P2142

Application of Fourier transform infrared spectroscopy for a neonatal *Kluyvera ascorbata* clinical outbreak

Miriam Cordovana[1], Jörg B. Hans[2,3], Arthur B. Pranada[4]

[1] Bruker Daltonics GmbH & Co. KG, Bremen, Germany; [2] National Reference Center for multidrug resistant Gram-negative bacteria, Department for Medical Microbiology Ruhr-University Bochum, Bochum, Germany; [3] Department of Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany; [4] MVZ Dr. Eberhard & Partner Dortmund, Department of Medical Microbiology, Dortmund, Germany

Background

Kluyvera species can be ubiquitously found in the environment, water, soil and sewage, as well as they are described as normal flora of the gastrointestinal tract. Among these species, *Kluyvera ascorbata* is the most commonly found in human infections. Despite rarely isolated, it is associated with a wide range of clinically significant infections, including UTIs, BSI, skin and soft tissue infections, pneumonia, peritonitis, abdominal abscesses. In addition, several reports show that *K. ascorbata* can cause severe infections in newborns (sepsis, meningitis). These might be hospital-acquired and can be generally transmitted within a hospital ward. Therefore, prompt recognition and tracking of the transmission are crucial.

Fourier transform infrared spectroscopy (FTIR) is a recently introduced method for microbial typing on different intraspecies levels with the potential of a near-time monitoring due to its speed.

In this study, we evaluate the **applicability of Fourier-transform infrared spectroscopy** methodology for the **typing of 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 the 4 species of the genus:

- **n=10 *K. ascorbata*** isolates collected during routine screening of a neonatal intensive care unit (NICU) in September 2022, that had not been observed there before.
- **n=3 *K. ascorbata* reference strains** from culture collections
- the type strains of K. *cryocrescens*, *K. georgiana* and *K. intermedia*, to ensure that all 4 species of the genus were represented

The screening isolates were typed by PFGE, performed by the National Reference Center in Bochum, Germany.

FTIR analysis was performed by the IR Biotyper® system (IRBT - Bruker Daltonics, Germany), following the manufacturers instruction (**Figure 1)**. The isolates were cultivated on Columbia blood agar (Becton Dickinson) at 37 ° C for 24±2 h, in 3 independent biological replicates. IR spectra were acquired from dried spots of bacterial suspensions in ethanol solution on the IR Biotyper sample plate, in three technical replicates.

Spectra acquisition, **processing** and **data analysis** were performed by the IR Biotyper® software V4.0. The spectral region 1300-800 cm^{-1} was used for the data analysis.

Exploratory data analysis was performed by HCA (Hierarchical cluster analysis), **PCA** (principal components analysis) and **LDA** (linear discriminant analysis).

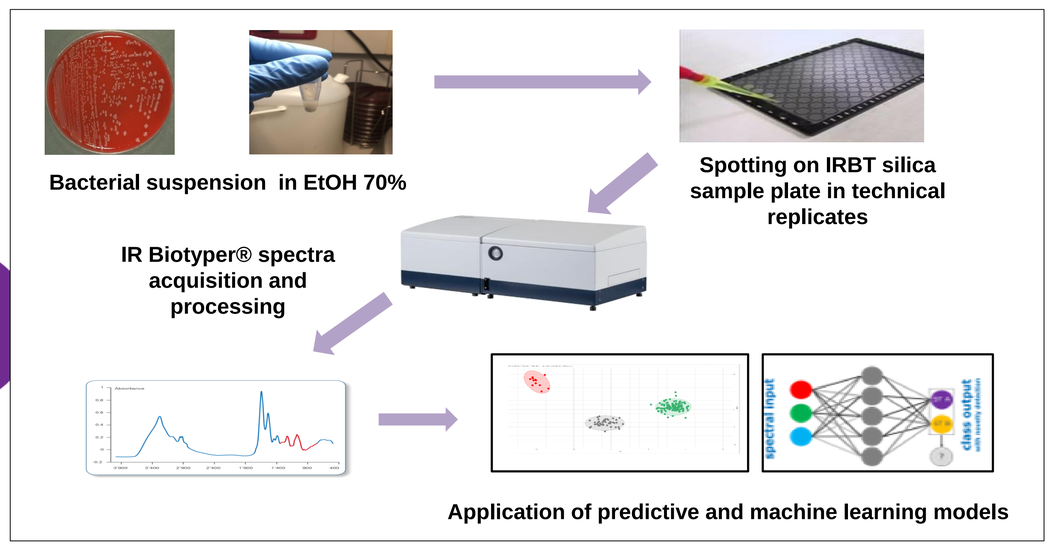

Figure 1. IR Biotyper® workflow

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*.

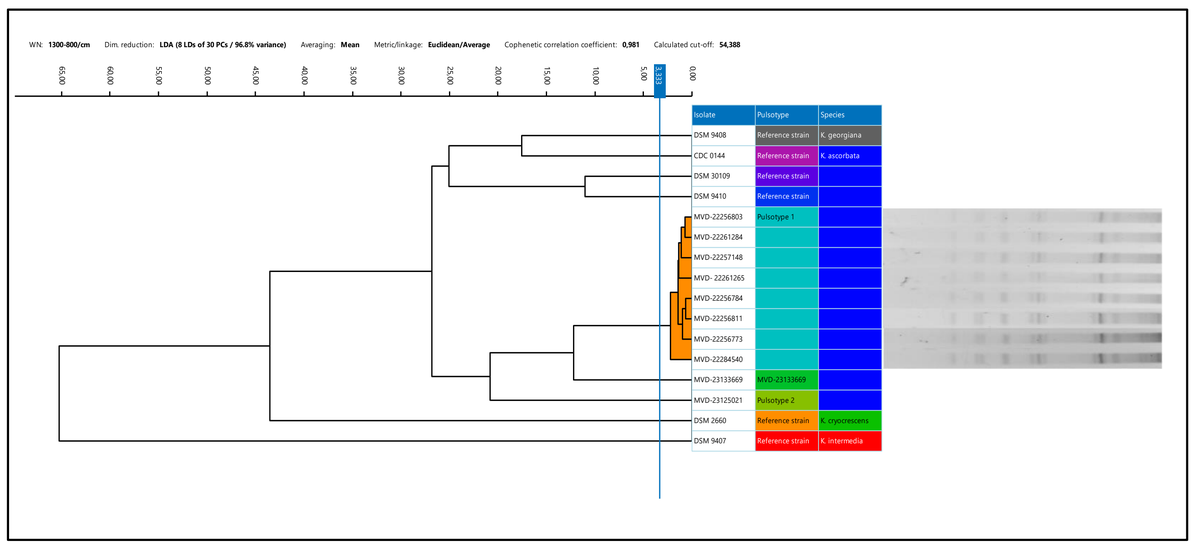

Figure 2. IR Biotyper dendrogram showing the clustering of the *Kluyvera* isolates, and the correspondence with the PFGE results. Each sample is depicted with its average spectrum, calculated from the single spectra represented by the technical and biological replicates. The clustering cut-off was automatically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

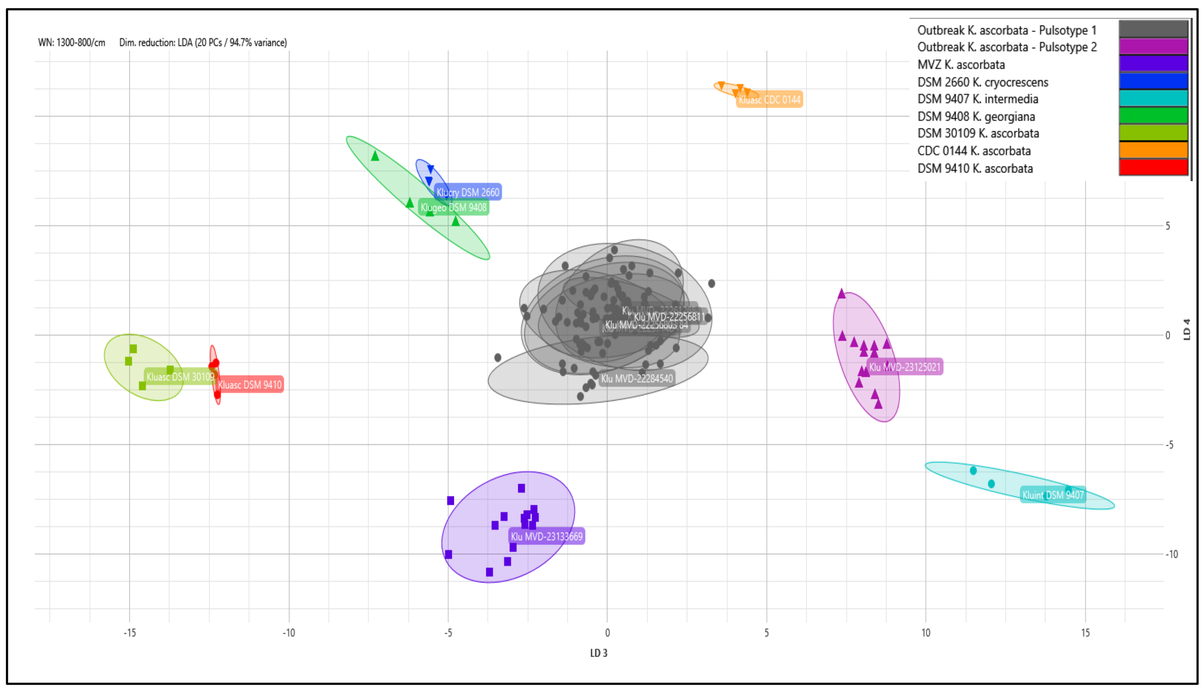

Figure 3. 3D LDA scatterplot showing the clustering on the *Kluyvera* spp. strains. Each geometric form represent a single spectrum. Each isolate is depicted with a different color. The isolates of the same pulsotype are depicted with the same color. The ellipses shows the 95% confidence interval.

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.

For Research Use Only - Not for clinical diagnostic usage

Contact: Miriam Cordovana (Miriam.Cordovana@bruker.com)

34th ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Barcelona, Spain
27 – 30 April 2024