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CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

Fourier transform infrared spectroscopy as a new tool for surveillance in local stewardship antimicrobial program: a retrospective study in a nosocomial *Acinetobacter baumannii* outbreak

Inmaculada Guerrero-Lozano[1] · Fátima Galán-Sánchez[1] · Manuel Rodríguez-Iglesias[1]

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Abstract

The aim of our study is to determine the discriminatory power of the Fourier transform infrared spectroscopy (FTIR), using multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) as molecular typing references. The study included seventeen isolates (OXA-23- and OXA-58-producing *Acinetobacter baumannii*) previously recovered from clinical specimens during the period May 2010–April 2011. Molecular typing was performed by PFGE and MLST. The specimens were analyzed in quadruplicate using the IR Biotyper (Bruker GmbH, Bremen, Germany). For each isolate, the average of the spectra was used for the analysis of the data. Comparing FTIR data with MLST, the results obtained by IR Biotyper are very consistent with those from MLST, since the software was able to differentiate the three ST assigned to the strains. Comparing FTIR data with PFGE, most results could be confirmed, as IR Biotyper clearly differentiated ST-80 SLV OXA-58-producing *A. baumannii* (pulsotype 3) from the rest of strains of OXA-58-producing *A. baumannii* (pulsotypes 1 and 2). All the OXA-23-producing *A. baumannii* isolates (pulsotype 4) grouped together by FTIR. FTIR proved to be an effective tool to investigate local epidemiology, and can achieve the same typeability and discriminatory power as genome-based methods.

Keywords *Acinetobacter baumannii* · Fourier transform infrared spectroscopy · MLST · PFGE · Surveillance · Nosocomial

Introduction

Acinetobacter baumannii behaves like an opportunistic pathogen with few virulence factors, which usually produces colonization more often than infection. However, the appearance of fulminant community pneumonias suggests that this microorganism can be highly pathogenic.

The most important role of these bacteria is as cause of nosocomial pneumonia [1]. *A. baumannii* is described mainly in the form of outbreak, and usually acquires mechanisms of resistance to antibiotics [2]. The most important feature of *A. baumannii* at the hospital level is the appearance of multidrug-resistant endemic and epidemic strains, usually introduced into the hospital through a colonized patient [3]. The appearance of multi-resistant and even pan-resistant strains occurs specially in intensive care units. It is also possible that they circulate in the community in small proportions and that they are selected in hospitals as a result of the selective pressure of antimicrobial use [4].

Infection control aims to limit the spread of multidrug-resistant bacterial strains. Such outbreaks could be controlled with enhanced hygiene control measures that are quickly implemented after early detection of cross-transmissions [2].

Recently, a new automated typing system based on Fourier transform infrared spectroscopy was commercialized, IR Biotyper (Bruker GmbH, Bremen, Germany). Fourier transform infrared spectroscopy (FTIR) generates spectra based on the absorption of infrared light by the different chemical components of the bacterial cell. Therefore, each microorganism has a highly specific infrared absorption signature correlated with genetic information that allows the identification of microorganisms at the subspecies level [5].

The aim of our study is to determine the discriminatory power of the FTIR, using multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) as molecular typing references, by analyzing an outbreak caused by OXA-23-producing *Acinetobacter baumannii* that

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✉ Fátima Galán-Sánchez
fatima.galan@uca.es

[1] Clinical Microbiology Laboratory, Hospital Universitario Puerta del Mar, Avda. Ana de Viya 21, 11009 Cádiz, Spain

occurred between 2010 and 2011 in Hospital Universitario Puerta del Mar, Cádiz, Spain.

Materials and methods

Bacterial strains

The study included seventeen isolates previously recovered from clinical specimens during a nosocomial outbreak that occurred in the period May 2010–April 2011, and stored at − 80 °C. All isolates were preliminary identified as *A. baumannii* by using the Wider I system (Soria Melguizo Madrid, Spain) and by matrix-assisted laser desorption/ionization time of flight mass spectrometry MALDI-TOF (Bruker Daltonics, GmbH, Bremen Germany). Partial DNA sequencing of *rpoB* confirmed the identification as *A. baumannii*. Multiplex PCR assays were used to detect the following beta-lactamase genes: $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24/40-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{KPC\text{-like}}$, $bla_{\text{GES-like}}$, $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$, and $bla_{\text{NDM-like}}$ [6].

Description of the outbreak: molecular genotyping

Before the introduction of an OXA-23-producing *A. baumannii* in May 2010, there was an endemic setting of nosocomial transmission of OXA-58-producing *A. baumannii* in the intensive care unit of the Hospital Universitario Puerta del Mar. During the period from May 2010 to April 2011, both clones (OXA-23- and OXA-58-producing) of *A. baumannii* coexisted. Molecular typing was performed by PFGE, and pulsotypes were determined according to international recommendations [6]. MLST was performed on the different pulsotypes according to Bartual [7].

All OXA-58-producing *A. baumannii* isolates ($n = 8$) were classified as ST-2 except one that showed a single-locus variant of ST-80 (ST-80 SLV) (data not published). OXA-23-producing *A. baumannii* ($n = 10$) were assigned to ST-1 [6].

A single pulsotype was obtained for OXA-23-producing *A. baumannii* and three pulsotypes for OXA-58-producing *A. baumannii* (data not published).

Characteristics of the strains included in the study are presented in Table 1.

Sample preparation for FTIR spectroscopy

Isolates were grown at 37 °C on Mueller–Hinton II agar (Becton Dickinson, Heidelberg, Germany) for 24 h. Samples were prepared according to the IR Biotyper manufacturer's instructions. An inoculation loopful (approximately 1 µl) of bacteria was suspended in 50 µl of 70% ethanol solution. Homogenization was obtained by vortexing the sample with metal beads (Bruker, Bremen, Germany) in 1.5-ml microcentrifuge tubes. An additional volume (50 µl) of deionized water was added to obtain a solution with a final volume of 100 µl. An aliquot (15 µl) of the suspension was placed on a sample plate device (Bruker, Bremen, Germany) and dried

Table 1 Characteristics of the strains included in this study

Isolate	Source	Isolation date	MLST	PLS	Resistance mechanism
Abau57	Blood	17/02/10	ST-2	2	OXA-58
Abau86	Blood	23/03/10	ST-2	2	OXA-58
Abau29	Tracheal aspirate	26/04/10	ST-1	4	OXA-23
Abau98	Central catheter	26/04/10	ST-2	2	OXA-58
Abau6	Tracheal aspirate	04/05/10	ST-1	4	OXA-23
Abau180	Blood	08/08/10	ST-1	4	OXA-23
Abau132	Central catheter	28/09/10	ST-1	4	OXA-23
Abau133	Tracheal aspirate	28/09/10	ST-1	4	OXA-23
Abau135	Tracheal aspirate	04/10/10	ST-1	4	OXA-23
Abau140	Tracheal aspirate	11/10/10	ST-1	4	OXA-23
Abau151	Blood	21/10/10	ST-2	1	OXA-58
Abau153	Bronchoalveolar lavage	26/10/10	ST-2	1	OXA-58
Abau146	Tracheal aspirate	11/10/10	ST-1	4	OXA-23
Abau168	Urine	29/10/10	ST-80 SLV	3	OXA-58
Abau181	Urine	20/12/10	ST-1	4	OXA-23
Abau187	Tracheal aspirate	12/01/11	ST-2	1	OXA-58
Abau211	Tracheal aspirate	28/03/11	ST-1	4	OXA-23
Abau217	Sputum	19/04/11	ST-2	1	OXA-58

PLS, pulsotype

at 35 °C for 30 min. The pellets were visualized to check for the absence of air bubbles before analysis.

The quality control Infrared Test Standards (IRTS 1 and IRTS 2) of the IR Biotyper Kit were resuspended in 90 µl of deionized water and 90 µl of absolute ethanol which were added and mixed. Twelve microliters of suspension was spotted in duplicate onto the IR Biotyper target, and let dry as described for the samples.

Cluster analysis

Principal component analysis (PCA) was performed applying the IR Biotyper Software version 3.0. The number of principal components used was 3 (95% variance). The specimens were analyzed in quadruplicate using the IR Biotyper with the default analysis settings, and only the region that corresponds to the region of carbohydrates was used. Spectra acquisition, visualization, and processing were performed in transmission mode in the spectral range 4000–600 cm^{-1} (mid-IR). The second derivative of the spectra was calculated using the Savitzky–Golay algorithm over nine data points. Spectra were then cut to 1300–800 cm^{-1} and vector-normalized to amplify differences between isolates, and correct variations related to spectra acquisition. The measurement for background and sample measurement was transmission; then, an absorption spectrum was calculated and in IR Biotyper version 3.0 was post-processed by the "make compatible scalar" function of OPUS 8.2.28 software (Bruker GmbH, Bremen, Germany), which interpolates exactly one data point per wavenumber. The resolution of the measurement, when using the default procedures, was 6 cm^{-1}. For each isolate, the average of the spectra was used for the analysis of the data.

Results

Cluster analysis by FTIR

FTIR showed that ST-2 OXA-58-producing *A. baumannii* were grouped in a large cluster, and OXA-23-producing *A. baumannii* in a second large cluster, with a cutoff of 0.341. One isolate appeared different in FTIR, ST-80 SLV OXA-58-producing *A. baumannii* (cutoff of 0.75) (Fig. 1).

Comparing FTIR data with MLST, the results obtained by IR Biotyper are very consistent with those from MLST, since the software was able to differentiate the three ST assigned to the strains (Fig. 1).

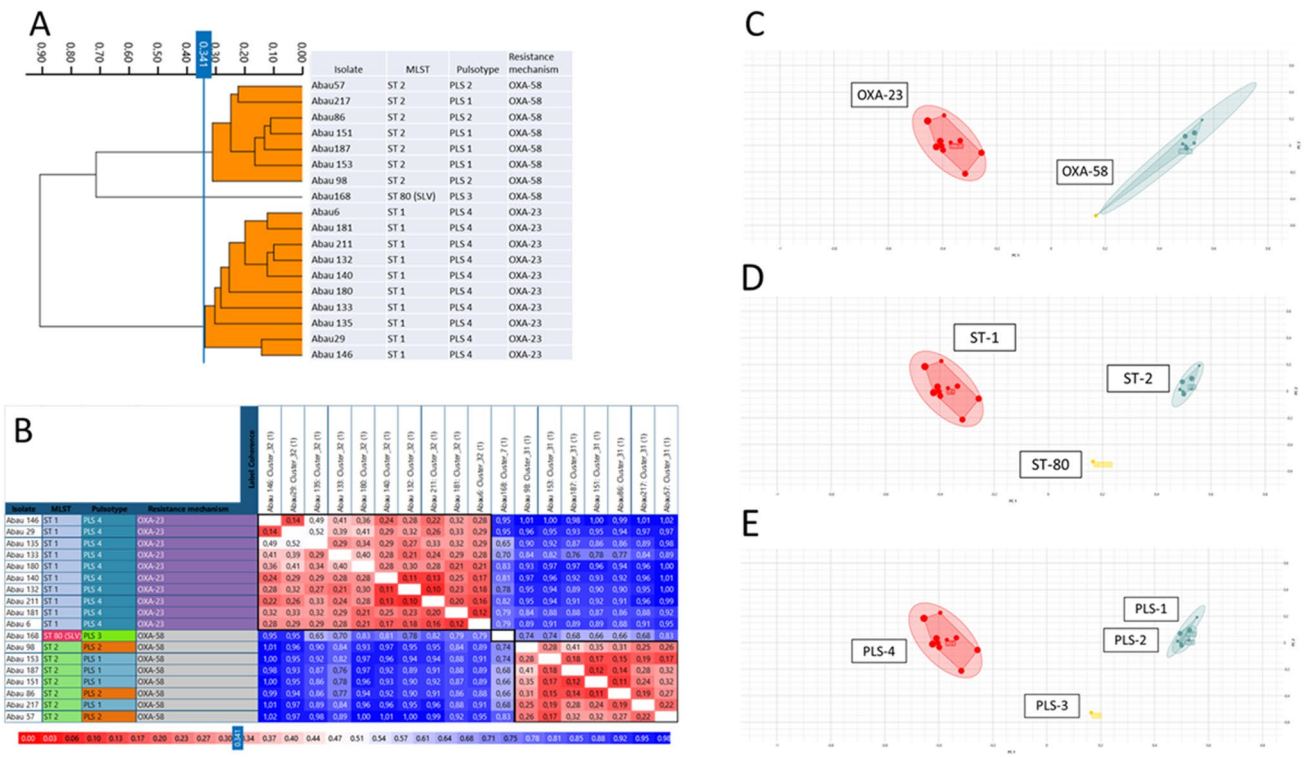

Fig. 1 **a** Dendrogram of all *A. baumannii* isolates (cutoff 0.341). **b**, **c**, **d** PCA plot showing the clustering. Analysis by PCA in two dimensions successfully grouped according to the mechanism of resistance (**b**), ST (**c**), and pulsotype (**d**). Number of principal components used: 3 (95% variance). **e** Distance matrix by Euclidean average linkage (cutoff 0.341)

Comparing FTIR data with PFGE, most results could be confirmed. IR Biotyper clearly differentiated ST-80 SLV OXA-58-producing *A. baumannii* (pulsotype 3) from the rest of strains of OXA-58-producing *A. baumannii* (pulsotypes 1 and 2). However, in this last group, FTIR found a high similarity (cutoff = 0.22) between isolates Abau 57 (pulsotype 2) and Abau 217 (pulsotype 1) and also (cutoff = 0.11) between isolates Abau 86 (pulsotype 2) and Abau 151 (pulsotype 1). All the OXA-23-producing *A. baumannii* isolates (pulsotype 4) grouped together by FTIR.

Discussion

In clinical practice, it is important which method to use for the interpretation of the results in the context of the occurrence of an outbreak, and thus be able to address it as soon as possible. The method used must be reproducible, with high discrimination, fast, and not too expensive.

IR spectroscopy is particularly attractive for the identification and classification of microbes as it is capable of monitoring strain typing. First studies using FTIR spectroscopy focused on foodborne pathogens [8]. Later, the use expanded to other pathogens with clinical or epidemiological importance. FTIR spectroscopy is a fast and cost-effective method to predict the capsular serotype of pneumococci, and probably for typing of other encapsulated species [9]. There are a few studies describing the use of Bruker IR Biotyper for outbreak investigation [9, 10] but, at our knowledge, this is the first one that focused on an *A. baumannii* outbreak, comparing FTIR and PFGE results.

In our study, the IR Biotyper proved to be an effective tool to investigate local epidemiology, assessing the clonal relationships between different *A. baumannii* isolates and providing results consistent with the reference DNA-based methods. It successfully identified an OXA-23-producing *A. baumannii* outbreak cluster, according to the results by PFGE. The clusters by spectroscopy perfectly matched with sequencing results (MLST).

These results are consistent with those reported by other authors for other multidrug-resistant microorganisms. Martak et al. [2] found that FTIR spectroscopy accurately clustered *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* isolates belonging to the same ST, and that the IR Biotyper can quickly detect the spread of clones of these microorganisms. However, for *A. baumannii*, they found that the optimal cutoff ranged from 0.495 to 0.530 with a lower global concordance with MLST, for which three isolates were misclassified, including one ST2 isolate that did not cluster with the other ST2 isolates. In our study, seven ST2 isolates were included, and all of them clustered together. To establish a reliable cutoff in *A. baumannii*, further studies with FTIR are needed. Our cutoff value and those reported by Martak et al. are higher than those found in *Enterobacteriaceae* [2, 10, 11].

Rakovitsky et al. [10] recommend that any laboratory planning to use FTIR biotyping for a given organism should initially validate the technical and analytical setup and results using a molecular or genomic approach. They propose complete concordance with whole genome sequencing (WGS) using the cutoff automatically generated by increasing the number of technical replicates to 12. As a limitation in our study, specimens were analyzed in quadruplicate, following manufacturer instructions but, since WGS was not performed, we cannot confirm complete concordance with this technique.

The main limitation of our study is that it was a retrospective analysis of isolates that were already known to be part of an outbreak. Lombardo et al. [12] applied the FTIRS-based IR Biotyper system for a real-time investigation of a carbapenem-resistant *A. baumannii* outbreak. IR Biotyper enabled a correct and accurate clustering of all the isolates in comparison with PFGE. The measurement of the samples on different days proved to have no influence in the IR Biotyper analysis. So, FTIR might be a screening tool for early assessment of a possible outbreak and could be integrated into routine surveillance.

We conclude that the FTIR Biotyper can achieve the same typeability and discriminatory power as genome-based methods. The results obtained by infrared spectroscopy (FTIR Biotyper) may offer a different way to identify a nosocomial outbreak very quickly, compared to other molecular techniques normally used. Fast, easy-to-apply, and economical, IR Biotyper can be a powerful tool for subtyping of multidrug-resistant microorganisms in local antimicrobial stewardship program.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42770-022-00774-6.

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Author contribution I.G.L., F.G.S., and M.R.I. planned the study. I.G.L. performed the FTIR analysis. I.G.L., F.G.S., and M.R.I. analyzed and evaluated the results. I.G.L. and F.G.S. wrote the manuscript. All authors revised the final version of the manuscript.

Data availability Raw data were generated at Hospital Universitario Puerta del Mar. Derived data supporting the findings of this study are available from the corresponding author on request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Publication has been approved by all co-authors as well as by the responsible authorities at the institute where the work has been carried out.

Competing interests The authors declare no competing interests.

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