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APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR TYPING IN A NEONATAL *KLUYVERA ASCORBATA* CLINICAL OUTBREAK

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Background

Kluyvera species are ubiquitous in the environment, and can be isolated from water, soil and sewage. Also, they are described as normal flora of the gastrointestinal tract. *Kluyvera ascorbata* is the most commonly *Kluyvera* species found in human infections.

Despite not commonly found in clinical samples, *Kluyvera ascorbata* has the potential to cause clinically relevant infections, among which BSI, UTIs, pneumonia, peritonitis, abdominal abscess, and skin and soft tissues infection. especially in healthcare settings. Particularly relevant are the severe infections that *K. ascorbata* can cause in newborns (sepsis, meningitidis), described in several reports.

As most of *K. ascorbata* infections are hospital acquired, and transmitted within a hospital ward, the prompt typing of the isolates, to allow the recognition and tracking of the transmission routes are crucial. Fourier transform infrared spectroscopy (FT-IRS) is a recently introduced method for microbial typing on different intraspecies levels, with the potential of a near-time monitoring due to its speed and user-friendliness.

In this study, we evaluate the **applicability of Fourier-transform infrared spectroscopy** methodology to type a ***K. ascorbata* outbreak** occurred in a **neonatal intensive care ward.**

Material and methods

A total of N=16 well characterized strains of *Kluyvera* spp. were included in this study, representing all the species of the genus:

- ✓ n=10 *K. ascorbata* isolates collected during the routine screening of a neonatal intensive care unit (NICU) in September 2022, that had never been observed there before;
- ✓ n=3 *K. ascorbata* reference strains from culture collections
- ✓ type strains of *K. cryocrescens*, *K. georgiana* and *K. intermedia* (total n=3), to ensure that all the species of the genus were represented

The strains collected during the screening underwent typing by PFGE, performed by the National Reference Center in Bochum, Germany.

FT-IRS analysis was performed with the IR Biotyper® system (IRBT; Bruker Daltonics, Germany). Spectra were acquired from 3 independent biological replicates, cultivated on Columbia blood agar (Becton Dickinson) at 37 ° C for 24±2 h. Spectra acquisition, processing and exploratory data analysis were performed by the IR Biotyper® software V4.0. The spectral region 1300-800 cm^{-1} was used for the data analysis.

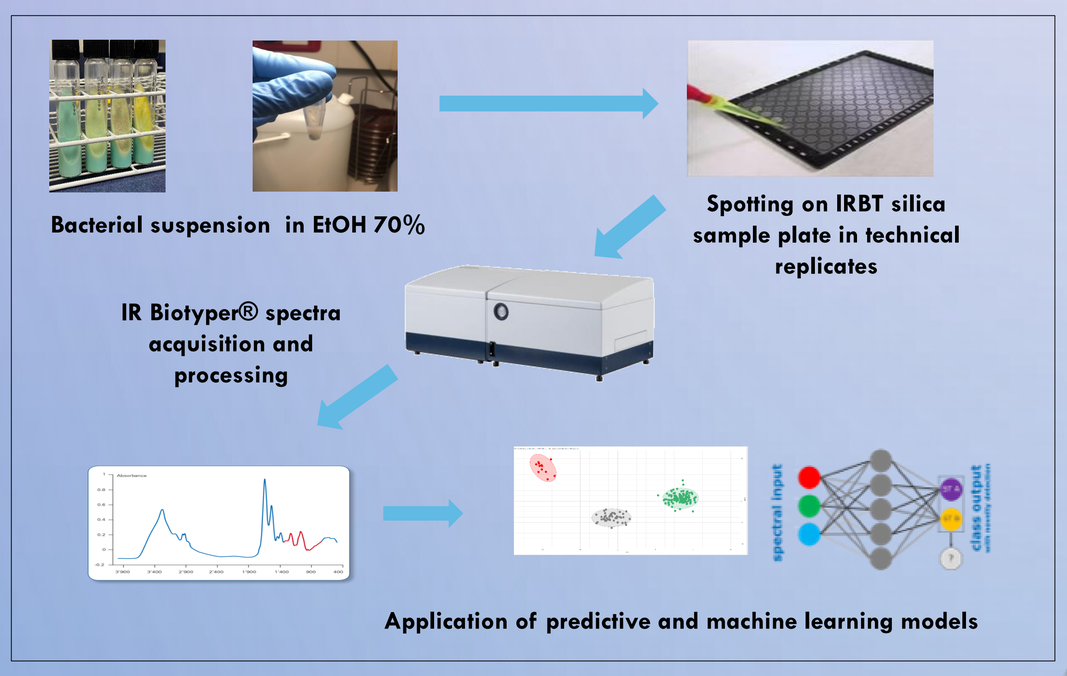

Figure 1. IR Biotyper® workflow

Results

- ✓ The typing by PFGE assigned n=8 *K. ascorbata* strains to the same pulsotype, while n=2 isolates were different.
- ✓ IR Biotyper cluster analysis showed that the *K. ascorbata* outbreak isolates clustered together, in complete agreement with PFGE results (**Figure 2**).
- ✓ The outbreak isolates resulted clearly differentiated from the two non-outbreak isolates, as well as from the three culture collection strains.
- ✓ Also, the type strains of the other *Kluyvera* species resulted well differentiated from *K. ascorbata*.

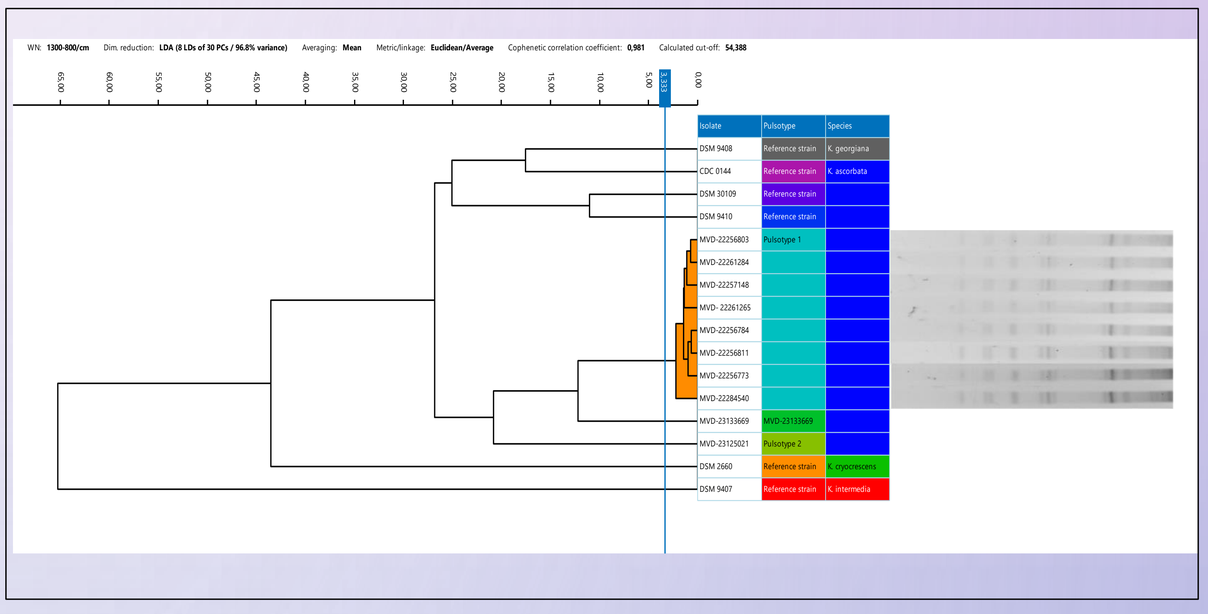

Figure 2. IR Biotyper dendrogram showing the clustering of the *Kluyvera* isolates, and the correspondence with the PFGE results. Each sample is depicted with its average spectrum, calculated from the single spectra represented by the technical and biological replicates. The clustering cut-off was automatically calculated by the software.

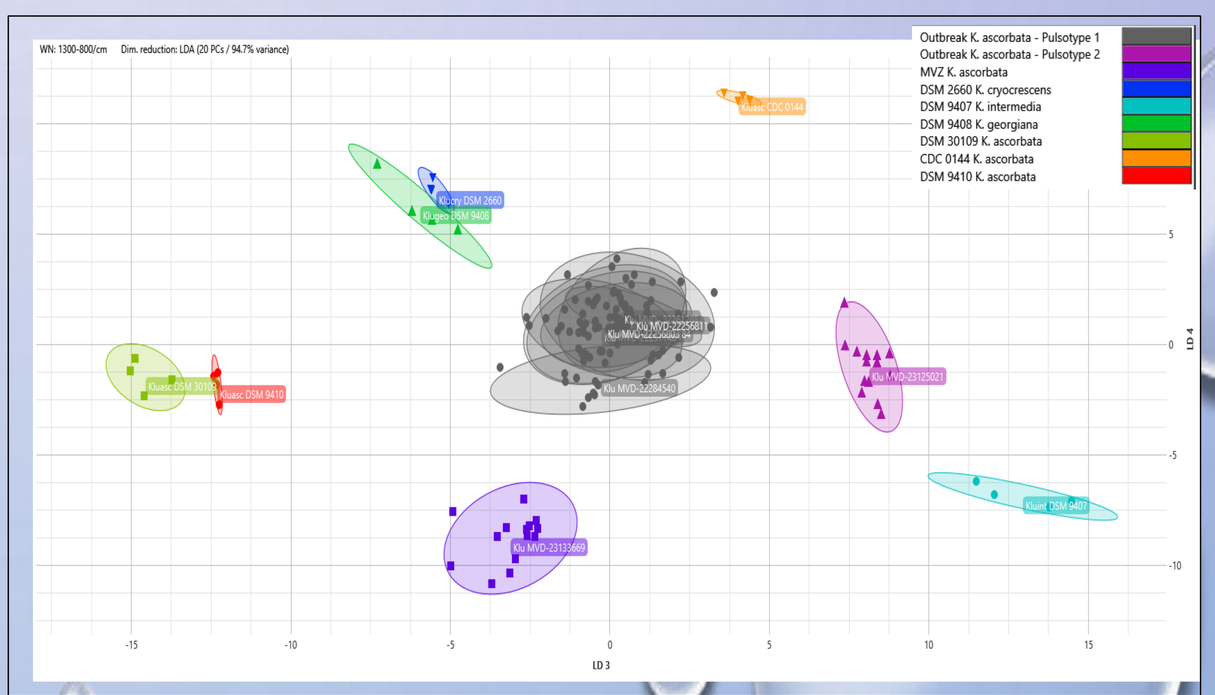

Figure 3. 3D LDA scatterplot showing the clustering on the *Kluyvera* spp. strains. Each geometric form represent a single spectrum. Each isolate is depicted with a different color. The isolates of the same pulsotype are depicted with the same color. The ellipses shows the 95% confidence interval.

Conclusion

In this study, **FTIR could successfully reveal the clonality among *K. ascorbata* outbreak isolates.** Due to its speed and ease-of-use, the method has the potential to be applied in a routine setting for near-time monitoring in hospital hygiene, enabling a **prompt detection of cross-transmission also for less common bacterial species.**

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