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Evaluation of a novel kNN-based approach for automated strain typing by Fourier-transform infrared spectroscopy

Miriam Cordovana[1], Norman Mauder[1], Paul G. Higgins[2,3], Olivier Join-Lambert[4], Donatella Lombardo[5], Simone Ambretti[5,6], Denise Dekker[7], Andreas E. Zautner[8,9], Alida C.M Veloo[10], Evgeny A. Idelevich[11,12], Roberta Migliavacca[13,14], Karsten Becker[11], Markus Kostrzewa[1]

1. Bruker Daltonics GmbH & Co. KG, Bremen, Germany 2. German Center for Infection Research, Partner site Bonn-Cologne, Cologne, Germany 3. Institute for Medical Microbiology, Immunology and Hygiene, University Hospital of Cologne, Cologne, Germany 4. Université de Caen, Normandie, Caen, France 5. Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy 6. Microbiology Unit, DIMEC, Alma Mater Studiorum Università di Bologna, Bologna, Italy 7. One Health Bacteriology Group, Bernhard Nocht Institute for Tropical Medicine Hambur, Hamburg, Germany 8. Institute of Medical Microbiology and Hospital Hygiene, Medical Faculty Otto-von-Guericke-University, Magdeburg, Germany 9. Institute of Laboratory Medicine and Microbiology & Vaccination Center, Klinikum Wuerzburg Mitte, Wuerzburg, Germany 10. University Medical Center Groningen, Groningen, the Netherlands11. Friedrich Loeffler Institute of Medical Microbiology, University Medicine Greifswald, Greifswald, Germany 12. Institute of Medical Microbiology, University Hospital Muenster, Muenster, Germany 13. Department of Clinical-Surgical, Diagnostic and Pediatric Scienses, Microbiology Unit, University of Pavia, Pavia, Italy 14. Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Background

Fourier-transform infrared spectroscopy (FTIR) is an innovative method for a reliable, quick, user-friendly and cost-effective microbial typing at different infra-species levels. However, the classical hierarchical clustering approach is subject to a certain degree of subjectivity and dependency on the dataset under investigation. It is challenging to define fixed, unambiguous and operator independent thresholds to assess the similarity between strains, which could allow FTIR to be implemented as a routine workflow method.

In this study, a novel approach for **FTIR automated similarity analysis**, based on the k-NN algorithm (IR Tracker™), implemented into the IR Biotyper® software (Bruker Daltonics, Germany), was investigated using **n=2109 genomically/epidemiologically well characterized** isolates belonging to the ESKAPEE group.

Material and methods

Sample set

Overall, **n=707 *K. pneumoniae***, **n=301 *P. aeruginosa***, **n=81 *A. baumannii,* n=281 *E. cloacae* complex**, **n=438 *E. coli***, **n=146** vancomycin-resistant ***E. faecium*** and **n=55 *S. aureus***, isolated in n=38 different outbreaks/surveillance contexts, in different hospital settings, in different countries, were investigated (n=1000 clonally related, and n=1109 unrelated).

Spectra acquisition

FT-IR analysis was performed by the IR Biotyper® system (IRBT - Bruker Daltonics, Germany), following the manufacturers instructions. IR spectra were acquired from cultures on Columbia/TSA sheep blood agar or Mueller-Hinton agar (different manufacturers), incubated for 18-24 h at 35 ° C, following the manufacturer's instructions. One single biological replicate was used for this study, to align with the intended use of the IR Tracker™ as real-time tool for prospective analysis.

IR Tracker™

The IR Tracker™ is a novel tool, based on the k-NN approach, which performs similarity analysis of IR spectra in a totally automated way. It uses fixed and universal thresholds (for each bacterial species), defined by the manufacturer, which define three categories of spectra similarity/probability of clonal relationship (high/moderate/low).

The IR Tracker™ delivers as a result the "best match" isolates list (the *nearest neighbors*), and a distance score value, depicted in a heat-map color-coding related to the similarity (orange: high; grey: uncertain; blue: low) – **Figure 1**.

In this study, the IR Tracker™ was applied on the different datasets separately. Results were compared with the reference method.

Results

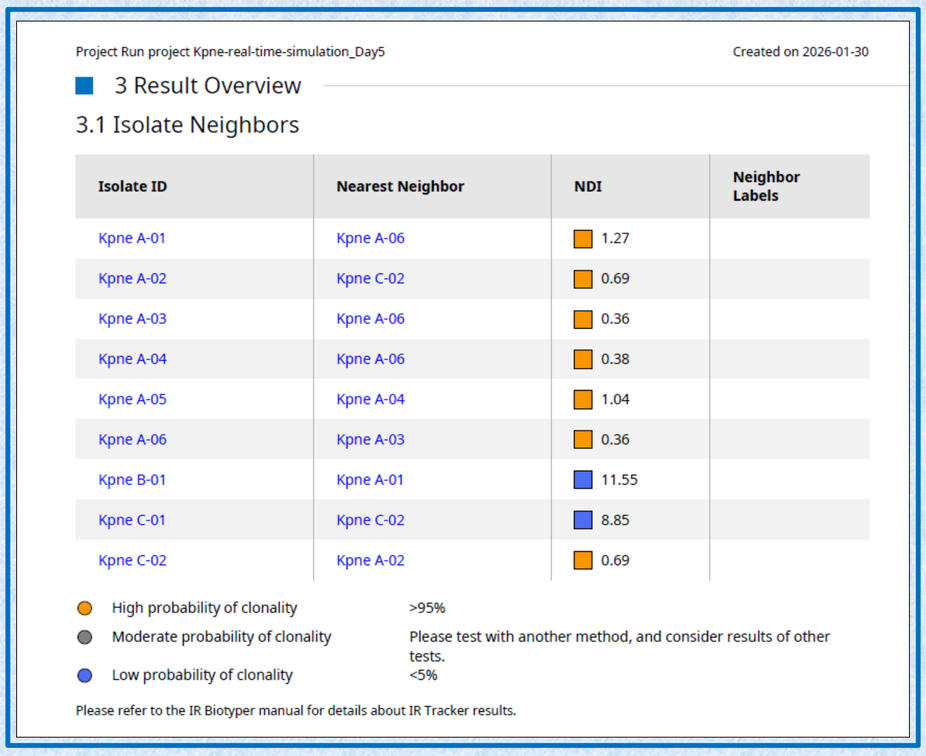
Overall, the IR Tracker™ delivered:

- ✓ **correct "best match" result, with the correct color-coding: 96.3% (2032/2109) of the cases** (from 90.3% of *S. aureus* to 98.7% of *P. aeruginosa*).
- ✓ **correct "best match" result**, but with an **"uncertain" color coding: 3.3%** (69/2109).
- ✓ **"false positive" result** (unrelated clonally related isolates typed as highly similar): **0.2%** (5/2109).
- ✓ **"false negative"** result (clonally related isolates typed as unrelated); **0.2%** (3/2109).

Detailed results are shown in **Table 1.**

ESKAPEE Species (tot)	Result IR Tracker correct (n,%)	Result IR Tracker uncertain (clonal isolates) (n,%)	Result IR Tracker uncertain (unrelated isolates) (n,%)	Result IR Tracker false positive (n,%)	Result IR Tracker false negative (n,%)
***K. pneumoniae* (707)**	685 (96.9)	7 (0.9)	13 (1.9)	0	2 (0.3)
***P. aeruginosa* (301)**	297 (98.7)	0	3 (1.0)	0	1 (0.3)
***E. cloacae* complex (281)**	270 (96.1)	4 (1.4)	7 (2.5)	0	0
***E. coli* (438)**	428 (97.7)	5 (1.2)	0	5 (1.1)	0
***S. aureus* (155)**	140 (90.3)	5 (3.2)	10 (6.5)	0	0
***E. faecium* (146)**	135 (92.5)	6 (4.1)	5 (3.4)	0	0
***A. baumannii* (81)**	77 (95.1)	0	4 (4.9)	0	0
Total (2109)	2032 (96.3)	27 (1.3)	42 (2.0)	5 (0.2)	3 (0.1)

Table 1. Details about the performance of the IR Tracker™ for the different bacterial species.



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

CONCLUSION

In this study, the k-NN-based automated FTIR approach showed very promising results. An extensive prospective validation in routine settings would be necessary, to assess the real-time performance, and could allow the refining of the thresholds, to optimize accuracy, sensitivity and specificity, if needed.

Not for use in clinical diagnostic procedures.

Contact: miriam.cordovana@bruker.com

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