Peaks of Promise and Peril: Screening for Antibiotic Resistance by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Neil W. Anderson

Department of Pathology and Immunology, Washington University in St. Louis, St. Louis, Missouri, USA

An article in this issue of the *Journal of Clinical Microbiology* (J.-H. Youn, S. K. Drake, R. A. Weingarten, K. M. Frank, J. P. Dekker, and A. F. Lau, *J Clin Microbiol* 53:35–42, 2015, <http://dx.doi.org/10.1128/JCM.01643-15>) describes the use of matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry for the detection of organisms carrying a *bla*_{KPC}-containing plasmid. This powerful and promising application highlights the challenges of using MALDI-TOF mass spectrometry for purposes other than organism identification.

Over recent years, the introduction of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has had a dramatic impact on the field of clinical microbiology. Bacterial, mycobacterial, and fungal isolates are now being identified in a much shorter time than previously, at a fraction of the previous cost, and often with greater accuracy (1, 2). The technology is easy to use and adaptable to laboratories with differing volumes and workflow. Given the profound success of MALDI-TOF as an identification platform, it is not surprising that investigators are now exploring alternative applications.

In this issue of the *Journal of Clinical Microbiology*, Youn and colleagues describe the use of MALDI-TOF for the screening of organisms for production of *Klebsiella pneumoniae* carbapenemase (KPC) (3). The approach taken by Youn and colleagues differs from previously described carbapenemase detection assays. Previous approaches have involved short incubations with antibiotic and then either measurement of hydrolyzed drug or indirect measurement of organism growth by MALDI-TOF. For their screening approach, Youn and colleagues use the presence or absence of a specific peak within the spectral profile correlating with the presence of a *bla*_{KPC}-containing plasmid. Their development of this technique was previously described in a 2014 publication in the *Journal of Clinical Microbiology* by Lau and colleagues (4). During an outbreak of carbapenemase-resistant *Enterobacteriaceae* (CRE) infection, the investigators identified an 11,109-Da MS peak unique to CRE isolated from patients at their facility. It was further elucidated that this peak corresponded to p019, the cleavage product of a gene located on *bla*_{KPC}-harboring plasmid pK-PQIL. In their latest publication, Youn et al. demonstrate how detection of the p019 peak can be used to distinguish characterized CRE from non-CRE isolates as well as retrospectively identify historic CRE isolates from their institute in a blind manner. Their results serve as a proof of concept, suggesting that this technique can be used successfully.

Previous studies have similarly demonstrated that individual peaks obtained through routine MALDI-TOF analysis can be used to track antibiotic-resistant isolates. A 2012 Australian study demonstrated that the presence of unique peaks can be used to distinguish *vanB*-positive *Enterococcus faecium* isolates from susceptible isolates with a sensitivity of 96.7% and a specificity of 98.1% (5). In 2014, a Japanese study illustrated high sensitivity and specificity of

individual peak detection for the tracking of extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* clonal groups (6). Much like the method described by Youn et al., these methods require the interrogation of spectra obtained through routine identification of organisms. As such, they can be implemented with minimal change to identification procedures already in place. Spectra can be analyzed prospectively or retrospectively months after a bacterial isolate has been discarded. Furthermore, through the use of robust analytical programs, these data can be queried rapidly in bulk. While these features make the implementation of such assays appealing, one must keep in mind their limitations. An important consideration is that many of these assays detect peaks that are surrogate markers of resistance, not direct measurements of resistance determinants. Regarding the assay described by Youn and colleagues, the peak being used to identify isolates is associated with a known protein product (p019) from a gene located on the pKpQIL plasmid in close proximity to the *bla*_{KPC} gene. While this close proximity may support the hypothesis of a strong association between the two genes, this association cannot be considered to be 100%. The detection of p019 does not preclude the presence of mutations in the *bla*_{KPC} gene. The association also applies only to PkPQIL plasmids harboring *bla*_{KPC}, therefore limiting the utility of this assay for the detection of KPC production from other plasmids. A 2014 New York study demonstrated that among 284 KPC-producing *K. pneumoniae* isolates, PkPQIL-like plasmids were identified in only 101 (35.6%) (7). One would not expect the detection of p019 to correlate well with KPC production in this population. Even regarding isolates that do indeed harbor PkPQIL plasmids, sequence analysis has shown that *bla*_{KPC} and p019 are located on different mobile genetic elements and

Accepted manuscript posted online 11 November 2015

Citation Anderson NW. 2016. Peaks of promise and peril: screening for antibiotic resistance by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol* 54:5–6. doi:10.1128/JCM.02490-15.

Editor: Y.-W. Tang

Address correspondence to nanderson@path.wustl.edu.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.

therefore may not always be associated with one another within these plasmids (8).

The data obtained by Youn and colleagues also demonstrate the technical challenges of interpretation of individual peak data. The presence of spectral peaks may be influenced by repeated use of the instrument. While recalibration with a manufacturer-provided calibrator can compensate for these changes, the measurement of peaks outside the typical range used for identification may require specific calibrators to ensure accuracy (9). There are also instrument-specific factors affecting peak acquisition that cannot be overcome by calibration. Youn and colleagues showed the positive effect that routine instrument maintenance had on their KPC screening assay (increasing sensitivity from 96% to 100%). If such an assay were to be implemented by other laboratories, all of these factors should be considered. While this also holds true for routine organism identification based on spectral profile, one could argue that assays based on the presence or absence of a single peak would be more vulnerable to small changes in peak acquisition. Laboratories may need to adopt more-stringent protocols, calibration, and maintenance requirements when using such assays. Well-defined positive and negative controls should also be implemented to promptly detect any change in assay function.

So what is the future role of such assays in the clinical microbiology laboratory? It seems clear that as long as these assays involve the detection of surrogate markers of resistance, confirmatory testing will be necessary. However, they may have utility as screening assays for resistance determinants. Youn and colleagues effectively demonstrated that they can successfully screen for KPC-producing isolates in a robust fashion within their institute by tracking the right marker in the right setting. They were able to use this approach because they had a clear understanding of the relationship between the marker that they were tracking and the resistance determinant that they were concerned about. Their assay would not be as successful in a setting where this relationship significantly differs. Therefore, laboratories should exercise caution prior to adopting this assay or others like it. Similarly to Youn and colleagues, laboratory clinicians must carefully prove that the assay has utility in their patient population prior to implementation. They should also adopt appropriate controls and protocols in order to address instrument-specific factors which might affect

assay performance. While Youn and colleagues have demonstrated that resistance determinants can be tracked efficiently by the presence or absence of individual mass spectrum peaks, much work is needed before this methodology can become widely applicable.

REFERENCES

1. Patel R. 2015. MALDI-TOF MS for the diagnosis of infectious diseases. *Clin Chem* 61:100–111. <http://dx.doi.org/10.1373/clinchem.2014.221770>.
2. Dingle TC, Butler-Wu SM. 2013. Maldi-tof mass spectrometry for micro-organism identification. *Clin Lab Med* 33:589–609. <http://dx.doi.org/10.1016/j.cll.2013.03.001>.
3. Youn J-H, Drake SK, Weingarten RA, Frank KM, Dekker JP, Lau AF. 2015. Clinical performance of a matrix-assisted laser desorption ionization–time of flight mass spectrometry method for detection of certain *bla*_{KPC}-containing plasmids. *J Clin Microbiol* 53:35–42. <http://dx.doi.org/10.1128/JCM.01643-15>.
4. Lau AF, Wang H, Weingarten RA, Drake SK, Suffredini AF, Garfield MK, Chen Y, Gucuk M, Youn JH, Stock F, Tso H, DeLeo J, Cimino JJ, Frank KM, Dekker JP. 2014. A rapid matrix-assisted laser desorption ionization–time of flight mass spectrometry-based method for single-plasmid tracking in an outbreak of carbapenem-resistant Enterobacteriaceae. *J Clin Microbiol* 52:2804–2812. <http://dx.doi.org/10.1128/JCM.00694-14>.
5. Griffin PM, Price GR, Schooneveldt JM, Schlebusch S, Tilse MH, Urbanski T, Hamilton B, Venter D. 2012. Use of matrix-assisted laser desorption ionization–time of flight mass spectrometry to identify vancomycin-resistant enterococci and investigate the epidemiology of an outbreak. *J Clin Microbiol* 50:2918–2931. <http://dx.doi.org/10.1128/JCM.01000-12>.
6. Hart PJ, Wey E, McHugh TD, Balakrishnan I, Belgacem O. 2015. A method for the detection of antibiotic resistance markers in clinical strains of *Escherichia coli* using MALDI mass spectrometry. *J Microbiol Methods* 111:1–8. <http://dx.doi.org/10.1016/j.mimet.2015.01.020>.
7. Chen L, Chavda KD, Melano RG, Jacobs MR, Koll B, Hong T, Rojzman AD, Levi MH, Bonomo RA, Kreiswirth BN. 2014. Comparative genomic analysis of KPC-encoding pKpQIL-like plasmids and their distribution in New Jersey and New York hospitals. *Antimicrob Agents Chemother* 58:2871–2877. <http://dx.doi.org/10.1128/AAC.00120-14>.
8. Partridge SR. 2014. Tn4401 carrying *bla*_{KPC} is inserted within another insertion in pKpQIL and related plasmids. *J Clin Microbiol* 52:4448–4449. <http://dx.doi.org/10.1128/JCM.02426-14>.
9. Jung JS, Popp C, Sparbier K, Lange C, Kostrzewa M, Schubert S. 2014. Evaluation of matrix-assisted laser desorption ionization–time of flight mass spectrometry for rapid detection of beta-lactam resistance in Enterobacteriaceae derived from blood cultures. *J Clin Microbiol* 52:924–930. <http://dx.doi.org/10.1128/JCM.02691-13>.