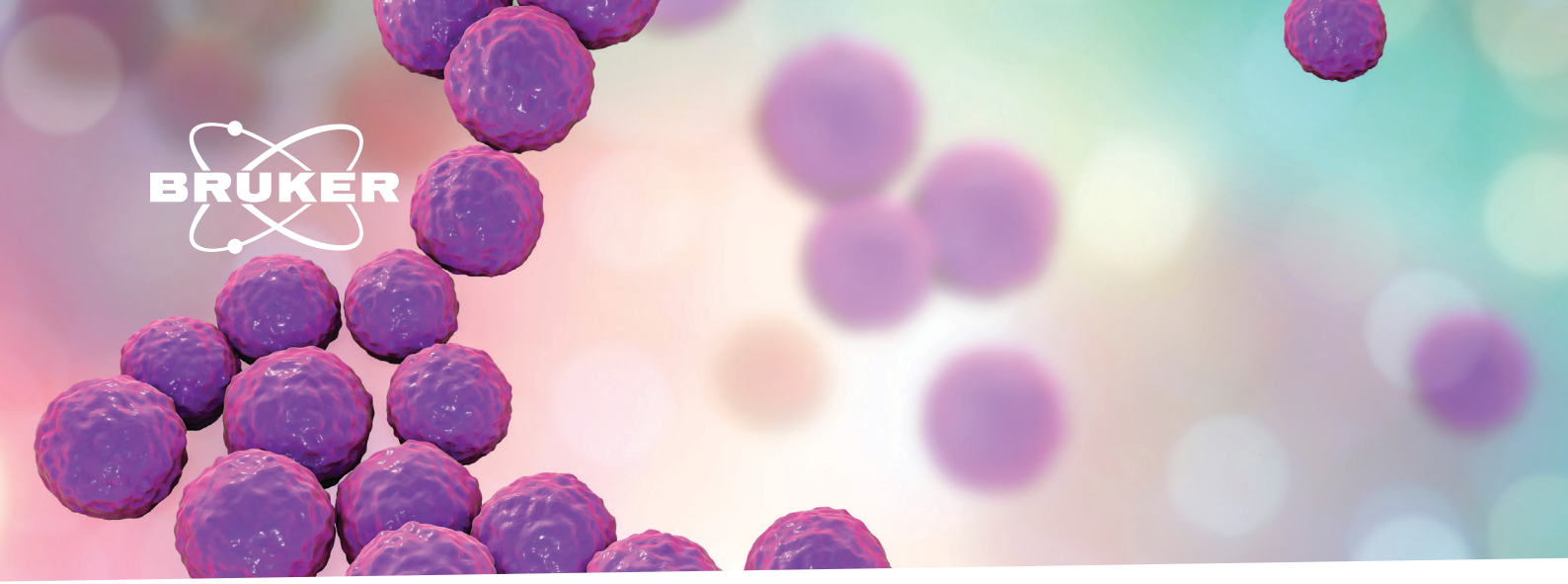


Outbreak Analysis of Vancomycin-Resistant *Enterococci* by the IR Biotyper®

Authors: Carlo Casolin, Anna Bertoncelli, Mattia Scarazzai, Alessia Nito, Davide Gibellini, Annarita Mazzariol, Department of Diagnostics and Public Health, University of Verona, Verona, Italy
Miriam Cordovana, Markus Meyer, Bruker Microbiology & Infection Diagnostics, Bremen, Germany

Introduction

Enterococci stand as prominent culprits behind a significant number of healthcare-associated infections (HAIs) on a global scale. The prevalence of multidrug resistance among these pathogens is alarming, contributing to prolonged hospital stays, escalated treatment expenses, increased risks of treatment failure, and elevated mortality rates. *Enterococcus faecium*, a resilient member of the *Enterococcus* genus, has emerged as a significant concern due to its remarkable ability to develop resistance against antibiotics. Originally regarded as a commensal organism inhabiting the gastrointestinal tract of humans and animals, *Enterococcus faecium* has progressively evolved into a formidable nosocomial pathogen, causing a wide range of infections, including urinary tract infections, bacteremia, endocarditis, and surgical site infections. Its adaptability to diverse environmental conditions, coupled with intrinsic and acquired mechanisms of resistance, poses substantial challenges for infection control and treatment within healthcare facilities.

The typing of bacterial isolates plays a critical role in identifying transmission routes and reservoirs within healthcare environments, facilitating the implementation of appropriate infection control measures. To achieve this, techniques capable of discerning the clonal relationships between bacterial isolates are essential. While various DNA-based methods, including pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and more recently, whole genome sequencing (WGS), have proven effective for this purpose, their widespread adoption is hindered by practical constraints. These methods are often prohibitively expensive, labor-intensive, time-consuming, and too slow to support real-time routine surveillance in most laboratory settings.

Here, we aimed to analyze a Vancomycin-Resistant *Enterococci* (VRE) outbreak, with a new typing method called Fourier Transform Infrared (FT-IR) spectroscopy, and compare this technology to the current established methods such as PFGE and MLST to evaluate its applicability for transmission route analysis.

FT-IR spectroscopy for microbiology

Ideally, methods for bacterial pathogen strain typing should be fast, reliable, inexpensive, and time efficient. FT-IR, which may meet the above criteria, is a vibrational spectroscopic technique, which has been widely used in chemistry to determine molecular structures.This technique analyzes the absorption of infrared light by molecules present in the sample, such as lipids, nucleic acids, carbohydrates, lipopolysaccharides, and proteins. This results in the generation of a specific FT-IR spectrum, reflecting the overall chemical composition of the specimen (see Figure 1). Applied to bacterial cells, this technique enables a fast and highly specific "fingerprint" of the bacterial surface in a non-destructive manner, and hence an evaluation of similarity between different isolates at strain level.

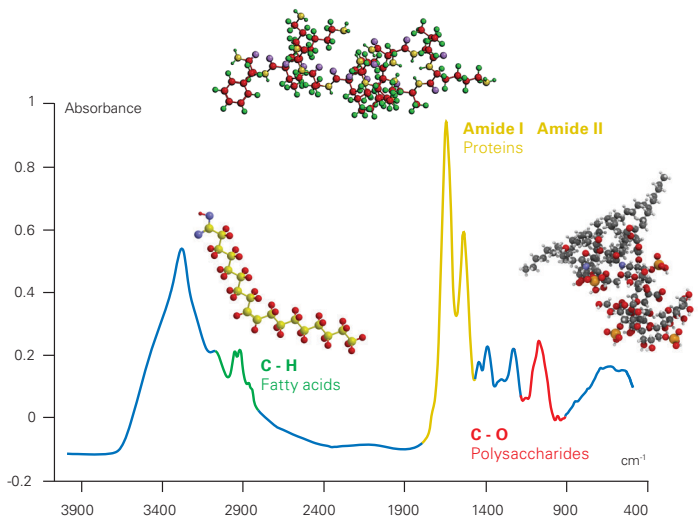


Figure 1
By default, the IR Biotyper analyzes IR spectra in the wavenumber range typical for carbohydrates (red), but other regions such as those indicating fatty acids (green) and proteins (yellow) can easily be selected for analysis in the software as well.

Materials & Methods

The present study included 28 VRE clinical strains isolated from the same clinical setting and 6 vancomycin-susceptible *Enterococci* as a control group. Vancomycin resistance was confirmed at genotypic level by PCR. Clonal relationship was determined with PFGE and for selected strains also with MLST. FT-IR analysis was performed with the IR Biotyper system (Bruker Daltonics, Germany) from strains grown on Blood Agar (37° C, 24 h, +/- 1 h). Data analysis was performed with the IR Biotyper software, applying hierarchical cluster analysis (HCA), Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). The whole workflow is visualized in Figure 2. Sample preparation has been performed according to manufacturer's documentation.

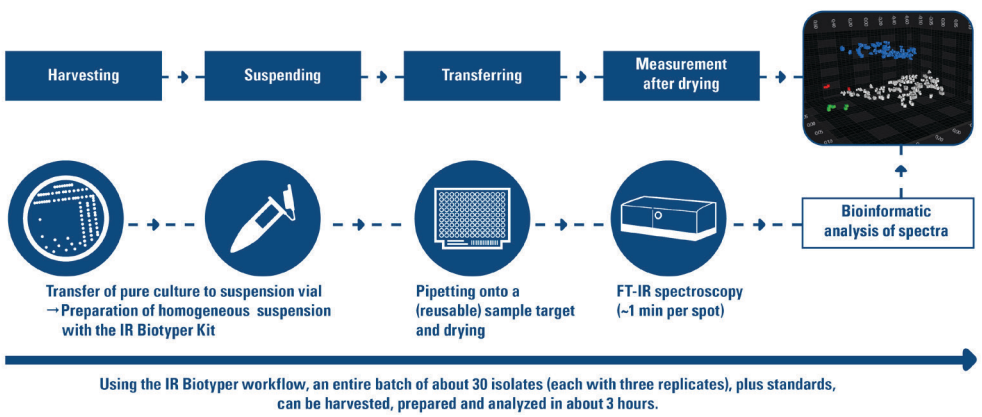
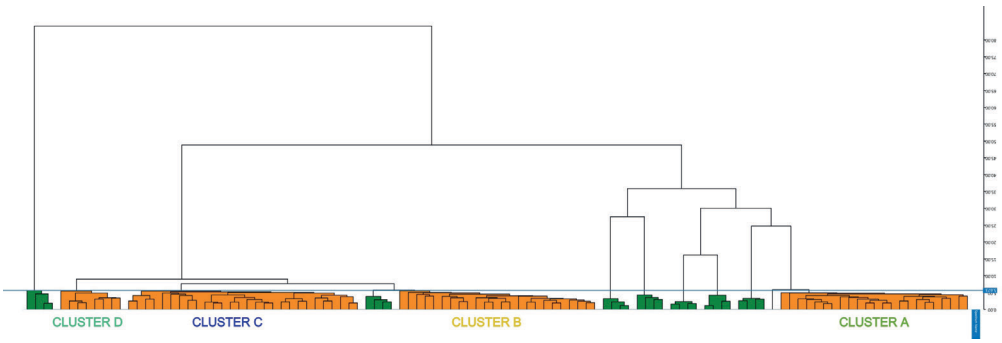


Figure 2
Schematic depiction of the IR Biotyper workflow.

Results

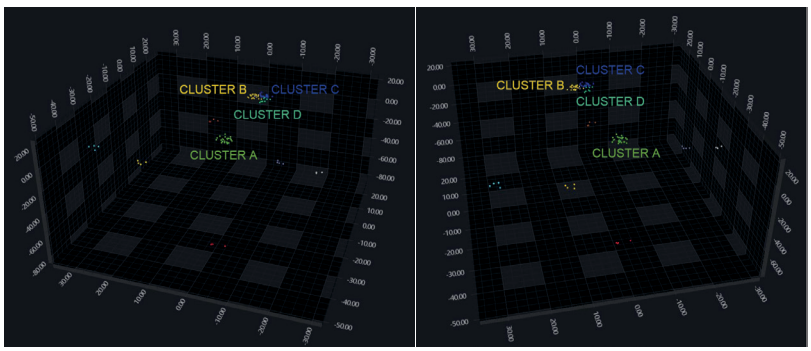
All 28 VRE strains investigated harbor the *van*A gene. The control approach with the PFGE method identified 4 clusters: namely A (6 strains), B (5 strains), C (8 strains) and D (2 strains). Seven strains out of the 28 showed no clonal relationship. FT-IR showed perfect concordance with the PFGE results by identifying the same 4 clusters and 7 strains that exhibit no clonal relationship to the others (Figure 3).

Figure 3
Clusters identified with HCA analysis, confirmed by PFGE analysis.



Additionally, FT-IR data can be visualized in a 3D plot by LDA (Figure 4). MLST could confirm the clusters observed by PFGE and FT-IR but showed less discriminatory power compared to the other methods.

Figure 4
LDA analysis visualized in a 3D scatter plot (x-axis: LD 1; y-axis: LD 2; z-axis: LD 3).



Conclusion

Fourier-transform infrared (FT-IR) spectroscopy emerges as a promising and valid alternative to traditional genetic typing methods such as MLST or PFGE. It is worth mentioning that FT-IR can help with further discimination of sub-clusters where traditional methods (such as MLST) might reach their limits of discrimination. From a practical standpoint FT-IR offers significant time savings (same day results after culturing) compared to conventional methods, reduces the necessity of a high technical expertise and is cost-effective.

This makes it a very accessible method that can be applied in routine analysis workflows. Even though the results of this study show a high concordance between FT-IR and genetic typing methods, it is important to mention that more data sets should be analyzed to underline these first comparisons. Nevertheless, FT-IR facilitates a real-time epidemiological surveillance holding considerable promise for preventing the widespread dissemination of multidrug-resistant (MDR) or highly pathogenic strains. FT-IR can become a game changer for rapid and cost-effective early outbreak detection, leading to faster responses and lowering the impact of HAI in many ways.

Not for use in clinical diagnostic procedures.
Please contact your local representative for availability in your country.

Online information
bruker.com/microbiology

Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0) 421-2205-0

info.md@bruker.com

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660