

## Harvard Catalyst | Predicting Antibiotic Resistance in Gonorrhea

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It is estimated that there were over one million cases of gonorrhea last year, both reported and unreported. With such a high burden of disease, there are highly effective diagnostic tests that can precisely detect presence of gonococcus. For some time, these tests have been able to determine the antibiotic susceptibility of a strain.

However, with cases and resistance on the rise, Dr. Yonatan Grad and his lab at the Harvard T.H. Chan School of Public Health would like to better identify where we may see antibiotic resistance. Dr. Yonatan Grad is an assistant professor in the Department of Immunology and Infectious Diseases at the Harvard T.H. Chan School of Public Health, and an attending physician in the Division of Infectious Diseases at Brigham and Women's Hospital. Dr. Grad, thank you for joining us. Welcome to the show.

Thank you very much for having me.

You study infectious diseases, and in particular, gonorrhea. Why is gonorrhea an important infection to study?

So I'll list a few different reasons. But very broadly, I think of it as an important infection to study because there is a large and increasing burden of disease. So there are over 550,000 cases reported in the US last year alone. And the true incidence is expected to be at least two times that. And globally, it is also a very common bacterial infection.

The second reason is that there is also an increasing burden of antibiotic resistance. So as we have seen in many other pathogens as well, gonococcus has regularly developed resistance to each of the antibiotics that we use to treat it. From first with penicillin and sulfonamides through most recently ceftriaxone and azithromycin. There have been treatment failures even with this dual regimen, and that is the current recommendation from the CDC and other institutions. And with that, I think the threat of resistance makes it particularly important.

Third, it seems like there are opportunities for different types of interventions. The way we approach gonorrhea clinically, first in diagnosis, is often through nucleic acid amplification tests. And those kinds of tests, while sensitive and thereby enabling us to identify infections, they don't inform on antibiotic susceptibility. So if we can develop new types of diagnostics that not only can be used point at the point of care, but also can inform on antibiotic susceptibility, that could enable us to have a greater arsenal of strategies for tackling antibiotic resistance. And in that way, gonococcus can also become kind of exemplar, or archetype of different strategies for trying to tackle the problem of antibiotic resistance that we could then think about applying to other types of bacteria. So in those ways, it really becomes a kind of model system that we can use to think about approaches to the great challenge of antimicrobial resistance.

And the nucleic acid amplification test that you mentioned, that's a test to identify gonorrhea in somebody? But it doesn't tell you whether it's resistant to antibiotics, or not.

That's right. So traditionally, the method for diagnosing gonorrhea, in fact, the first kinds of approaches involved bacterial culture. Where you take a sample from an individual you suspected might have an infection. And this is, for example, someone who might come in with, in the medical or clinical jargon, [? prurient ?] urethritis. Basically, pus coming from the penis, from the urethra, and pain with urination, burning sensation. They would swab the material and plate it out on media that would facilitate growth of the bacteria.

They could then see, does gonococcus, which is another name for *Neisseria gonorrhoeae*, the bacteria that causes gonorrhea-- they could see if it grows. That growth then becomes a basis for testing antibiotic susceptibility. Once you have the bacterium culture, you can test whether it is sensitive to a panel of different antibiotics. That was the traditional approach for diagnosis. And that's the way we can characterize the resistance phenotype. Or basically, what drugs the bacteria are susceptible to.

The nucleic acid amplification test is a more recent introduction. It's a very sensitive kind of approach, and one that can be used noninvasively. So you don't have to use a swab that goes into the urethra. You could take a sample of urine, for example, and look to see, is there DNA from *Neisseria gonorrhoeae*? It's very sensitive. So we can see, is there any evidence of gonococcus present?

And from that, if there is, we know that wherever it's present, we think of it as an obligate human pathogen. Wherever we find it, that's an indication that there is an infection. And that becomes a basis for treating. But it does not tell us on its own about antibiotic susceptibility. For that, at least to date, what we've required for phenotypic assessment-- again, the physical manifestations. Can it actually grow or not? That requires culture.

Over about the past seven or so years, cases have been going up. It's interesting, actually, just yesterday, the CDC numbers came out and showed a 5% increase from 2017 to 2018. But from 2016 to 2017, there was an 18% increase. And in the UK, the increase to 2018 was over 20%. So it has been increasing fairly dramatically over the past few years. So after many years of decline, we have seen a rapid increase such that the numbers are about as high as they've been in 30 years.

Why do you think that is?

So there are a few ways to think about the rates of infection for various infectious diseases. Generally, we think about whether there are ecological phenomena that lead to cycling of rates of infection. Things like population wide immunity can contribute to the fluctuation in rates. But for STIs, or sexually transmitted infections, one of the things we also think about is whether there are changes in behavior that might contribute.

For gonorrhea, it's not clear how much immunity actually exists in the population. But it is clear, at least from a behavioral perspective, that there are some clues that might be-- that behavior, changes in behavior might be what's at the root here given that syphilis and chlamydia rates have also been increasing. So with the rise of all the STIs concurrently, that points towards behavioral changes as one of the drivers, potentially, for the rapid rise in gonorrhea incidents. That change in behavior may also be related to advances in the care and prevention of HIV.

Right.

As we have seen that undetectable levels of HIV-- so when your HIV viral load is totally suppressed with antiretroviral therapy, we know that is, as people, say U equals U. That undetectable means untransmissible. So that is, people have been taking their antiretrovirals, and suppressing their HIV viral loads, it seems to be the concern for HIV acquisition is diminished. And this is also augmented by the widespread uptake of pre-exposure prophylaxis for HIV. So the use of Truvada, for example, as pre-exposure prophylaxis limits one's risk. And so as there has been behavior change with less condom use, there is more opportunity for the spread of sexually transmitted infections other than HIV. So there is speculation that this shift of the advances in HIV prevention may also be associated with a rise in STIs.

And so with a rise in STIs, and with more people getting infected with gonorrhea, does that lead to increased chance of resistant strains of gonorrhea? Or the development of resistant strains?

Sorry, does the higher incidence?

Yeah, more cases mean higher probability? Or more mutation into antibiotic resistant strains?

Yeah, not necessarily. There is some modeling work that suggests that with more treatment and more antibiotic pressure, we'll see the emergence of more resistance. But it's not entirely clear that that's necessarily the case. What we have seen is more resistance. And there are all sorts of possible reasons why that's the case. So one of them is that we're treating more. So we identify more cases. We use more antibiotics. We're driving resistance that way.

Another possibility is that gonorrhea spreads globally. And that much of our resistance may actually start in other places in the world where there is heavy antibiotic use. And then with the global spread of gonorrhea, as people move from place to place, we could start to see resistant strains emerging from other places as well.

Could you tell us about some of the work your lab is doing around gonorrhea and antibiotic resistance?

Yes. I would be delighted to. So we're asking a variety of questions, thinking about how we approach the problem of antibiotic resistance from several perspectives. First, on just a population level, what's happening? What are the trends in resistance? What are the trends in antibiotic use that might be driving that resistance? Mechanistically?

And then we use interdisciplinary methods, including genomics, mathematical modeling, and epidemiology to try to make inferences from what happens at a population level, and understand at a molecular level. For example, what is the basis for antibiotic resistance? Why is it that within the diversity of the gonococcus species, some lineages seem more able to acquire and maintain resistance than others?

We then work in the wet lab to try to validate those hypotheses. And then think about how we take those conclusions, and apply them back at the population level through the development of diagnostics, therapeutics, or public health interventions. How do we do surveillance, for example, for new types of resistance? So we try to ask questions at each of these levels.

Earlier on, we talked about how people are using nucleic acid amplification tests to identify gonococcal infections. One idea is that if we can not only look at DNA to find the presence of gonorrhea, but can also use the DNA to identify the presence or absence of resistance determinants. Maybe we can rapidly predict from the genotype the resistance phenotype. And if we can confidently say that a individual's infection is going to be susceptible to a particular antibiotic, it would enable us to use that antibiotic rather than treating empirically with the last line antibiotics that are all that remain to us.

For example, ciprofloxacin, a fluoroquinolone antibiotic, used to be one of the antibiotics we would use to treat gonorrhea, empirically, because it was expected that ciprofloxacin would cure most if not all infections, but resistance to ciprofloxacin increased in the population. And it increased so much that we stopped using it because more than 5% of cases, and in fact now somewhere around 30% of cases, turn out to be resistant at least in the US. In other places in the world it's even much higher than that.

But we know that resistance to ciprofloxacin is achieved through a particular mutation. In this case, it's a mutation in the gene gyrase A. It's one of the-- The product of *gyrA* is targeted by ciprofloxacin. There's a mutation in position 91, and if that mutation is present we expect that the bacteria will be resistant. But if that mutation is not present, then we expect that the bacteria will be susceptible to Cipro.

So if we could have a rapid diagnostic that identifies what's going on at that position we can take Cipro back off the shelf and now use it where before we weren't using empirically. Now we can actually use it because we would be confident that the bacteria are susceptible.

So if we can develop and expand beyond just that one example to think about predicting susceptibility for a variety of different antibiotics-- Even going back to penicillin perhaps now with greater choice among different antibiotics-- we can not only slow the emergence of resistance to our last line antibiotics because we'd be using them less, but perhaps by having a range of choice we can slow the emergence of resistance generally.

To do that we need to know what the genetic basis is of resistance and how well we can predict susceptibility. So that means not only being able to identify those that are resistant, but being able to confidently identify those that are susceptible. Our lab has been working on trying to do that. So both to ask how well do our known resistance determinants account for resistance and then what we call the negative predictive value. So in the absence of those, how well do they actually identify susceptibility.

We have in the process that used the population genomic data-- So this is where we go from population levels and use statistical inferential methods to ask first, how well can we account for what we observe, the resistance that we observe, through known resistance determinants. And then if there are ones where we haven't been able to explain their resistance, can we take those to the lab or use statistical methods to generate hypotheses as to which variants, which mutations, might explain the resistance. Test those in the lab and through that kind of approach we've identified new pathways to antibiotic resistance.

Now with that kind of characterization those can be incorporated into new diagnostics along the lines of what I described for ciprofloxacin. We've also been thinking about, then, how do you apply this kind of approach at a population level given that we expect-- You know we're up against nature and natural selection. There will always be evolution of new pathways to resistance. If we're using genotypic method, that's fundamentally a prediction-approach. It's not actually testing the susceptibility. It's trying to predict from the genome what the susceptibility is.

We might expect to find that there are new pathways to resistance emerging or there are ones that have simply been under the radar of our current surveillance approaches that will blossom once we apply more selective pressures. So we've also been looking at what would a surveillance system look like? How many samples would you have to test? Are there ways to make this kind of approach efficient?

So that's one example of the kind of work that we've been doing. Where we go from population level down to molecular mechanism and then think about how that applies. We've also in the course of doing that work stumbled on something that I think is also incredibly interesting. As we've been trying to characterize resistance and what explains resistance we found that there is actually-- And this is work that we have, that we're just preparing to put into a manuscript and publish, hopefully sometime soon. Not only could we identify variants that contribute to resistance, but we actually also saw that for a number of isolates the amount of resistance that we predict by the genome is much higher than their actual resistance. In other words, they are more susceptible than we would have predicted based on looking at their genome. They have resistance determinants, but they're still susceptible. So what's going on there?

It turns out that there is actually another genetic element that we've now identified that confers susceptibility. So it seems to be a suppressor of antibiotic resistance that has arisen multiple times in the gonococcal population. And we've been characterizing why that might be, but it points to another kind of idea for a therapeutic strategy, which is to combine something that can mimic this genetic phenomenon of suppressing antibiotic resistance with an antibiotic.

So in the same way that Augmentin or amoxicillin plus clavulanic acid contains clavulanic acid, which basically blocks the enzyme that breaks down amoxicillin and makes the bugs susceptible to amoxicillin again. Here we can think about other strategies where there are perhaps a molecular mimic of the suppressor that we've identified, combining it with an antibiotic we can render resistant isolate susceptible again. So that's a therapeutic strategy we're now also trying to explore.

OK. So the suppressor exists in the same genome as the resistant gene.

Correct.

So you could have a strain that has-- So you might look at the strain and say, oh it's resistant, but for some reason it's not. When we attack it with antibiotics it dies.

Exactly.

And so that's something that's new that your lab has discovered.

Yes. Yeah. So we're-- And we have-- So I should say that, in fact, the observation of antibiotic hyper-susceptible clinical isolates of neisseria gonorrhoeae actually was first reported in a paper in nature in 1978. But at the time they had two questions. One was what actually is the mutation here? And the second is, why does it appear in clinical isolates at all? You would think that there is just pressure for continued aggregation of resistance, not to become susceptible again.

Susceptibility, right.

So really the innovation, I think, that we have in this work is to show that this susceptibility, this suppression of resistance, has appeared many times in the gonococcal genome. We can estimate just what fraction of isolates have it. But the other thing that we show here is that it's associated, we believe, with the site of infection.

The site of infection in the body?

Correct.

OK.

Yeah. So that gets to the question of why would these suppressors appear? It must be that there is some selective pressure that drives some change in the genome. And it just so happens that that change also impacts antibiotic susceptibility. Our hypothesis was that this has to be something to do with site of infection because there aren't that many other things going on that would apply selective pressures to gonococcus. It can only live in a few different places in people.

And the antibiotics are-- There's a few different antibiotics. So we know about that.

That's right. And we also know that antibiotics are not a constant pressure.

Right. Right.

People can have asymptomatic infections. So in fact, when I mentioned before that the incidence, the true incidence of gonorrhea is expected to be at least two times higher than the reported incidence, it's because of these asymptomatic infections. So people can carry gonorrhea, in fact, most cervical and rectal and pharyngeal infections we think are asymptomatic. So people don't have symptoms when infected in those sites.

And what that means is that they're carrying gonorrhea without antibiotic pressure for the most part, unless they're getting treated for something else. And so the bugs have to adapt to those niches. We hypothesized that something in one of those niches may apply pressure that leads to a change and that change has a consequence on antibiotic resistance independent of the antibiotic pressures.

So it turns out that this variant that we observed that is associated with suppressing antibiotic resistance is more commonly found in cervical specimens. So we are guessing that there is something in the cervical niche, something about that environment that leads to, that selects for this particular change, and that that change just so happens to also increase susceptibility to antibiotics. And that's really I think the interesting intersection between the antibiotic pressure that we apply through treatment and the selective pressures that the bacteria experience through natural infection.

And you mentioned that you see this kind of work as sort of a template for looking at antibiotic resistance in other organisms. Could you talk about how you might look at antibiotic resistance generally based on the work you're doing with gonorrhea?

Yeah. So there are a couple of examples here. One is on the relationship between antibiotic use and resistance. We had a paper earlier this year where we asked whether the use of the antibiotic azithromycin in the population might actually be driving resistance in gonorrhea through something that-- in work that we've done with Mark Lipsitch, in fact that Mark has led-- is termed bystander selection.

So here the idea is that the antibiotic exposure experienced by a particular pathogen is in part attributable to disease caused by that pathogen, but it might be predominantly actually from disease caused by other pathogens. For gonococcus that can be carried asymptotically you can imagine that if you have gonorrhea in one of these mucosal sites, but you don't have symptoms you're not taking antibiotics to treat it, but let's say you have a strep throat at the same time. You will get antibiotics for your strep throat and those antibiotics will then also apply a selective pressure, not only to cure your infection, the throat infection, but also will cause an antibiotic pressure on gonococcus. So this is what we termed the bystander effect.

We looked at for azithromycin-- One of the antibiotics used to treat gonorrhea, but also one of the most commonly prescribed antibiotics in the population-- what happens over the course of a year. We know that there is seasonality to antibiotic use, particularly for azithromycin, where it peaks in the winter and tapers in the summer. And winter use is two to three times that of summer use. So we have these nice sinusoidal waves looking over years of azithromycin use.

If antibiotic use drives antibiotic resistance then we'd expect that resistance is the derivative of use. So derivative of sine is cosine and looking at the sinusoidal waves that peak in January, cosine is 90 degrees off or in this case for a 12 month period it will be three months away. So we would expect that resistance would peak three months after the peak in use.

And in fact, when we look at the-- using data from our collaborators at the CDC-- when you look at the cycling of resistance over the course of a year, we see not only does it cycle, but it does peak or around March, April in keeping with expectation. So something that population-wide antibiotic use seems to be driving resistance in gonococcus as well, indicating the presence of bystander selection.

Others have looked at data sets from Europe and have also identified population-level relationships between use and resistance. So this is one example of how work we're doing with gonococcus might be applicable to other pathogens. And then we're thinking about gonococcus again is an exemplar for some of these methods for identifying new resistance determinants that we could feed into diagnostics, and also how we can best do surveillance for the emergence of new resistance determinants. So those are just a few examples of how we're hoping some of the methods that we're developing and demonstrating with gonococcus might also be applied to other pathogens.

Well, Dr. Grad, thank you very much for coming in. It's was a pleasure to have this conversation with you.

Thank you. It's been really fun.

Next time on ThinkResearch.

We are now using next generation sequencing to say which treatment we should use on a patient that sort of matches the mutation to the treatment [INAUDIBLE] of the tumor. What we'd love to get to though is you know you're able to look at the sample, predict who's going to get disease, and then do something so they never get it.

Dr. Georg Gerber discusses his approach to studying c difficile, combining genomic sequencing, machine learning, and precision medicine.

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