Clinical Chemistry

Identifying Clinically Relevant Bacteria Directly from Culture and Clinical Samples with a Handheld Mass Spectrometry Probe

Article:
Sydney C Povilaitis, Ashish Chakraborty, Lindsey M Kirkpatrick, Rachel D Downey, Sarmistha B Hauger, and Livia S Eberlin.
Identifying Clinically Relevant Bacteria Directly from Culture and Clinical Samples with a Handheld Mass Spectrometry Probe.
Clin Chem 2022;68(11): 1459–70. https://doi.org/10.1093/clinchem/hvac147

Guests: Dr. Livia Eberlin is an Associate Professor in the Department of Surgery at Baylor College of Medicine in Houston and Dr. Lindsey Kirkpatrick is an Assistant Professor of Pediatrics in the Ryan White Center for Pediatric Infectious Disease and Global Health at the Indiana University School of Medicine and the Riley Hospital for Children at IU Health in Indianapolis.

Bob Barrett: This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. When it comes to dealing with bacterial infections, rapid and accurate pathogen identification is critical to allow selection of targeted antibiotic treatment and improve outcomes for patients. Broad-spectrum antibiotic regimens can lead to adverse effects including allergic reactions, bacterial resistance, and opportunistic infections. However, specific and targeted antimicrobial regimens can only be administered if the pathogen is accurately identified. Primary methods for bacterial identification rely on isolating bacteria from specimens by culturing them in media prior to biochemical testing and that can take up to more than five days.

A paper appearing in the November 2022 issue of *Clinical Chemistry* describes the development and evaluation of a handheld mass spectrometry-based device called the MasSpec Pen that enables rapid analysis of clinical samples to help address that very issue. We are pleased to have two of the authors of that paper with us as guests in this podcast.

Dr. Livia Eberlin is an Associate Professor in the Department of Surgery at Baylor College of Medicine in Houston, and Dr. Lindsey Kirkpatrick is an Assistant Professor of Pediatrics in the Ryan White Center for Pediatric Infectious Diseases and Global Health at the Indiana University School of Medicine and the Riley Hospital for Children at IU Health in Indianapolis. Dr. Eberlin, let's start with you. How does the MasSpec Pen work and what changes did you make to the system identifying bacteria in clinical specimens?

Livia Eberlin: Yeah, so we developed the MasSpec Pen with the intention to be a very user-friendly device. So, we call it a pen even though it doesn't write. I say this a lot of times, it actually reads molecular information and the way that we developed the technology was it, to make possible for clinical

professionals and medical professionals to take advantage of mass spectrometry in a way that hadn't been done before.

So the device itself is pretty simple. It feels like a pen, it's not a pen. It's like a probe that anyone can just hold in their hands. It's connected to a mass spectrometer, which is really the main analytical instrument doing the molecular analysis, and the pen itself is fully made of plastic materials. So it's disposable, it can be autoclaved if needed for sterilization. Once the user touches this pen onto a sample surface, which could be let's say a tissue that has an infection or it could be just bacteria on a glass slide, then you click a foot pedal and that click triggers the delivery of a discrete droplet of solvent to the tip of this pen. So the pen is contacting the surface, then that solvent, which in most of the cases we use water, will interact with the sample and extract molecules from this sample surface or from this tissue, or from the bacteria.

And this process of the chemical extraction, I like to say is one of the most basic chemical experiments because we'll use solvents a lot to extract molecules from complex samples. I drink coffee a lot, so I always say that's like a chemical experiment, because you use water to extract all these wonderful molecules that make up our coffee with it, which end up being our samples.

So in the same way, we use that droplet of solvent to extract molecules from, let's say bacteria, and then we open this vacuum system and then that droplet that now contains all of this rich molecular information, is directly transferred to the mass spectrometer and the mass spec, it does its job, it's really an amazing analytical instrument that reads this molecular information. So the output of this analysis is a graph that shows what are the molecules that were on this sample that you just analyzed.

So yeah, the use of the device is very simple for a user that is a non-expert. What you need to do is grab the probe, touch it onto the sample that you want to analyze, click the foot pedal, and then automatically it will deliver that droplet, extract the molecules, shoot it to the mass spec that does the analysis, and then you end up with a graph, which is the mass spectrum that contains the molecular information. So for this application with bacteria, we actually did not have to do a lot of changes. We played a bit with the extraction time, so how long we sit the droplet on the sample. We tested a bunch of different solvents to see which one works best for bacteria and we also played a little bit with like the length of the tubing and some of the parameters of the mass spectrometer. But generally, it was a pretty straightforward optimization process to be able to get these really wonderful molecular profiles from bacteria.

Bob Barrett: Dr. Kirkpatrick, why would there be a need or interest to apply this method for bacteria identification when MALDI mass spec methods are so well developed for identification of bacteria?

Lindsey Kirkpatrick: Yeah. So I will say this--MALDI is an amazing tool that really has benefited the clinical laboratory and clinicians in so many ways. I mean, I think overall it reduces time to specimen collection and diagnosis of the offending pathogen. It's highly accurate and also the pre-analysis processing is simple and reproducible. I think overall, that's very beneficial to the laboratory workflow. However, one thing holds true for MALDI: there must be culture growth and this takes time; this takes days to be exact. So when it comes to MALDI, MALDI also doesn't provide any susceptibility data. So you're still waiting to figure out what antimicrobial agent is the best therapy for that particular pathogen.

So while there are culture-independent workflows for CSF and urine for MALDI, they really are not readily deployed in a CLIA certified laboratory due to tedious sample prep and stringent regulations. So I think overall, regardless of how great MALDI is for the clinical laboratory and how much it supports us as far as our clinical decision-making, I think there needs to be faster methods in order for us to identify the offending pathogen for an infection and also provide susceptibility data when maybe it's an unusual pathogen that has high resistance rates or something along that line.

Bob Barrett: What are some of the advantages for detection of bacteria directly from biological samples?

Lindsey Kirkpatrick: So, to give a little foundation, the diagnosis of a pathogen responsible for a bacterial infection relies on culture-based techniques, and really culture is the gold standard in a clinical laboratory today. At baseline it usually takes anywhere from 24 to 48 hours, or maybe longer, for identification and susceptibility testing for pathogens to be available to the clinician, because we must wait essentially on culture growth for any of these test to be performed. We do have some systems in place to help us make more rapid identification and susceptibility testing available that's in our laboratory. We use BioMérieux VITEK systems as well as MALDI from an identification standpoint and then we use VITEK from a susceptibility standpoint, as well as our traditional techniques that might take a little bit longer.

There are also phenotypic and genotypic based tests that have been applied to a positive blood culture to aid in rapid identification and determination of antimicrobial susceptibility of common pathogens. But these commercialized tests overall don't extend to other specimen types and not every

laboratory employs them for a number of different reasons. Even when newer methods of pathogen identification for other specimen types, like next-generation sequencing and PCR, you are still waiting on a result, often from a reference laboratory, for identification, and you're still waiting on culture growth for confirmation and susceptibility data.

Another issue is that some organisms may not be easily isolated or grown by conventional culture methods due to many factors. There's really too many to name because it can be very pathogen-specific overall, and then you throw in pre-treated patients, where we may have sterilized the source and nothing grows on culture and then you get even more trouble. So I can honestly spend all day going over this and the methods and what are the limitations with them. But I think what is lacking overall is a methodology that can extend across many specimen types, that aids in rapid and direct identification of pathogens, and provide some resistance and susceptibility data with accuracy, sensitivity, specificity, without needing to wait on culture growth.

I think that's where the MasSpec Pen really comes into play and it has many advantages in that way. A method that can be deployed near point-of-care in the clinical laboratory is even more beneficial so we as clinicians aren't really waiting days for answers. I think, overall the MasSpec Pen can fill this gap. From a clinical standpoint, if I can know very quickly that the *Staph. aureus* species infecting my patient is methicillin-sensitive, I can target that quickly with a beta-lactam and keep them off vancomycin at the start, which is a nephrotoxic drug. Or maybe I have a critically ill patient with an osteoarticular infection that was pretreated due to their shock. Then perhaps I can pinpoint the culprit utilizing this method as its footprint is still there, despite no culture growth, and target the best antibiotic regimen to that pathogen overall.

Let's say there's a patient living in a long-term care facility, has spinal fusion hardware, and they got the spinal fusion four years ago and develops an infection and I find *Acinetobacter*, a nasty little pathogen that's often resistant, with this method, then maybe I can broaden out my coverage until I get more information from traditional methods. So overall I think, the benefits to the clinical care aspect are astronomical. I think also from a cost standpoint, a lot of these tests that we utilize in the clinical laboratory are very expensive. If we can consolidate to one methodology and one instrument, or a couple different instruments and I think overall it will benefit not only the laboratory, but the patient.

Bob Barrett: Well, those are the advantages. There have to be some limitations though, can you tell us about that?

Lindsey Kirkpatrick: I mean, I think there's always limitations to any method and any test. I think from a standpoint of detection and identification and complex matrices, I think you must always consider the effects of the body matrix on the pathogens, such as the presence of inflammation of our own cells, nucleases, proteases, and how this will affect the accuracy of identification and information gleaned in terms of susceptibility resistance. We also must be very careful in our interpretation. Some infections are traditionally, or can be polymicrobial, so when you obtain unusual or indeterminate results utilizing this method, I think that's something that must be thoroughly explored before we could potentially explore that box.

Overall, knowing the limitations of your tests and where problems may arise is key, and I think, Livia can probably speak better to this in terms of matrix effects and ionization efficiency and complex matrices and their effect on direct identification and gleaning some susceptibility data as well as the potential limitations when multiple microbes are present utilizing this method. So, I'm going to go ahead and just push the question to her and I'll put her up to maybe going over some of those things.

Livia Eberlin: Yeah, absolutely. One of our main intended uses of the MasSpec Pen is to directly analyze, let's say an infected specimen that's coming out of the OR. We actually originally developed the technology to help in surgical decision-making. Our primarily focus when we started this all was in oncology, so we want to help surgeons in cancer surgeries identifies tissues. And then through our collaboration, we identified new bacterial infections, especially osteoarticular infections in pediatric patients, as being a very important and significant opportunity to apply this technology.

But as Lindsey said, with mass spectrometry, one of the incredible things that we can do is we analyze really tens to hundreds, if not thousands of molecules in a single analysis. It's very sensitive, which is great for detecting molecules that we want to see. But also, when we're dealing with very complex samples, let's say if you have a piece of bone, human bone that's infected with bacteria, there's going to be a mixture of molecules that are being all detected at the same time. So that complicates our analysis. It's something we're still investigating, what are our limit of detections in terms of the bacteria mixed within other biological matrixes? How does that change the graph or the mass spectra that we are acquiring? How will that impact the performance of the method? So these are all variables and unexplored really, questions that we have now that we're currently pursuing through some of the RB clinical studies that we're pursuing now in collaboration with Lindsey, but also other collaborators at Texas Medical Center.

Bob Barrett: So why is it important to identify the pathogen so quickly, particularly in osteoarticular infections?

Lindsey Kirkpatrick: So, I deal with a lot of osteoarticular infections on the clinical side. I think the benefits of many in terms of the monomicrobial osteoarticular infections, which are generally in children particularly due to hematogenous spread. So say like, anytime you scratch a mosquito bite, bacteria gets into your bloodstream, your immune system typically takes care of it. Any time we eat a cracker, bacteria gets flicked off into our bloodstream and we can handle it. Well, every once in a while, bacteria is able to set up shop for whatever reason. A story I get often is, "My kid they were playing on the monkey bars. They fell, they hit their hip, and then a week later they can't walk and their hip, it's hot red and swollen." We find an infection there.

I think one of the biggest benefits will be in terms that the antimicrobial stewardship and avoiding potential side effects and cost from an unnecessary antibiotic from the start, or starting an antibiotic that may be needed to treat an unusual pathogen found, and can reduce overall morbidity. Because really with osteoarticular infections, especially in the joint space, like a septic joint -- damage starts within the first few hours of that infection. So by the time we clean it out and start targeting antibiotics, we're already behind. So I think the quicker we can get to the best antibiotic for that infection, then the quicker we can get to getting them on an oral regimen potentially, the better in terms of chronic osteoarticular infections, which do happen. Even in healthy children, we see them often in our chronically ill children, but our healthy children will also get them. Identification may aid in less hospital days and transition to oral regimens sooner.

In addition, targeted treatment can reduce the risk of developing infections like seeded colitis, which in my immunocompromised patients, and my patients with chronic medical issues that require antibiotics, quite often can be quite devastating. And then also, we want to prevent resistance. Certain bugs or more apt to cause resistance with certain antibiotics. So if we can avoid those antibiotics and avoid resistance, especially in children that may or may not need other courses of antibiotics, that would be ideal.

Livia Eberlin: Yeah, I think that time from the identification to prescribing antibiotic is just critically important. I'm not a doctor myself.

Lindsey Kirkpatrick: I agree. I would agree.

Livia Eberlin Lindsey is much more eloquent on that. But the MasSpec Pen, you're literally seconds, so it's extremely quick. So even if we don't get to the point where we can identify all levels of

the bacteria infection, even just a quick understanding of what is this bug, could help start that treatment process. I think that's really one of the great things about mass spectrometry in general, with the MasSpec Pen, it's just an easy to deploy technology and the result is so quick that it can really make an impact in a way that other technologies may be limited.

Lindsey Kirkpatrick: I think too, like going back to those unusual bugs that may not grow on culture, I think one of the bugs that we worry about in pediatric osteoarticular infections is *Kingella*, and typically it's PCR that we utilize to identify that. Sometimes I'm scratching my head. I have a kid with no culture growth, maybe they got one or two days' antibiotic before but they're within that age range of when they might get *Kingella,* which is upwards of like 12 months of age to like 5 years old, is usually the range. I think about it in terms of, "Okay. Well, do I need to send them home on dual regimen to cover not only for *Staph. aureus* and *Kingella*, or can I just target the *Kingella?*" which would be ideal, right?

We want to use our most narrow regimen possible, not only to avoid side effects, but also to get our best bactericidal response to killing that bacteria. So I think overall I agree with Livia. It's critical that we put them on the right antibiotic as quickly as we can to reduce damage and also to reduce side effects from other potential antimicrobials that they don't need. Vancomycin, I've seen so much nephrotoxicity associated with that and if I can avoid that, I would love to, and the quicker we can get that answer, the better.

Bob Barrett: Well finally, are there diseases or uses of this technology in microbiology or perhaps other departments in the clinical laboratory?

Lindsey Kirkpatrick: Yeah. So I can speak from an infection standpoint. Livia can speak to the rest of the sky. I think overall, I think this methodology can be developed to identify pathogens and provide some susceptibility data in adults and children that have infections and traditionally sterile sites and are typically monomicrobial, so not only osteoarticular infections, but bloodstream infections, skin and soft tissue infections, other musculoskeletal infections, like psoas abscesses, central nervous infections like meningitis, or even like lung empyemas or closed hardware infection.

So I think overall, I think the application it broad. Of course when you think about different sites and what bugs might be there or pathogens might be most common in those sites, that definitely changes, so that would require an expansion of the library and further testing. I could see this methodology being utilized for fungal detection in sterile sites as well. Cultures can take up to six weeks to grow, if at all,

or other fungal testing like PCR or antigen testing or biomarker testing overall is not the best in terms of sensitivity and specificity.

I also saw a paper where I could see this being used for viral testing. There was some work done with COVID-19 and the identification of using lipid-rich profiles in order to identify that. I was there for the beginning, the early pandemic, where we had no tests or very limited reagents, how beneficial it would have been to be able to apply this methodology to that. I think Livia can speak to the other potential applications much better than I can. But from an infection standpoint, I think that's what I think about.

Livia Eberlin: Absolutely, one of the very interesting applications that came up here in my department -- so I'm in the Department of Surgery and one of my collaborators mentioned the diabetic foot infections. We have a big VA Medical Center here in Houston and these are probably microbial infections, right Lindsey? So they're harder to identify what exactly the group of bacteria is, but that's another case where the doctors are waiting for days, sometimes over a week. I've heard here in this huge medical center to get some understanding of what is the infection, and how they're going to treat it. Of course it's critically important to get those patients under medication as soon as possible.

In the clinical lab, we've been exploring applications in toxicology, that's very much chemical analysis. So I'm a chemist and Lindsey is too. So that is really exciting to me, the ability to detect drugs and compounds that shouldn't be in our biological systems, and the MasSpec Pen can offer opportunities there to expedite screening of drugs and so forth that are so critically important in toxicology type of assays.

And then just for disease detection, we've been really broadly looking at several types of different cancers, of subtyping cancers, looking at response to therapy, but also some benign conditions like endometriosis, and helping identification of lesions and so forth. So there's really just a lot of exciting opportunities for this technology.

Bob Barrett: That was Dr. Livia Eberlin from the Department of Surgery at Baylor College of Medicine in Houston, and she was joined by her co-author, Dr. Lindsey Kirkpatrick from the Ryan White Center for Pediatric Infectious Diseases and Global Health at the Indiana University School of Medicine and the Riley Hospital for Children in Indianapolis. They have been our guests in this podcast on identifying clinically relevant bacteria directly with a handheld mass spectrometry probe. Their paper on that topic appears in the November 2022 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.