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Concise Communication

Analysis of four carbapenem-resistant *Acinetobacter baumannii* outbreaks using Fourier-transform infrared spectroscopy

Hadas Kon BEng[1], Elizabeth Temkin DrPH[1], Polet Elmalih BSc[1], Alona Keren-Paz PhD[1], Debby Ben-David MD[2], Ronza Najjar-Debbiny MD[3], Tamar Gottesman MD[4] and Yehuda Carmeli MD[5]

[1]National Institute for Antibiotic Resistance and Infection Control, Israel Ministry of Health, Tel Aviv, Israel, [2]Wolfson Medical Center, Holon, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [3]Carmel Medical Center, and Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, [4]Rabin Medical Center, Hasharon Hospital, Petach Tikva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel and [5]National Institute for Antibiotic Resistance and Infection Control, Israel Ministry of Health, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Abstract

We used Fourier-transform infrared (FTIR) spectroscopy to analyze 4 carbapenem-resistant *Acinetobacter baumannii* outbreaks. FTIR distinguished between isolates from different hospitals and uncovered the relatedness between isolates from acute-care hospitals and a post–acute-care hospital. Using higher cutoffs reveals more distant relationships and lower cutoffs support analyses of recent events.

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Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a nosocomial pathogen ranked as an "urgent threat" by the US Centers for Disease Control and Prevention.[1] Typing of CRAB isolates is important for understanding the local epidemiology of CRAB and for controlling outbreaks. Different methods for typing *A. baumannii* have been developed.[2] Methods based on DNA banding patterns, such as pulsed-field gel electrophoresis (PFGE) and rep-PCR, are usually the methods of choice for outbreak investigations due to their high discriminatory power, but they are slow and labor intensive. Multilocus sequence typing (MLST) is considered the gold standard for population structure (ie, distant evolutionary origin) studies, but its lower discriminatory power reduces its usefulness for local and short-term outbreak investigations. Whole-genome sequencing (WGS) offers the highest resolution but is limited by expense, low speed, and complex methodology.

Fourier-transform infrared (FTIR) spectroscopy is a new typing method with discriminatory power similar to that of PFGE, rep-PCR, and MLST and approaching that of WGS,[3] but it is faster, less expensive, and requires less expertise.[4] FTIR quantifies the absorption of infrared light by molecules in the bacterial cell, generating a spectrum that serves as an isolate's molecular fingerprint. Accompanying software proposes a cutoff value that determines up to which distance spectra can be grouped into a cluster. Unlike rep-PCR and PFGE, FTIR can be used prospectively to build a repository, with samples continually collected during an outbreak and compared to previous isolates.[5,6] Experience with FTIR in outbreak investigations is limited. In this retrospective study, we describe the use of FTIR as a tool for analyzing CRAB outbreaks.

Author for correspondence: Yehuda Carmeli, E-mail: yehudac@tlvmc.gov.il

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Methods

In total, 110 isolates were collected during 4 CRAB outbreaks in Israel. One outbreak took place in a post–acute-care hospital (PACH), hospital A, in January 2019, with 26 patient samples. The next outbreak occurred in an acute-care hospital, hospital B, in April–May 2020, with 6 patient samples and 12 environmental samples. An outbreak occurred in acute-care hospital C in June–July 2020, with 12 environmental samples. And the final outbreak occurred in acute-care hospital D in February–March 2021, with 17 patient samples and 37 environmental samples. Samples or isolates were shipped to the reference laboratory, where they were identified to the species level using VITEK MS (bioMérieux SA, Marcy l'Etoile, France). Antibiotic susceptibility was determined using VITEK 2 (bioMérieux).

FTIR was performed as previously described.[4] Isolates were grown at 35±2°C on blood agar plates (HyLabs, Rehovot, Israel) for 24 hours. Samples were prepared according to the IR Biotyper (Bruker, Leipzig, Germany) manufacturer's instructions. Four replicates per sample were analyzed using the Biotyper default settings. Spectra were analyzed using OPUS version 7.5 software (Bruker). Hierarchical cluster analysis (displayed as a dendrogram) was generated by OPUS 7.5 software with the Pearson correlation coefficient option. We chose cutoff values that defined a cluster by inspection of the resulting dendrogram. We compared cutoffs within the range recommended by the manufacturer for *A. baumannii*: 0.25–0.4.

Results

The dendrogram is displayed in the Figure 1. A cutoff of 0.3 defined 9 clusters of 2–45 isolates, leaving 4 singletons. Seven clusters were single-institution clusters. The other 2 clusters each represented 2 institutions: cluster II, composed of isolates from hospital B and PACH A, which often share patients; and cluster IV, composed

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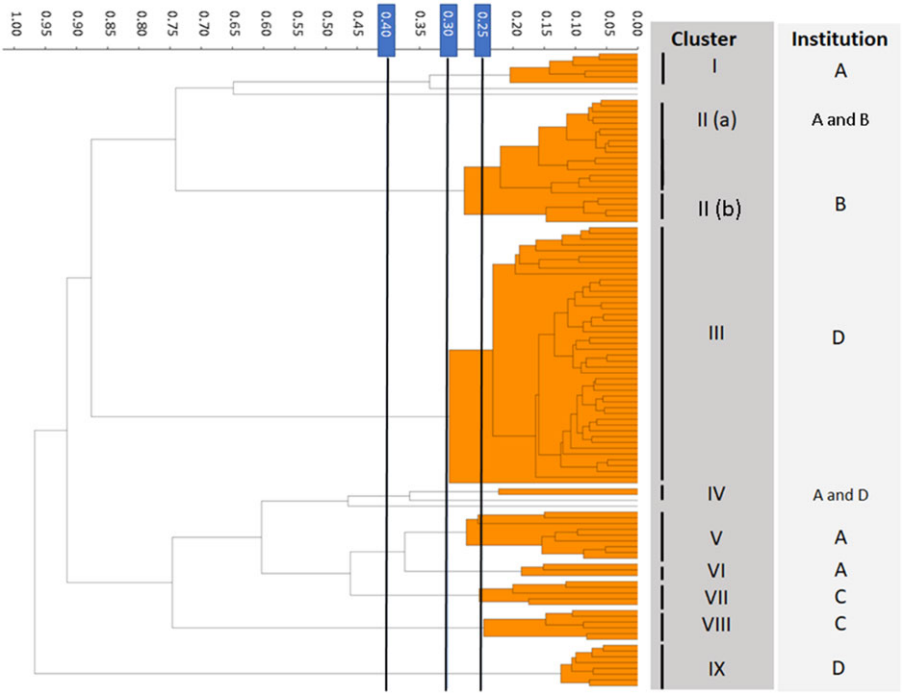

Fig. 1. Dendrogram of carbapenem-resistant *A. baumannii* isolates from outbreaks in 1 post–acute-care hospital (hospital A) and 3 acute-care hospitals (hospitals B–D).

of 1 isolate each from hospital D and PACH A, which rarely share patients.

Raising the cutoff to 0.4 resulted in 8 clusters and 2 singletons. One isolate that was a singleton using the 0.3 cutoff was united with cluster I, its own institution's cluster, and another singleton was united with cluster IV, a small mixed cluster. Clusters V and VI, both composed of isolates from PACH A, merged into 1 cluster.

Lowering the cutoff to 0.25 yielded 12 clusters and 6 singletons. Cluster II was split into 2 distinct clusters, one with isolates from PACH A and hospital B and another with isolates from hospital B only. Clusters V and VII, each representing 1 institution, were divided into 2 clusters each. One isolate previously grouped with isolates from its own institution in cluster V became a singleton.

Thus, for this 4-institution analysis, the 0.4 cutoff best described isolates' institutional origin. Isolates from hospital B belonged to a single cluster, and both hospitals C and D were represented by 2 clusters. In contrast, isolates from PACH A were scattered over 5 clusters, some unique to PACH A and others shared with acute-care facilities. Lowering the cutoff to 0.25 revealed within-institution patterns. In hospital B, all isolates belonged to cluster II, and the 0.25 cutoff was able to discern 2 subclusters based on sampling date. Isolates in cluster II(a) were collected on 1 day in April 2020, and those in cluster II(b) were collected 1 month later.

Two other within-institution patterns were detected at all 3 cutoffs. First, in the 2 hospitals that had both environmental and patient samples, both isolate types belonged to the same clusters. Second, in hospital D, all isolates from cluster IX came from 1 ward, whereas cluster III comprised isolates from 7 wards.

Discussion

FTIR biotyping distinguished between isolates from different hospitals and revealed the complex epidemiology of CRAB. In only 1 hospital was the outbreak caused by a single cluster; using the 0.25 cutoff, the Biotyper distinguished between 2 sampling dates 1 month apart. In the other 2 acute-care hospitals, 2 unrelated clusters were observed. In hospital D, one of the clusters was spatially defined (ie, a single-ward outbreak) and the other involved multiple wards. For the latter, traditional epidemiological methods combined with WGS would be needed to determine whether this diffuse spread was mono- or polyclonal and could be explained by transfer of patients or contaminated equipment or by less obvious "transmission opportunities" between patients in different wards (eg, shared consultants).[7] Coexistence of multiple multidrug-resistant *A. baumannii* clones within an institution is common, resulting from both recombination and diversification of circulating clones and importation of new ones.[7,8]

Unlike the acute-care hospitals, the pattern in PACH A was complex. Multiple clusters were observed, likely reflecting importation of unrelated clones from various hospitals that transfer patients there, augmented by nosocomial spread within the PACH. Moreover, 2 isolates from PACH A belonged to the same clusters as isolates from hospitals B and D. Previous studies have noted the overlap of *A. baumannii* clones in different hospitals in the same country, indicating a shared ancestor or clonal dissemination via patient transfers.[9] The importance of PACHs and long-term care facilities in CRAB dissemination has been described.[10] These findings suggest that regional interventions to identify, track, and coordinate transfers of carriers may be more effective than institution-level interventions to control CRAB.

This study had several limitations. We lacked patient data to support the biotyping results, and we did not validate the FTIR results by comparing them to WGS, as done in some studies.[3,4,6]

Our experience using FTIR confirmed the advantages of this technology, including ease, speed, discriminatory power, and high throughput capabilities and that FTIR can be used to build a repository over time. This repository allowed us to uncover the relatedness between isolates from acute-care hospitals and a PACH. We have shown that different cutoffs for cluster definition

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serve different purposes, with higher cutoffs (ie, more specific) revealing distant relationships and lower cutoffs (ie, more sensitive) enhancing resolution, and supporting the analysis of recent events. FTIR biotyping is a useful tool for studying *A. baumannii* outbreaks at the local and regional levels.

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