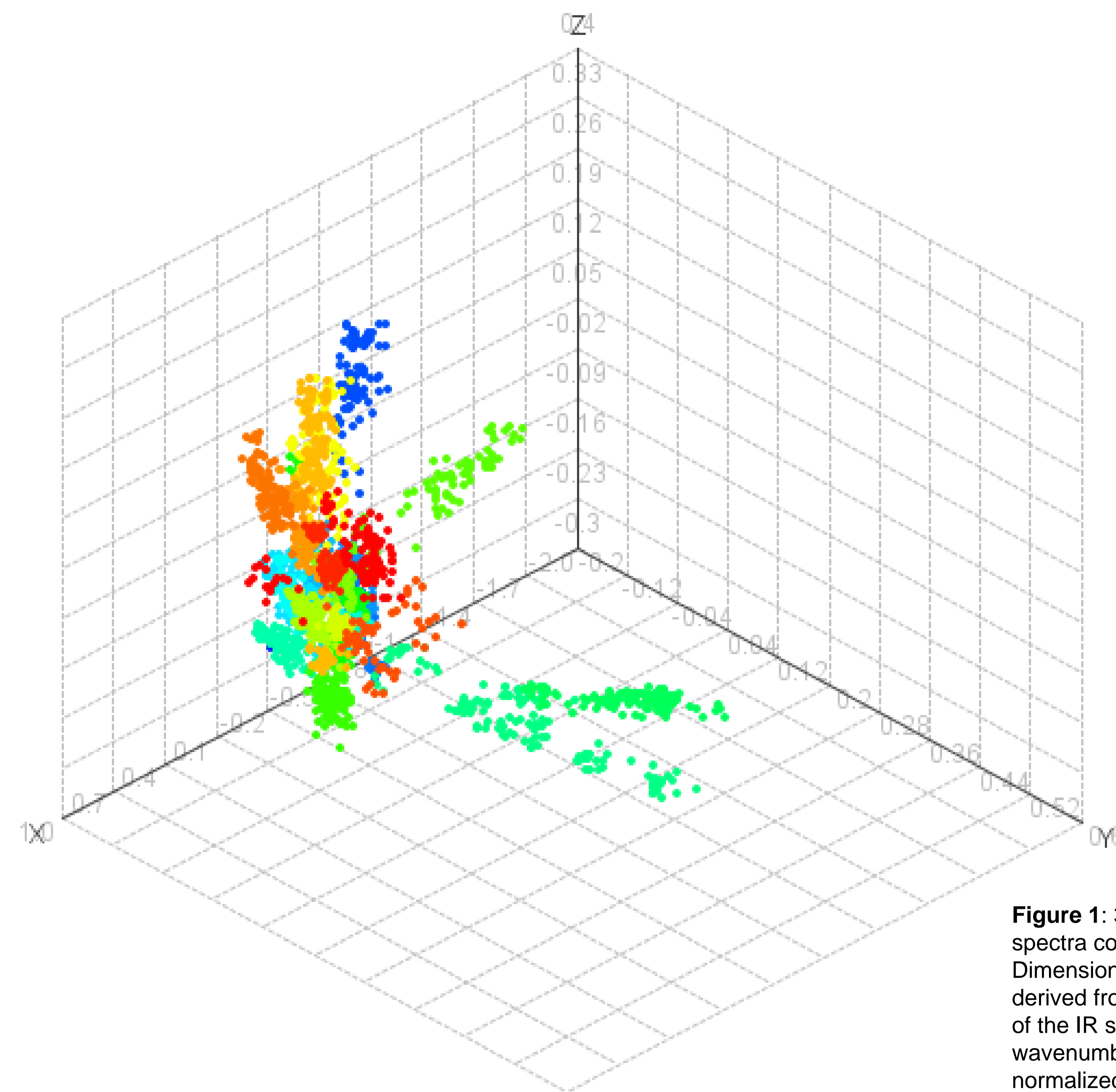


Figure 1: 3D scatter plot of 2060 infrared spectra colored by serotype/class. Dimensions are the first three components derived from a principal component analysis of the IR spectra (2nd derivative, wavenumbers 800-1300 /cm, vector-normalized).

Conclusions

1. FT-IR is a new and promising technology for serotyping of pneumococci.
2. FT-IR is faster and less laborious as conventional methods.
3. FT-IR results are reliable and highly discriminatory.