Retrospective analysis of a healthcare-associated *Elizabethkingia anophelis* outbreak using IR Biotyper and concordance with WGS

Rocca María Florencia[1], Cipolla Lucía[1], Gianecini Ariel[1], Nievas Jimena[2], Prieto Mónica[1]
[1] Servicio de Bacteriología Especial, INEI-ANLIS Malbrán, Buenos Aires, Argentina
[2] Instituto Quirúrgico del Callao, Buenos Aires, Argentina

Elizabethkingia anophelis is an environmentally persistent, multidrug-resistant non-fermenting bacillus associated with healthcare outbreaks, particularly in neonatal and intensive care units. Strain-level typing remains challenging due to taxonomic complexity.
In 2020, a neonatal unit outbreak occurred in Buenos Aires, with clonal relatedness confirmed by whole-genome sequencing (WGS).
Following installation of the IR Biotyper (IRBT) at the National Reference Laboratory, a retrospective evaluation was performed.

OBJECTIVES

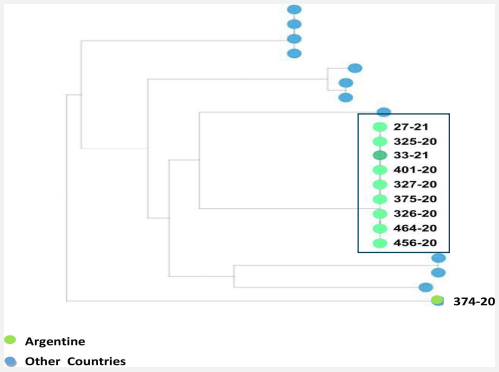
- Evaluate IRBT performance for identifying outbreak-related isolates.
- Assess intra inter-day reproducibility.
- Determine concordance with WGS.

METHODS

- Isolates: 8 clinical samples (rectal swabs, blood, respiratory specimens) and one environmental isolate (33-21, faucet aerator).
- WGS: MiSeq instrument using the Nextera XT DNA library preparation kit (Illumina)
- IRBT analysis: standardized preparation on silica plates.
- Replicates: technical and biological replicates across three independent days.
- Clustering: Euclidean distance using UPGMA and Ward algorithms.
- Analysis: label coherence, intra-isolate clustering stability and cut-off optimization.
- Multivariate analysis: Principal Components Analysis (PCA) and Linear Discrimination Analysis (LDA).

RESULTS

- Single-day analysis showed high variability and suboptimal clustering.
- Three-day replicates significantly improved robustness: >95% intra-isolate coherence achieved.
- Full concordance with WGS-defined clonality.
- All outbreak isolates, including environmental source, clustered together. **Fig 1 and Fig. 2**
- Scatter Plot PCA showed natural outbreak cluster separation **(Fig. 3)** and LDA maximized discrimination with 96% variance explained. **Fig. 4.**
- Processing time: less than 60 minutes per run, with low costs.



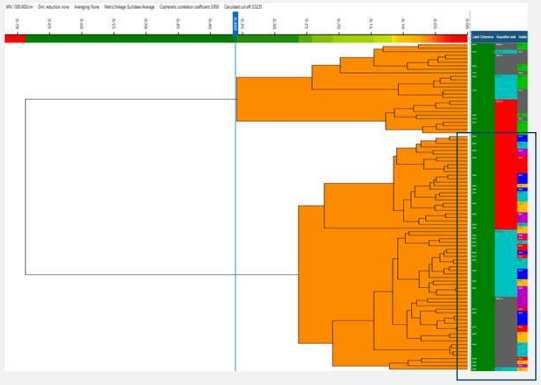


Figure 1. Phylogenetic tree showing the relationship of *E. anophelis* isolates (box), constructed from the analysis of SNPs in the central genome of isolates from Argentina and other countries.
The unrelated isolates correspond to *E. anophelis* recovered from clinical samples in Argentina in recent years.

Figure 2. FT-IR dendrogram with hierarchical clustering of *E. anophelis* isolates based on spectral similarity using Euclidean distance and average linkage. Outbreak-related isolates clustered consistently (box).

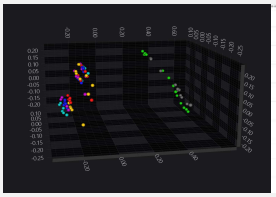


Figure 3. Scatter plot 3D PCA (3PCs-96,8%).

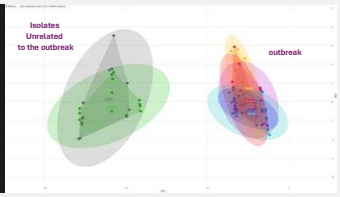


Figure 4. Supervised multivariate method grouping LDA

CONCLUSIONS

IR Biotyper reliably reconstructed outbreak-related clustering of *Elizabethkingia anophelis* and showed strong concordance with WGS.
Multi-day analysis substantially improved analytical performance.
IRBT is a fast, reproducible and accessible tool for outbreak investigation and epidemiological surveillance in reference laboratories.

ESCMID Global
Congress of the European Society of Clinical Microbiology and Infectious Diseases