

Fourier-transform infrared spectroscopy reliably differentiates capsular types in hypervirulent and classical *Klebsiella pneumoniae*

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Background

Hypervirulent *Klebsiella pneumoniae* (hvKp) is an evolving pathotype, which is more virulent than classical *K. pneumoniae* (cKp) including multidrug-resistant (MDR) strains. hvKp isolates are primarily involved in community-acquired infections typically characterized by multiple foci of infection and/or metastatic spread in otherwise healthy individuals. The spread of hvKp and, more recently, of convergent lineages that combine resistance genes with virulence markers necessitates awareness and, especially, their rapid detection. In addition to the presence of certain biomarkers, some capsule types are also strongly correlated with hvKp.

In this study, a phenotyping Fourier-transform infrared spectroscopy (FTIRS) approach was investigated to assess its potential application in the **differentiation of hvKp from cKp strains** in comparison with genotypically determined virulence traits

Material and methods

- ✓ N=171 non-duplicated, previously geno- and phenotypically characterized Kp isolates, including **classical (n=158), hypervirulent (n=11), and convergent (n=2)** strains pathotypes.
- ✓ The strains belonged to **48 capsule types** and **76 sequence types**.
- ✓ The **hvKp strains belonged to the 6 different K types**: KL1 (n=1), KL2 (n=6), KL5 (n=1), KL20 (n=1), KL114 (n=1) and KL27 (n=1). All these 6 capsule types included also cKp.
- ✓ The two convergent strains belonged to the K types KL102 and KL103. Both types include also cKp.
- ✓ All strains were analyzed by the FTIRS-based IR Biotyper® system (IRBT - Bruker Daltonics, Germany), following the manufacturers instruction (**Figure 1**). Three independent biological replicates on Columbia blood agar incubated for 24 ± 2 h at 35 ± 2 ° C were included.

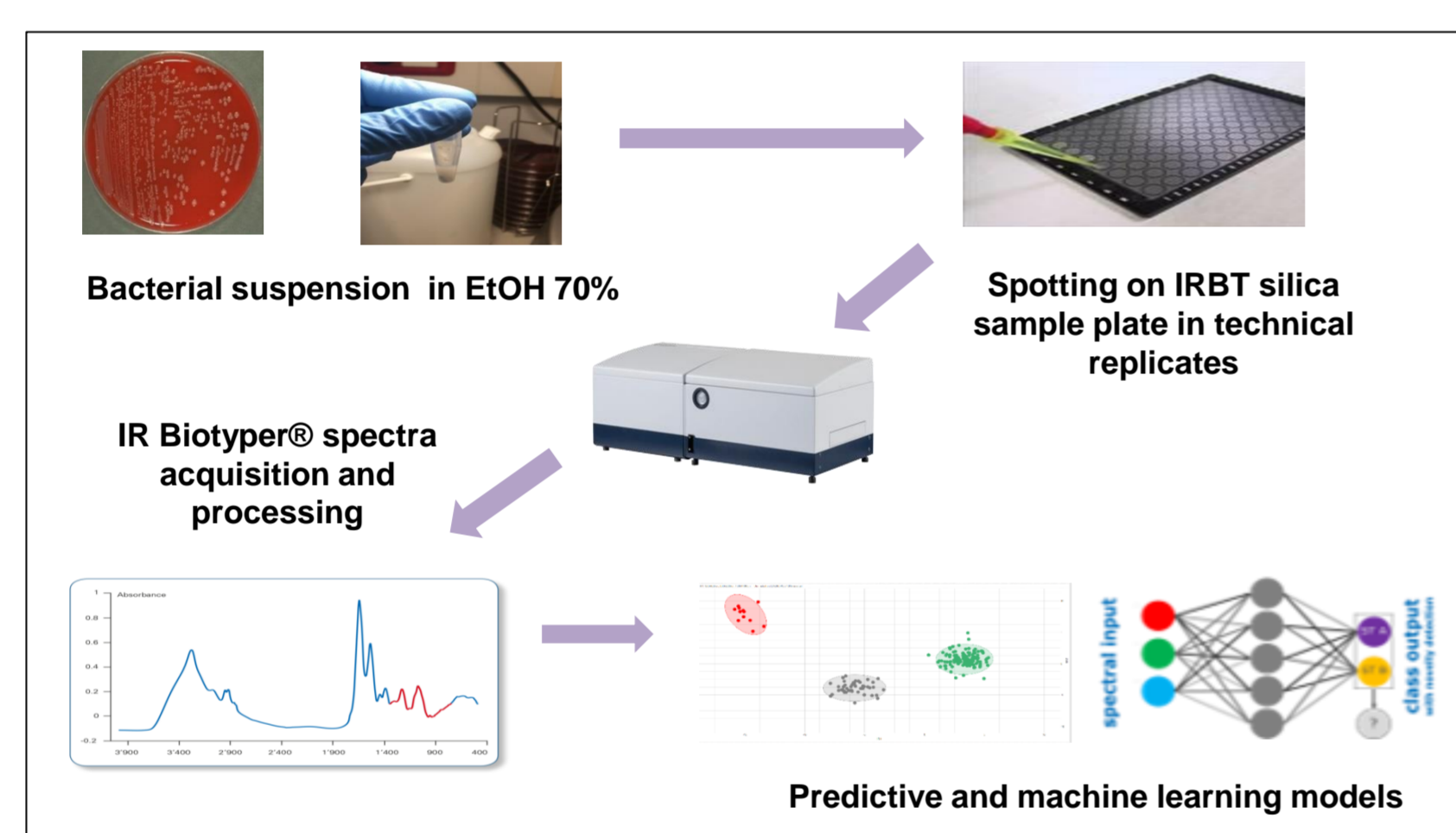


Figure 1. IR Biotyper® workflow

- ✓ **Spectra acquisition, processing, data analysis, and machine learning applications** were performed by the IR Biotyper® software V4.0 (Bruker Daltonics GmbH & Co. KG, Bremen, Germany).
- ✓ **Exploratory data analysis** was performed by hierarchical cluster analysis (HCA), using Euclidean metric and UPGMA linkage type. Different wavenumber ranges were used to evaluate the discriminatory power of the IRBT system. To assess the potential in the delineation of the pathotypes, a subset including all the strains of those capsule types for which both hvKp/convKp and cKp were available (n=30), was investigated.
- ✓ **Machine learning** was applied to create an **automatic classifier**, tailored by the results of HCA.

Results

HCA clustering using the **IRBT default wavenumber range (1300-800 cm⁻¹)** showed a **high concordance with capsule (K) types** (adjusted Rand index = 0.958, 95% CI), irrespective of the pathotype – **Figure 2**.

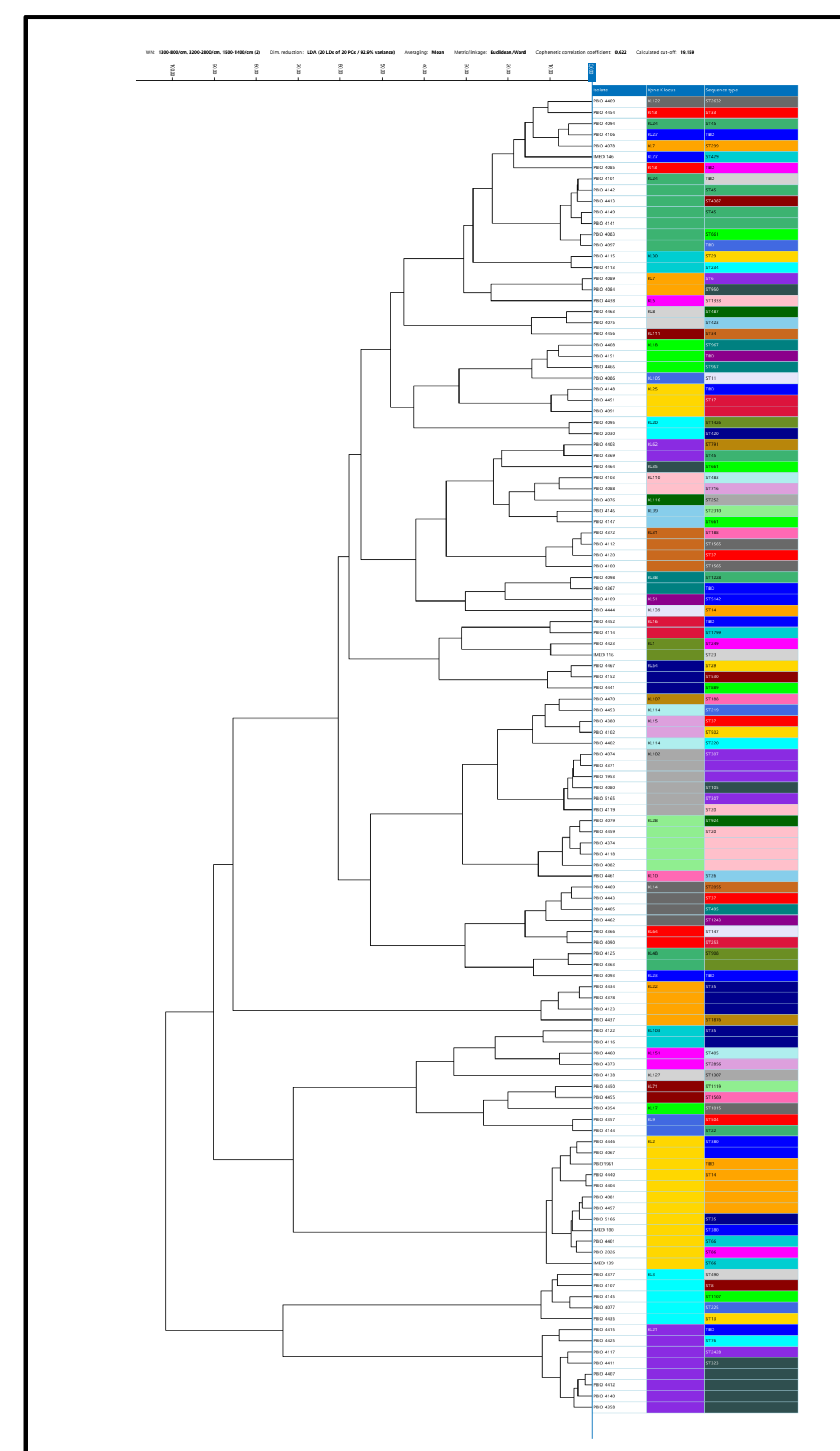


Figure 2. Dendrogram showing the IRBT clustering of Kp isolates in relation to the capsule type. On the right side of the figure, isolate name, K locus and sequence type are reported, displayed in different colors. On the left side of the figure, the IRBT clustering is shown.

An **automated classifier to discriminate K types** was built training an artificial neural network, using n=111 isolates, randomly selected and representing the 48 sequence types.

The classifier showed an overall accuracy of **96.7%** on the testing set (58/60 strains correctly delineated), with errors limited to the cKp group (one unclassified K27 isolate and one K151 misclassified as K114).

Using the **wavenumber region corresponding to proteins and lipids (3000-2800/1700-1400 cm⁻¹)**, HCA shows a clustering with **perfect concordance with the pathotype**, irrespective of the K locus (**Figure 3**).

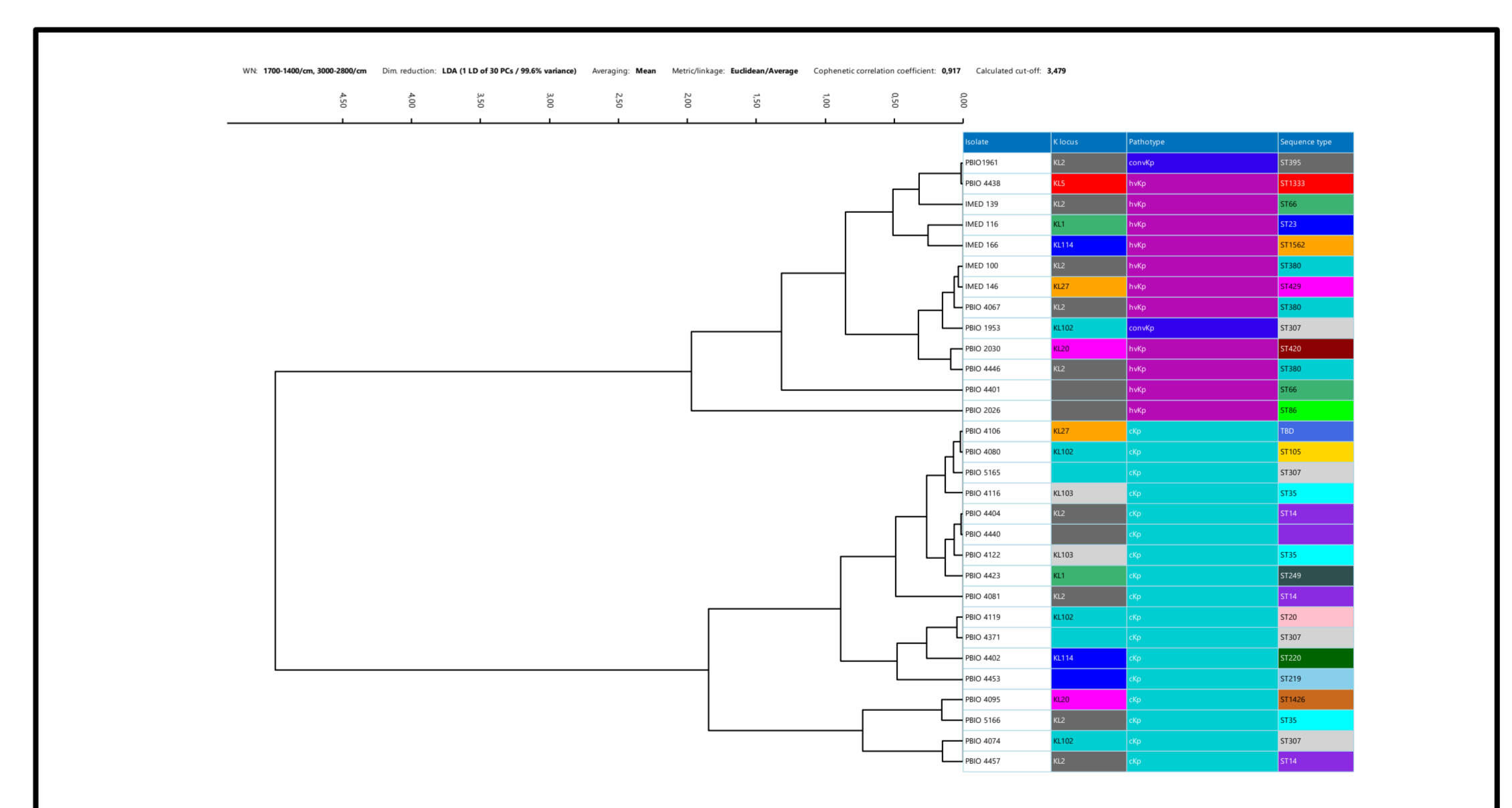


Figure 3. Dendrogram showing the IRBT clustering in relation to the pathotypes. On the right side of the figure, the K locus, the pathotype and the sequence type are reported, displayed in different colors (hvKp in violet, convKp in blue and cKp in cyan). On the left part of the figure, the IRBT clusters are shown.

Conclusion

- ✓ FTIRS provided a **reliable discrimination between the *K. pneumoniae* capsule types**.
- ✓ A **differentiation between pathotypes was also possible**, using different wavelengths.
- ✓ FTIRS could enable a novel approach for **rapid and automated indication for hvKp strains**, applying machine learning algorithms in a hierarchical fashion.
- ✓ Further studies are demanded to evaluate the robustness and the “universal” applicability of this innovative approach.

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