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Ready, Set, Type! Proteomics vs Agglutination for Escherichia coli H Antigen Confirmation

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Guests:
Dr. Mari DeMarco and Dr. Christopher Lowe of the University of British Columbia in Vancouver, Canada.

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.

Foodborne outbreak surveillance systems rely on clinical microbiology laboratories as sentinels, given their role in the initial detection and reporting of pathogens in people seeking medical care. Prompt investigation of an outbreak requires rapid identification of the organism responsible. The June 2016 issue of *Clinical Chemistry* includes a study by Keding Cheng, Yi-Min She, and colleagues, describing a novel proteomic method that compliments and updates existing workflows for detecting and differentiating E. coli strains during outbreaks. An editorial by Dr. Christopher Lowe and Dr. Mari DeMarco accompanied that article and provided additional context about the implications and the challenges of implanting this mass spectrometry based method in a clinical laboratory, and they join us in this podcast.

Dr. DeMarco is a clinical chemist at St. Paul's Hospital and a clinical assistant professor at the Department of Pathology and Laboratory Medicine at the University of British Columbia in Vancouver, Canada. Dr. Lowe is a medical microbiologist and infection prevention and control physician at Providence Healthcare. He is a Clinical Assistant Professor in the Department of Pathology and Laboratory Medicine at the University of British Columbia in Vancouver, Canada.

And Dr. Lowe, could you describe public health investigations around potential foodborne outbreaks.

Dr. Lowe: Outbreak investigations are complicated. They are multidisciplinary and require a significant coordinated effort to essentially detect and resolve foodborne outbreaks. So, at the risk of hopefully not oversimplifying the process, the key really is in planning and prevention. And so with any foodborne outbreak, public health and microbiology labs, and reference laboratories really need to coordinate and work together for surveillance systems. How do we detect

outbreaks, and how do we detect pathogens of significance, are really, really the key for outbreak management and detection.

I think the first part really is dependent on the front lines. So, healthcare workers, physicians, microbiology laboratories, work with public health to provide publicly reportable diseases so that public health can go further to investigate. So, the onus really is on the front line and the need for rapid testing from microbiology labs to work up these pathogens of significance. But when there is really a concern about foodborne outbreaks, you do need further investigations, and that's where reference laboratories and where larger or more national laboratories come into play in terms of confirmatory testing. And that might involve DNA fingerprinting such as pulsed field gel electrophoresis to determine the genetic relatedness of bugs such as E. coli. And further on, if we need final confirmation, that's one of the things that were discussed really in this article in *Clinical Chemistry*, that there is really a need for this flagellar antigen testing or confirmation to identify the flagellar type, in this case H7. That's kind of when the final confirmatory steps to determine an E. coli O157:H7.

Now throughout all this, it's not just the microbiology lab and the reference labs that are investigating this outbreak. As the sentinel cases are reported by physicians or microbiology lab, public health officials, whether they're public health nurses, physicians, or epidemiologist would start the outbreak investigation in terms of case definitions, case finding, investigating potential source of outbreaks, and then ultimately trying to hypothesize and identify the source and confirm these outbreaks.

So, it's really a multipronged approached and a fairly complex approach to identifying and resolving them, and this MSH technology is just one component that is very exciting, but again one component in improving how we manage outbreaks.

Bob Barrett: So Dr. DeMarco, talk about the innovation to the traditional workflow that's presented in the June issue of *Clinical Chemistry* by Doctors Cheng and She and colleagues.

Dr. DeMarco: Well, as Dr. Lowe has touched on, methods used at either end of the outbreak investigation spectrum have really evolved considerably in the last decade, starting with initial bacterial identification by MALDI-TOF MS, and finally confirmation of clonality by next generation sequencing. However, the intermediate steps of identifying possible outbreak organisms with O and H antigen typing have remained relatively unchanged since the 1940s. It is in these intermediate steps where Cheng and colleagues have

focused their efforts and have devised the mass spectrum metric H antigen typing protocol which they call MSH.

This mass spec method is a qualitative proteomics approach with a bit of a twist on the standard tryptic digestion protocol. In this method, bacteria isolates are cultured overnight. They're resuspended in water and then trapped on a point two micron syringe filter. To this filter, trypsin is added and proteolytic digestion is carried out right on the filter.

At the completion of the digestion step, the syringe filter is flushed with a buffer and now the digested pieces of bacterial proteins including the H antigens can pass through the filter and are collected for analysis by nano liquid chromatography tandem mass spectrometry. For H antigen peptide ion scanning and fragmentation, data dependent acquisition was used and the specimen is so called typed by searching a curated database containing flagellin sequences.

Bob Barrett: What are H antigens and why is their identification important?

Dr. DeMarco: H antigens are the flagellin protein sub unit that polymerize in a helical fashion to form the bacterial flagella. Based on their abundance and their location on bacteria, they are the prime recognition single for the innate immune system and are fundamental to the virulence of pathogenic E. coli. There are 53 different E. coli antigens that have been identified to date, and all are included by a single gene, the fliC gene and knowing these possible flagellin sequences, one can connect mass spec data on tryptic peptides from an E. coli isolate to known flagellin sequences in order to identify the particular H antigen.

Bob Barrett: And how does mass spectrometry based H antigen typing compare to conventional serotyping techniques?

Dr. DeMarco: Well, when Cheng and colleagues compare their mass spec, H typing method to conventional serotyping, it performed quite well. Mass spec correctly identified approximately 96% of specimens and compared to conventional serotyping which identified approximately 84% of H antigens correctly. In this case, they relied on whole genome sequencing results as the tiebreaker when any discrepancies arose between the MSH and conventional serotyping.

In addition to examining the analytical and clinical performance metrics, we should also consider differences in workflow within the laboratory. Serotyping requires culturing on motility-inducing agar prior to analysis, followed by a series of a agglutination reaction utilizing antisera specific to the 53 known E. coli antigens. Most probable

antigens are targeted first, but one could imagine having to stuff through a long series of agglutination reactions before finding a match. Workflow can be time consuming on a scale of a couple of days to maybe over a week. Serotyping can also yield inconclusive results due to cross reactions among E. coli strains and ineffective motility induction even after multiple subcultures.

In contrast, MSH method requires the initial culture step but does not require motility induction for identifying the H antigen. Although this method does depend on a complex workflow involving nano fluid chromatography which can be tricky and prone to column clogging and carryover issues, it utilizes high resolution ion trap mass spectrometer which generates spectra with greater complexity relative to MALDI-TOF data commonly employed for bacterial identification and subsequently uses bioinformatics tools to identify the antigen.

For smaller labs, depending on the expertise, equipment, and throughput, this may not be a practical replacement for serotyping. But for a large public health lab, the advantages of skipping motility induction and improved accuracy are really attractive.

Bob Barrett: Doctor, in recent years, the application of MALDI-TOF mass spectrometry has revolutionized clinical microbiology laboratories. Yet this novel application relies on, and this is a mouthful, let me get to it, nano flow liquid chromatography electrospray ionized high resolution mass spectrometry. What are the benefits and challenges of using this system?

Dr. DeMarco: Well firstly, on behalf of chemists everywhere, I would just like to say how jealous we all are of our microbiology colleagues who have until recently, been able to rely on liquid chromatography free mass spec methods like MALDI-TOF. While it sounds innocuous, adding chromatography to your workflow does add considerable complexity. Fortunately, the benefits are also considerable in that you can separate complex mixtures prior to analysis and with an outflow method, you can collect a lot of data with very little specimen. Coupled to a high res instrument, the setup lets you select, fragment, and identify hundreds of peptides in a single injection.

Bob Barrett: So how does one develop quality control metrics for the MSH technique?

Dr. DeMarco: Well, quality control metrics are a challenge for clinical proteomics methods and that that doesn't exist decades of experience within clinical lab setting using such metrics. For instance, how do you monitor instruments and overall

method performance and potential sample carryover on a nano LC system. One metric you'll commonly hear applying to proteomics experiments is sequence coverage, which unfortunately will plateau after certain thresholds of bacterial biomasses use. And as Cheng and colleagues have found, it alone was insufficient as a quality control metric for their MSH method. Fortunately, back in 2005, Ishihama, Mann, and colleagues published an algorithm, the exponentially modified protein abundance index, to estimate peptide and protein abundance in level three proteomics experience.

In contrast to sequence coverage, there's no upper bound on the exponentially modified protein abundance index, and when applied to MSH, this metric was a useful tool to track sample overloading and identify potential carryover between sample injections. I think this is a really nice example of how we can utilize and adopt proteomics techniques for the clinical lab.

Bob Barrett: So Dr. Lowe, back to you. How does MSH technology advance outbreak investigations?

Dr. Lowe: Well, as we'd mentioned previously, the outbreak investigations are complex, and I think one of the most important things is regarding turnaround time, and really time to detection, or even a suspicion of a possible foodborne outbreak. That's where a lot of areas in microbiology, as Dr. DeMarco had mentioned, have really improved over time or at least modified or adopted new technology to decrease the time to detection. For example, acute care microbiology laboratories have incorporated the techniques such as mass spec or the MALDI-TOF without the chromatography aspect to decrease the turnaround time for identification of E. coli. And of course even in the acute care labs, O antigen testing is already done with rapid agglutination.

So, the front line microbiology labs can contribute to decreasing time to detecting the sentinel cases. In this case for MSH technology, that would help improvement of confirmation of these outbreaks by reference labs. Some reference labs have been using the DNA fingerprinting for pulsed field gel electrophoresis to relatedness of the organisms and further confirmation takes time.

So, as Dr. DeMarco also mentioned, flagellar antigen confirmation is not entirely an easy process. It does require potentially multiple subcultures or passages, and even in best case scenarios, there are some isolates that may not be typable because they're non-motile. So there are significant challenges to the traditional way that we do flagellar antigen testing. And I think the ability of this MSH

technology to improve throughput and to improve accuracy and to improve turnaround time for this confirmatory testing, will be an important component of resolving or confirming outbreaks. Confirmation is again now the key component of Canadian surveillance systems. The Canadian National Reference Lab processes as they know it in excess of 500 isolates per year. And so the requirement for traditional H antigen testing to take days to weeks for confirmation compared to MSH testing can improve the process and prevent a hindrance for stalling potential ongoing outbreak investigations.

Bob Barrett: So finally doctor, let's look ahead. Where do you see this MSH technology being applied in the coming years?

Dr. Lowe: It's really an interesting technology, and Dr. DeMarco really outlined a lot of some of the technical aspects and improvements in terms of identification accuracy. I mean, there's certainly a lot of benefit that you could see for H antigen testing in particular, with respect to potential cost reductions, sample preparation workflow, improved throughput and faster time to detection. These are all really appealing aspects to any laboratory process.

In addition, mass spec technology has really been increasing adopted by microlabs for very, very similar reasons. And if further work tends to be done to adopt this technology, for example to typing bacterial strains, that would be a significant opportunity to further improve outbreak investigations or even detection. Currently, it seems like one potential bottleneck in initial outbreak investigations is trying to identify whether bacterial strains are genetically related. At any given time, multiple labs may be sending a reference lab, several E. coli 0157 isolates. But the number of E. coli 0157 does not necessarily imply an outbreak, and really it needs to be further confirmatory testing, in most cases from pulsed field gel electrophoresis to confirm. Otherwise, that would result in potential unnecessary public health investigations and excess work.

At this point, pulsed field gel electrophoresis is most frequently utilized because there are standardized protocols through the CDC PulseNet guidelines. In addition to the standardized protocols, there are abilities to compare strains from different reference labs because they're all using the same technique. In addition, whole genome sequencing has potentially been used as a modality to determine genetic relatedness. But each of those modalities can be time consuming. It could also be labor intensive and expensive. If MSH technology or mass spectrometry methodology can be adopted for quick bacterial typing, that could greatly improve the time to initiation of public health investigations

for potential outbreaks. I think this can also be true for outbreaks beyond foodborne infections.

In general, there are really similar principles that apply for other outbreaks, in particular, if we're worried about outbreaks or potential transmission of bacteria within an acute care facility. Particularly this is with reference to some of our more concerning bacteria, are multidrug resistant bacteria such as MRSA, the Methicillin-resistant Staph Aureus, or VRE, Vancomycin-resistant enterococci, or really the most concerning one that's received most attention lately, is the carbapenamase-producing enterobacteriaceae. Now these bacteria tend to be resistant to our last line of antibiotics, including carbapenems. And so it's clearly an area of need, and within hospitals, when we were trying to identify clusters or outbreaks of these organisms, it also still does rely on DNA fingerprinting as well as epidemiological investigations to determine whether an outbreak exists, to determine whether transmission has actually occurred.

At this point, these DNA fingerprinting tools to determine relatedness really aren't easily accessible to many acute care hospitals throughout North America, and they need to be referred to reference laboratories. And because of that, that could potentially delay confirmation again because it does take time to do pulsed field gel electrophoresis, there are transport issues. And so when investigating an outbreak, those potential delays, whether with respect to transport or with respect to time needed to confirm through pulsed field gel electrophoresis or whole genome sequencing, can again delay the process of outbreak identification and taking action to prevent transmission.

However, with availability of mass spec technology in many acute care laboratory hospitals these days, there is a potential for quick typing to be done locally and that could be a significant benefit in that it can quickly inform local infection control practices to prevent further spread of these very concerned organisms. So, this technology can potentially be applied in a lot of different perspectives, whether you're a public health official, whether you're a reference laboratory, or an acute care hospital facility, this technology really kind of has the potential to speed up the turnaround time for outbreak investigation, detection, and surveillance.

Bob Barrett: Dr. Christopher Lowe is a medical microbiologist and infection prevention and control physician at Providence Healthcare. He is also Clinical Assistant Professor in the Department of Pathology and Laboratory Medicine at the University of British Columbia in Vancouver, Canada. Dr. Mari DeMarco is a Clinical Chemist at St. Paul's Hospital and a Clinical Assistant Professor at the Department of Pathology

and Laboratory Medicine at the University of British Columbia in Vancouver. They have been our guests in this podcast from *Clinical Chemistry*. I'm Bob Barrett, thanks for listening!