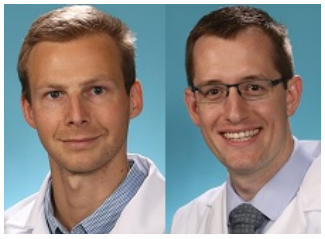
Clinical Chemistry

Genomic Prediction of Antimicrobial Resistance: Ready or Not, Here It Comes!



Article:
Eric M Ransom, et al.
Genomic Prediction of Antimicrobial Resistance: Ready or Not, Here It Comes!
Clin Chem 2020; 66:1278-89 https://doi.org/10.1093/clinchem/hvaa172

Guests: Dr. Robert Potter and Dr. Eric Ransom are both clinical and public health microbiology fellows at Washington University in St. Louis.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Antimicrobial resistance is one of the greatest concerns to human health worldwide and clinical microbiology laboratories are tasked with generating clinically relevant results, including antimicrobial susceptibility profiles to guide important patient care decisions. Antimicrobial susceptibility testing has evolved over the years and now laboratories have access to genomic approaches like whole genome sequencing and metagenomic next generation sequencing. A review article appearing in the October 2020 issue of *Clinical Chemistry* examined genome-based antimicrobial resistance prediction in relation to clinical microbiology: how it is performed, when it may be useful, and opportunities for the future. We are pleased to have two authors of that review article as guests in this podcast. Dr. Robert Potter and Dr. Eric Ransom are both clinical and public health microbiology fellows at Washington University in St. Louis.

Dr. Potter, we'll start with you. Could you please describe for our listeners what exactly is meant by genomic prediction of antimicrobial resistance?

Robert Potter: Thank you. I'd be happy to answer that. First, I feel like it's important to add some background information for our listeners in regards to infections. So, whether it's a sore throat, pneumonia, skin wound, everyone has experienced some type of interaction with a microbe and everyone wants it to heal as quickly as possible. Clinical microbiology laboratories play a critical role in this process by detecting the pathogen and then once we detect the pathogen, physicians want to know what drugs have the best activity against that organism. And there are two mains ways that our laboratories will predict drug resistance. The first is by exposing the pathogen to the drug in the lab to see if it survives. This method is called phenotypic susceptibility testing and the second gets back to your original question, which is genomic resistance prediction. For this, the lab looks into the pathogen's genome for resistance genes or resistance mutations that can be used for prediction if a

specific antibiotic will work. Essentially, from conducting whole genome sequencing and analysis of an organism, we could computationally identify all the antibiotic resistant determinants and we use determinants to encompass those horizontally acquired individual genes and also vertically transmitted target site mutations, and then use that information to predict how phenotypically susceptible an organism will be for a given antibiotic or class of antibiotics. And today, our discussion will focus on genomic prediction of resistance genes from a microorganism that is recovered in culture, not those directly identified from a clinical specimen.

Bob Barrett: Okay. So, Dr. Ransom, let's go to you. Are there some examples you could share with us on how genomic resistance prediction is currently being used in clinical microbiology laboratories?

Eric Ransom: Yeah. Absolutely. Genomic resistance prediction is most often used in the clinical microbiology subdiscipline of virology for viruses like HIV, cytomegalovirus, hepatitis viruses B and C. Labs routinely look for genetic mutations associated with treatment failure. Resistance predictions can also be done on bacterial genomes. The best example of this and most common is for MRSA, or methicillin-resistant staphylococcus aureus. Labs can use a variety of molecular approaches to find this corresponding resistance gene, usually mec-A. The final example I'll share with you is for tuberculosis. The microbe responsible for this is mycobacterium tuberculosis. This pathogen can be extensively multidrug-resistant often requiring at least three drugs to reach a cure. To make things worse, mycobacterium tuberculosis also takes an extraordinarily long time to grow in the laboratory making phenotypic testing less appealing. This can take weeks or even months. Fortunately, newer technologies can provide an initial drug resistance assessment in only a few hours by looking for a genetic mutations in the gene rpoB.

Bob Barrett: What benchwork and what computational analysis do these techniques require and which is more important, the lab work or the data analysis? Dr. Potter.

Robert Potter: So, I'd say really both the lab work and the data analysis are important. Computer scientists like to use the phrase "garbage in garbage out" to describe extrapolating information when your input isn't great. So, exactly similar to every other area of lab medicine, the pre-analytical stages are just as important as the analysis and the post analytical interpretation.

In regards to the benchwork, for the type of sequence analysis that we discuss in our review article, we're essentially starting with purified bacterial isolate that's been grown on any sort of culture. The next step is that that isolates has its entire nucleic acid content, which for most bacteria will include their chromosome plus any number of plasmids, extracted and made into sequencing libraries and those sequencing libraries are specific for the platforms that different laboratories will use for sequencing. You're essentially taking the bacterial DNA, chopping it up into fragments, and then adding known sequences called adapters and barcodes onto those ends of that DNA. And the libraries can then be pulled together with lots of different bacterial isolates, and this is really important from a cost-effective standpoint since all of those isolates can then be analyzed that one time. You put those libraries on whatever sequencing platform you want to use and out comes the series of DNA reads that you'll start interpreting computationally.

So, following generation of that sequencing data those adapters and barcodes, that we added right after the DNA was extracted, are computationally removed and those raw bacteria reads then undergo some other cleaning steps which include removing low quality or short reads and also any sort of contaminating human DNA that may be there. And then after these steps those reads can be used finally for analysis. So, once you have those reads, you could use them directly for identification of antibiotic resistance genes by mapping them to any number of curated databases of known antibiotic resistance genes or, and what's more commonly used I would say, is that these clean reads can be used to de novo assemble a bacterial genome and then the protein coding sequences that compose this bacterial genome can then be identified and again compared to a database of known antibiotic resistance genes. And that's important because you can use this method more readily to identify antibiotic resistant mutations and not just the individual genes.

And so that covers sort of the computational part involved in resistance prediction. One technology that we have not discuss extensively in this review is metagenomic next generation sequencing. So, instead of culturing the organism, we can just sequence all of the DNA present in the clinical sample. Obviously, this would lead to a lot more human DNA contamination that would have to be removed computationally and could present a HIPAA problem, but it could shorten the time needed to deliver resistance prediction if used in specific clinical context. Another concern is possibly decreased sensitivity for detecting microbial DNA given that in these samples there's just going to be a lot higher abundance of the human DNA.

Metagenomic sequencing could also be useful for heterogeneous populations of bacteria where we're really seeing multiple strains of the same species, all kind of masquerading as different bacteria, and these are not often resolved well using the de novo assembly approach that I just discussed.

Bob Barrett: So, Dr. Ransom, what limitations are preventing widespread use of this approach?

Eric Ransom: This is a really important question. There are several key reasons limiting widespread use of genomic resistance prediction using whole genome sequencing at this time. First, there's not always a clear correlation between genotype and treatment success. More basic research is needed to better identify and unitypically explain antibiotic resistance, especially for the vast number of uncharacterized microbes out there. One way this will get overcome hopefully in the future will be to use machine learning approaches to decipher these complex resistance mechanisms. A second reason for non-widespread use is turnaround time. In most circumstances, genomic resistance is slower than conventional methods which typically take under 72 hours. And then third is cost and with that the equipment resources necessary. Conventional methods have been used for decades and have been optimized fiscally and those are significantly cheaper than current genomic approaches right now. And then the last thing limiting widespread use is the trained workforce necessary to implement it. So, full implementation would require a larger number of trained molecular technologists and bioinformaticians that are currently just not yet available.

Bob Barrett: Finally, and I'd like to hear you both answer this question: how do you see next generation sequencing in clinical microbiology evolving in the next few years, maybe even out five or ten years from now?

Robert Potter: So, I'd say, I am a little biased because of my background in next-generation sequencing, but I believe that it will become an important adjunct to conventional lab testing especially for large academic labs, reference labs, and any high-volume public health lab. One often heralded use of next generation sequencing that we don't explicitly focus on in our review is organism identification. I think that the biggest hurdle for this within the realm of at least cultural organisms is that MALDI-TOF is an amazing technology with the chief limitation that it really works best with a pure culture, but it is a big hurdle for next-generation sequencing to get over. I think therefore one potential use for NGS is in the metagenomic application for parallel organism identification and elucidation of all those antibiotic-resistant

determinants without actually having a bacteria that you need to grow. And among the number of institutions are making some great progress in the use of metagenomic next generation sequencing as a lab developed tests, including UCSF, Mayo, and Johns Hopkins, and it's an exciting time to be in the field.

I think some technological advancements on the horizon are going to continue to improve sequencing costs and turnaround time. Generally, I'd say right now Illumina sequencing is considered the most cost-effective and for the applications of identifying antibiotic resistance genes or mutations discussed in a review, the draft genomes produced by the shorter Illumina reads are accessible for use, but one emerging technology is the Oxford Nanopore sequencing platform and its uses in clinical setting. An advantage of this system is that the reads come off the sequencer in real time, whereas for Illumina technology its batch for 24 hours to 48 hours, which means that you're able to process these reads immediately and therefore can give almost instantaneous identification of the organism and elucidation of the antibiotic resistance genes. And these reads are also long which can help resolve difficulties in determining whether or not your antibiotic resistance gene is on a plasmid or a chromosome and we would argue that this plasmid born resistance genes are very important from an infection control standpoint.

Eric Ransom: Yes, I agree with what Robert said that genomic resistance prediction will play an increasingly important role in clinical microbiology labs moving forward. With that being said, I do not anticipate traditional phenotypic approaches going away anytime soon. The field of clinical microbiology has undergone impressive changes over the last several years and phenotypic testing has proved itself over and over again. So, I think while there will be dramatic changes to the field coming up, I think you'll see an important role for both of these technologies moving forward, and what an exciting time to be in the field of clinical microbiology.

Robert Potter: So, I'd say, to sum things up for our listeners, genomic resistance prediction is still in its infancy in regards to clinical microbiology. But I believe that it's already shown some utility in specific situations. The role of genetic resistance prediction will hopefully continue to expand as our understanding and appreciation of this technology grows and as the workflow and computational resources become more aligned with routine clinical use.

Bob Barrett: That was Dr. Robert Potter and he was joined by Dr. Eric Ransom in this podcast. They and other colleagues from the Washington University in St. Louis co-authored a review article titled, "Genomic Prediction of Antimicrobial

Resistance: Ready or Not, Here It Comes!" You can find that in the October 2020 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.