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Evaluation of Fourier transform infrared spectroscopy, developed for typing of *Klebsiella pneumoniae* complex isolates involved in hospital outbreaks, with clinical and environmental isolates from Tanzania

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Background

Klebsiella pneumoniae (Kp), frequently associated with nosocomial infections, is considered as a global major health threat. Rapid and reliable typing methods are crucial for outbreak detection/tracking and cross-transmission investigations.

Fourier Transform Infrared Spectroscopy (FTIR) proved to be a reliable method for Kp typing, however, the cellular elements which allow FTIR-based differentiation are not completely clarified yet.

Aim

In this study**, correlations between FTIR spectral relatedness and K-locus-based *in silico* serotype, sequence type and SNPs/allelic mismatches were investigated** with a collection of Tanzanian clinical and environmental Kp isolates.

Material and methods

Sample set

Overall, **N=97** extensively characterized Kp isolates, collected from children and livestock, were included in the study.

- **n=55** different **sequence types** (ST)
- **n=44** different **K-locus (KL) capsule types**
- **n=52 clonal** isolates (**18 different clones**, **<15 SNPs**)
- **n=45 unrelated isolates**, either sharing ST/KL with the clonal isolates or being totally different

Spectra acquisition

FT-IR analysis was performed with the IR Biotyper® system (IRBT - Bruker Daltonics, Germany), following the manufacturer's instructions. IR spectra were acquired from isolates cultivated overnight at 35 ° C on Columbia sheep blood agar (Becton Dickinson, Heidelberg, Germany). One single biological replicate was used for this study, to align with the intended use of the IR Biotyper as a real-time routine tool.

Data analysis

Classic **hierarchical cluster analysis (HCA)** and a **kNN- (k-nearest neighbor)-based approach (IR Tracker™)** were used for the FTIR/WGS comparison.

The IR Tracker™ is a novel tool which enables IR **spectra similarity analysis in a totally automated way**. For each microbial species, **two fixed and universal thresholds** (inlier and outlier), defined by the manufacturer and calculated on the basis of statistical parameters, delineate **three categories of spectral similarity/probability of clonal relatedness** (high – moderate (uncertain) – low), depicted with a **heat-map color-coding**. The IR Tracker™ delivers a result in a "best match" isolates list (the "*nearest neighbors*"), accompanied by the spectral distance score value and its categorical allocation (**Figure 1**).

Correlation between FTIR and WGS was performed using the Adjuster Rand Index (ARI) and Pearson's correlation (r).

Results

- ✓ **IRBT HCA** showed a **very high correlation between FTIR and the combination ST+KL** (ARI=0.914) – **Figure 2.**
  - Correlation with KL capsule types and ST were 0.883 and 0.705, respectively.
- ✓ **IR Tracker™ accuracy was 97.9% (95/97).**
  - One isolate having a cluster-specific KL but a different ST, was classified by the IR Tracker™ as highly related.
  - One isolate clonally related to others was classified as not related.
- ✓ **IR Tracker™ best match spectra distance** values showed a **high correlation with genetic relatedness** (**r=0.86**).

Figure 1 (below). Example of IR Tracker™ results, showing the best match result, accompanied by the spectral distance (NDI, neighbor distance index) and depicted with its corresponding color-code.

Project Run project Kpne-real-time-simulation_Day5 — Created on 2026-01-30

3 Result Overview

3.1 Isolate Neighbors

Isolate ID	Nearest Neighbor	NDI	Neighbor Labels
Kpne A-01	Kpne A-06	1.27	
Kpne A-02	Kpne C-02	0.69	
Kpne A-03	Kpne A-06	0.36	
Kpne A-04	Kpne A-06	0.38	
Kpne A-05	Kpne A-04	1.04	
Kpne A-06	Kpne A-03	0.36	
Kpne B-01	Kpne A-01	11.55	
Kpne C-01	Kpne C-02	8.85	
Kpne C-02	Kpne A-02	0.69	

High probability of clonality	>95%
Moderate probability of clonality	Please test with another method, and consider results of other tests.
Low probability of clonality	<5%

Please refer to the IR Biotyper manual for details about IR Tracker results.

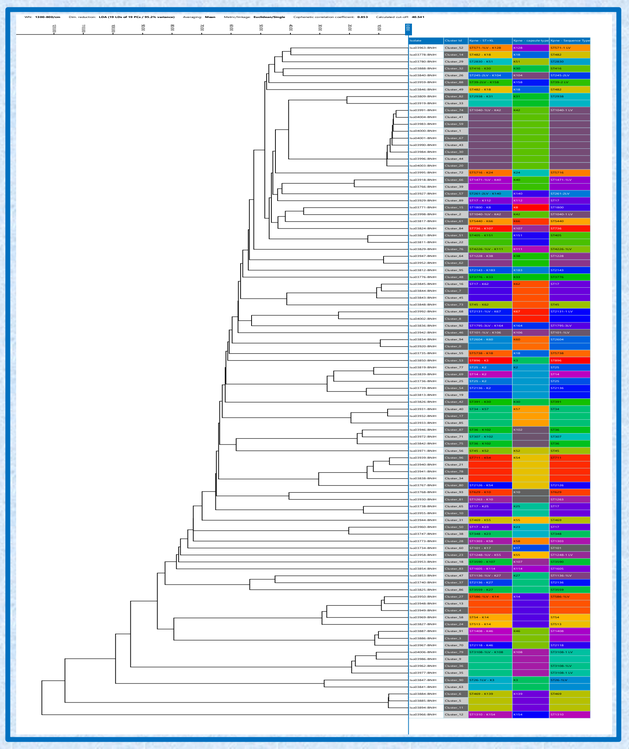

Figure 2. Dendrogram showing the IRBT clustering (Euclidean single linkage). On the right side of the picture, from left to right, isolate name, cluster ID, ST+KL, KL capsule type and ST are shown.

Conclusions

- ➢ In this study, the **FTIR differentiation of *K. pneumoniae*** complex indicated **strong associations with the combination of ST and KL**, while the correlation with either ST or KL was lower.
- ➢ IR Biotyping showed a **resolution power close to WGS for *K. pneumoniae* complex.**
- ➢ Isolates sharing the **same ST+KL**, but **with genetic distance >15 SNPs**, were recognized as **not clonally related.**
- ➢ Isolates with the **same KL but different ST** were **classified as different.**

Not for use in clinical diagnostic procedures.

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