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Typing of Nosocomial Outbreaks of *Acinetobacter baumannii* by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been evaluated for the identification of multidrug-resistant *Acinetobacter baumannii* nosocomial outbreaks in comparison with the repetitive sequence-based PCR DiversiLab system. The results suggest that MALDI-TOF MS can be used for real-time detection of *Acinetobacter* outbreaks before results from DNA-based systems are available.

Acinetobacter baumannii has become a major nosocomial threat, causing infections with high rates of morbidity and mortality (1–4).

Acinetobacter baumannii hospital outbreaks have been assessed with various DNA typing methods (5–8). Very few typing methods assess outbreaks in real time. Recently, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been developed for the rapid identification of pathogens (9). There are a growing number of reports showing the utility of MALDI-TOF MS to type different bacterial species for easy and early detection of nosocomial outbreaks (10–16).

The aim of this study was to assess the potential utility of MALDI-TOF MS to detect the nosocomial spread of *A. baumannii* isolates. For this purpose, 35 multidrug-resistant strains of *A. baumannii*, isolated from colonized or infected patients hospitalized in a general hospital in Italy in 2010, were studied using the repetitive sequence-based PCR (rep-PCR) DiversiLab system (DL; bioMérieux, Marcy l'Etoile, France) and MALDI-TOF MS system (Bruker Daltonics, Bremen, Germany), and the results were compared. After storage at −70°C, the isolates were thawed out and cultured simultaneously on MacConkey agar.

DiversiLab rep-PCR (bioMérieux) was performed according to the manufacturer's instructions (5, 17). Briefly, DNA was extracted from a bacterial culture, amplified using rep-PCR, loaded in LabChip, and run using an Agilent 2100 Bioanalyzer (Agilent Technologies). The results were analyzed by DiversiLab with the Pearson correlation (PC) coefficient to emphasize peak intensities more than peak presence or absence. The strains were considered indistinguishable, similar, or different if the similarity was ≥97.5% without differences in the fingerprinting pattern, >95% and <97.5%, or ≤95%, respectively.

For MALDI-TOF MS, bacteria were cultured for 24 h at 37°C on MacConkey agar and extracts were prepared as described previously (18, 19). For isolate identification, the row spectra were compared with those in the Biotyper database and a log(score) of ≥2.3 was considered to represent a secure species identification. Spectra were acquired with a microflex LT mass spectrometer (Bruker Daltonics) and recorded in the positive linear mode at a laser frequency of 20 Hz, ion source 1 voltage of 20 kV, ion source 2 voltage of 8.5 kV, and mass range from 2,000 to 20,000 kDa, as described elsewhere (18). Reference spectra of newly created main spectra were added to the original Biotyper database. To evaluate the spectrum variation within each strain and to estimate the mass spectral variance of biological replicates, a principal component analysis (PCA) was performed; two biological replicates from six randomly selected *Acinetobacter* strains were tested, as described above, and spectra for each selected strain were acquired in the same experiment on the mass spectrometer. Technical mass-spectral variance was also evaluated, analyzing five technical replicates from each *Acinetobacter* independent culture on the same MALDI target plate. For main spectral projection construction, a total of 5,000 shots were acquired from 10 technical replicates, each spectrum was visually inspected by Flex analysis 3.0 software (Bruker Daltonics), and peak profiles with variable occurrence among strains were removed. Reference spectra of newly created main spectra were added to the original Biotyper database. A log(score) value higher than 2.3 was obtained for all spectra from biological and technical replicates of the six different *Acinetobacter* strains matching their own newly created reference main spectra. PCA performed on spectra from biological and technical replicates within each *Acinetobacter* strain selected revealed discrete clusters, indicating little variation between replicates and supporting the reproducibility of the method described. Hierarchical clustering of the spectra was performed by applying the average linkage algorithm to a distance matrix containing the Hamming distance between all pairs of peak profiles.

The discriminatory power of each typing method was assessed using Simpson's index of diversity (SID), calculating the probability that two unrelated strains sampled from the test population will be placed into different typing groups (20), and the 95% confidence intervals (CI) of the SID values were calculated as described previously (21). The quantitative concordance between

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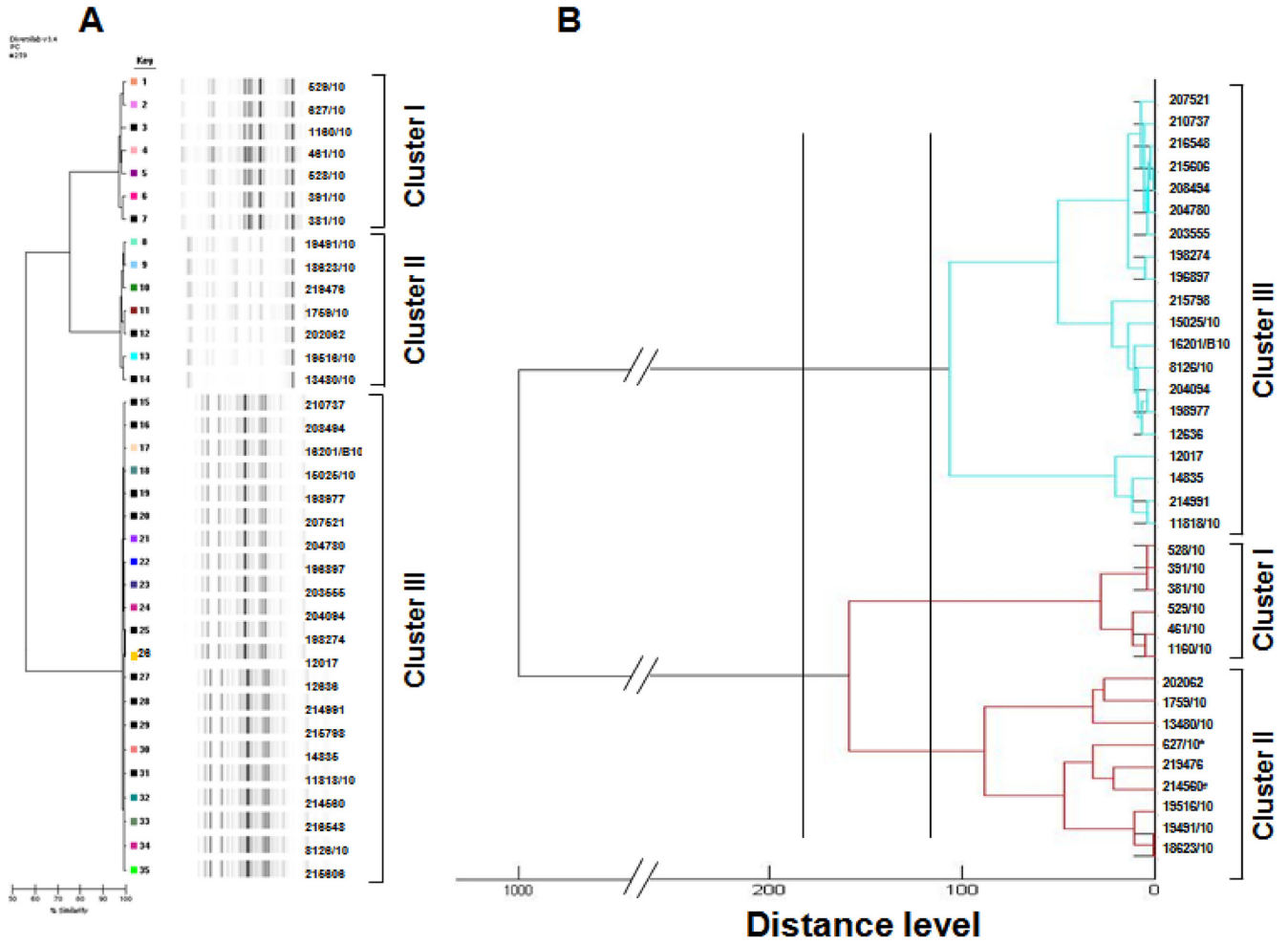

FIG 1 Hierarchical clustering of *Acinetobacter baumannii* isolates by rep-PCR and MALDI-TOF MS. (A) Dendrogram describing percentage similarities and banding patterns of *A. baumannii* by repetitive PCR, interpreted by the Pearson correlation coefficient. (B) Dendrogram representation of hierarchical cluster analysis provided by MALDI-TOF MS. The vertical lines indicate the similarity cutoff values around 200 and 100 used arbitrarily for MALDI group definition. Isolate assigned to cluster I by the DiversiLab system is indicated by an asterisk. Isolate assigned to cluster III by the DiversiLab system is indicated by a number sign.

typing methods was analyzed by using adjusted Rand and Wallace coefficients (22).

The rep-PCR system identified three different clusters of *A. baumannii* isolates (Fig. 1A): cluster I, 7 indistinguishable isolates, with an average genomic similarity ratio of >98.5%; cluster II, 7 indistinguishable isolates, with an average genomic similarity ratio of >98.5%; and cluster III, 21 indistinguishable isolates, with an average genomic similarity ratio of >98.5%. The three clusters were significantly different from each other in that the percentage of similarity between clusters I and II was around 75% and was around 55% between clusters I and III and clusters II and III (Fig. 1A).

All isolates were identified as *A. baumannii* by MALDI-TOF with a log(score) of ≥2.3. The hierarchical clustering of MALDI-TOF peak profiles identified three different clusters of *A. baumannii* isolates, substantially interchangeable with those obtained with the rep-PCR system (Fig. 1B), except for isolates 627/10 and 214560, which were assigned to different clusters (clusters I and III) with rep-PCR and included in the same cluster (cluster II) with MALDI-TOF.

The statistical analysis of the data showed that the rep-PCR system (Simpson's index, 0.595; 95% CI, 0.477 to 0.713) and MALDI-TOF MS system (Simpson's index, 0.576; 95% CI, 0.444 to 0.709) provided similar results, with a good concordance between the two methods (adjusted Rand's coefficient, 0.858) and a high probability of MALDI-TOF MS to predict rep-PCR results (Wallace coefficient, 0.892). Arbitrarily chosen cutoff values around 200 or 100 for the MALDI-TOF MS distance level seem to match with the 55% or 75% similarity obtained by rep-PCR (Fig. 1B).

A rapid and accurate detection of nosocomial outbreaks of *A. baumannii* is essential for the appropriate management of and timely intervention in infection control. Nowadays, sequence-based methods available for typing of *Acinetobacter* spp., such as pulsed-field electrophoresis (8, 23) and multilocus sequence typing (MLST) (17), are rather time-consuming, laborious, and expensive and require a long period of time before generating a typing result, useful for outbreak investigation and control (24, 25). The use of DL rep-PCR has been intensively investigated for detecting outbreaks of nosocomial infections. This method is con-

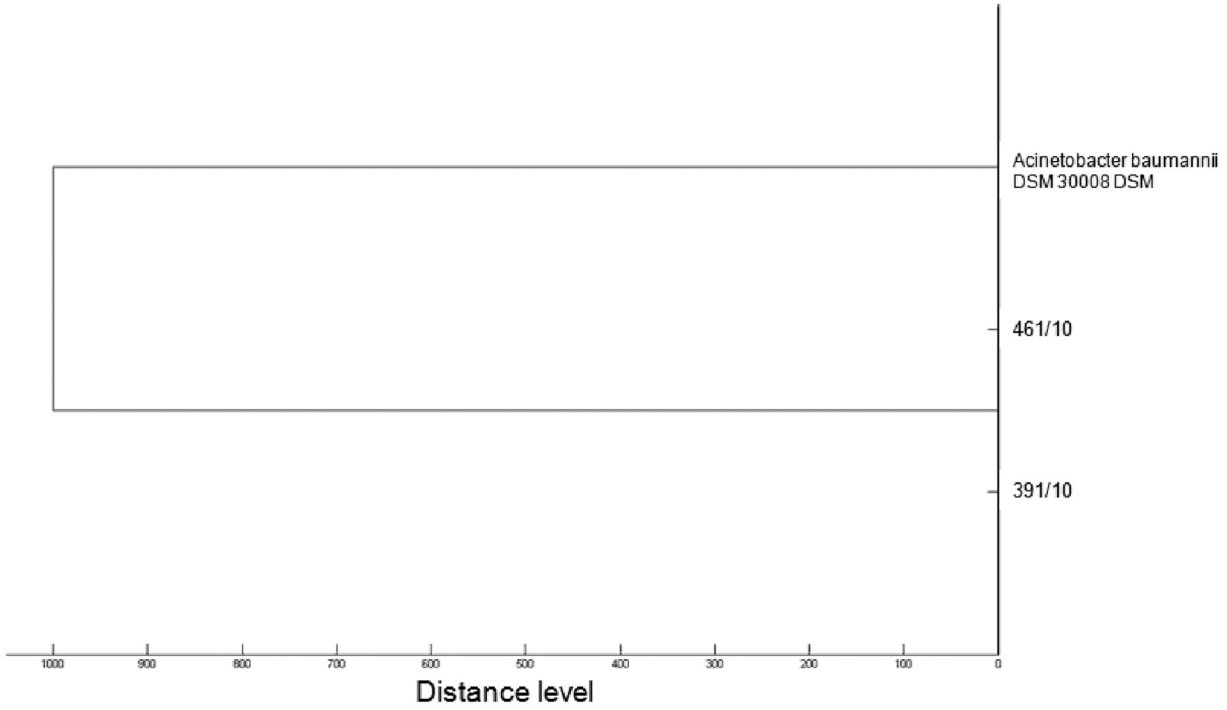

FIG 2 Dendrogram representation of hierarchical cluster analysis of *A. baumannii* isolates 461/10 and 391/10 and unrelated strain DSM 30008 DSM.

sidered comparable with the above-described sequence-based methods for different bacterial species, including *A. baumannii* (5, 17, 23). Moreover, it is an affordable method for laboratories of clinical microbiology.

To verify if the relatedness between isolates observed with MALDI-TOF MS and DL rep-PCR was evidenced also by a reference method such as MLST, two *A. baumannii* strains (461/10 and 391/10), classified in cluster I by both MALDI-TOF MS and rep-PCR, were assessed using an MLST scheme based on fragments of the seven housekeeping genes *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD* (http://pubmlst.org/abaumannii/). *Acinetobacter baumannii* reference strain DSM 30008 DSM was added as an unrelated strain. MLST analysis was performed as described previously (26), and amplicons were sequenced using an ABI Prism 3130xl sequence analyzer (Applied Biosystem, Foster City, CA). Sequences were analyzed using Chromas Lite 2.1 software (Technelysium) and compared with the *A. baumannii* database at the MLST website (http://pubmlst.org/abaumannii/). The two isolates belonged to sequence type 128, while the reference strain number DSM 30008 DSM belonged to sequence type 13 and was classified by MALDI-TOF MS as a unique isolate (Fig. 2). This finding could be particularly relevant, as often the clinical question is to know if a new isolate is an outbreak strain or a unique isolate.

The accuracy and speed of data acquisition which can be achieved using MALDI-TOF MS make this a potentially important tool for a rapid and sensitive identification of nosocomial outbreaks caused by various alert organisms. In line with recent studies (5, 12, 17), the results of the present work suggest that MALDI-TOF MS can be used for a rapid and optimal detection of nosocomial *A. baumannii* outbreaks, as described for other microorganisms (10, 11, 13–16).

In this study, MALDI-TOF MS classified two isolates (isolates 627/10 and 214560) in different clusters from the ones they were classified in with rep-PCR. These results were out from three separate experiments, in which the two isolates were analyzed together with all the other isolates. A possible explanation for this result could be that the quality of the spectra obtained for these isolates was not clear enough to allow an exact typing. Indeed, different technical or biological causes, such as the quality of the spectra (27, 28) and the incubation time of cultures, can affect the MALDI-TOF results (29). Bacteria adapt rapidly to environmental changes, modifying the production of proteins or other cellular processes when they are stored, handled, or cultured over different time periods. This makes it important to carefully control all the above-described variables in experiments aimed at bacterial typing with mass spectrometry.

In conclusion, the present study suggests that MALDI-TOF MS can be successfully used in routine clinical microbiology for real-time identification of nosocomial outbreaks from *A. baumannii* isolates before results from DNA-based systems are available.

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