MYCOPLASMA GENITALIUM LABORATORY DIAGNOSIS: NEED FOR PRECISION BASED TREATMENT APPROACHES IN THE ERA OF RAMPANT ANTIBIOTIC RESISTANCE

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8/22/2018
Mycoplasma Genitalium Laboratory Diagnosis: Need for precision based treatment approaches in the era of rampant antibiotic resistance

[video transcript]

[00:00:05] Thank you Dr. Urban for this wonderful opportunity. I've never presented in a venue of this sort, so this is incredibly interesting and seems like the way towards the future. And the topic again is the diagnostic landscape for mycobacterium, sorry Mycoplasma Genitalium diagnostic testing, especially with its emergency as an important STI and particularly, in the era of rampant antibiotic resistance. And I'm using the word very carefully. Don't emphasize rampant.

[00:00:46] I've no relevant financial relationships or conflicts of interest.

[00:00:54] So in preparing for this talk I reached out to Dr. Urban and asked her, "What are the kind of issues that primary care providers and clinicians would be most interested in with relation to this topic?", and I got some feedback from her. So most of my talk is designed around these topics but I think it's worthwhile to know, to discuss this and to keep these points in mind as I go through this presentation. So this is this is the response from Dr. Urban, non-gonococcal urethritis in men often gets treated. Syndromically. If persistent, empiric treatment with multiple course of antibiotics is genuinely used. Many providers would like to test for Mycoplasma Genitalium up front and avoid the multiple courses of antibiotics. And then when treatment actually fails, the providers are sometimes left wondering is this actually resistance or is it noninfectious urethritis. There are similar issues in women, although this is less common. What will be of interest would be to review the current available options for testing Mycoplasma Genitalium for commercial and research use only type tests, and also discuss the resistance and resistance testing issues. More specifically, what are the options available in New York and also what are the ideal specimen types for testing? This is the first time that I heard about this but Dr. Urban also mentioned that now there are home test kits available for STD testing. And, you know, is there any information out there in terms of their efficacy? There are companies like myLAB, and a bunch of other ones that send you these kits; most of them are, again, molecular tests. And what about testing of other genital mycoplasmas? Example, Ureaplasma. So I'm hoping that by the time the presentation is complete I'll have answered most of these questions.

[00:03:12] Some of the main learning objectives for this presentation towards the end of this talk. The participants should be able to describe the basic characteristics of Mycoplasma Genitalium and other genital mycoplasmas, recognize the impact of this organism on reproductive and sexual health of both men and women, and identify their margins of drug resistance. And discuss the, they should be able to discuss the need and methodologies for accurate diagnostics of Mycoplasma Genitalium. So, mycoplasmas belong to this class of bacteria known as mollicutes, these are very special kind of bacteria; they are bacteria without cell walls. So essentially, an entire class of antibiotics, the B-lackams, are completely ineffective against them. The clinically relevant genital mycoplasma's include Mycoplasma Hominis, Mycoplasma Genitalium, Ureaplasma Urealyticum, and Ureaplasma Parvum.
These genital mycoplasmas are characterized by very small genomes. And they are thought to arise from other gram positive bacteria, like clostridia by genome reduction. I wanted to point this out because when their genome, small genomes, they have fairly limited biosynthetic capabilities. So when you're trying to grow them it's actually quite difficult. Many are actually unculturable, axenically. Which means you cannot grow them on your typical clinical microbiology labs, on plates and media. And the ones that you can, you need very special media for it. Just wanted to put this out there, there are there are way way more thick in shape. This is this is actually Mycoplasma Genitalium. Mycoplasma Genitalium is the new kid on the blocks. Compared to the other genital mycoplasmas, it was discovered more recently. This is in 1981 and this is isolated from two men with symptomatic NGU. You can clearly see a flashy morphology of this particular bacterium; so, something unique about these mycoplasma. As I mentioned, these bacteria have very small genomes, as a matter of fact, Mycobacterium, Mycoplasma Genitalium, sorry, is the smallest genome of all bacteria that are out there. About less than, a little bit more than half a million base pairs including about 525 genes. And compare comparing and contrasting with E.coli, E.coli is about eight times bigger and about 4000 plus genes. The natural habitat of the genital mycoplasmas is the human genital urinary tract. They adhere to the mucosal surface epithelial lining, generally not found outside this environment except in a few instances such as an immunodeficient setting, as in the case with very low birth, preterm, premature infants. One thing that I wanted to point out is genital mycoplasmas, especially Mycoplasma Genitalium, has the ability to vary the immunogenic proteins on the cell surface. And this is a very important property that allows them to avoid the immune response and persist from months to years in the same host. This just a lazer scanning, confocal microscopic image of infected uroepithelial cells. Where you the blue stain corresponds to the nucleus and that green stain corresponds to the Mycoplasma Genitalium. So what about Mycoplasma Genitalium and disease? So, I mean, in men there's been a lot of evidence for quite some time now, almost decades, that it is, or more, that this is this is one of the leading causes of Nongonococcal Urethritis. Up to 15 to 25 percent of NGU cases are because of Mycoplasma Genitalium and in case of persistent NGU the numbers are as high as 30 percent. In women, previously this topic was in terms of the morbidity of this organism. This was a little controversial and less clear. But newer studies have shown that, o. Have studies worldwide that have shown a strong link between Mycoplasma Genitalium and diseases like cervicitis, PID, and infertility. A study meta-analysis of these many of these publications has demonstrated up to a two-fold increase in risk for some of these diseases. So there is growing evidence that this is also very important pathogen in women and not just a bystander bystander. There is a strong association between both HIV acquisition and transmission, and Mycoplasma Genitalium infection. Essentially, the Mycoplasma Genitalium infection causes a lot of inflammation of the genital tract and this supports, basically, HIV acquisition and transmission. So what about in general and mycoplasma's? So this is. Just one second. I cannot see it. So what about other genital mycoplasmas? So this slide is a little bit busy but towards the left, you see the different conditions of the different genital mycoplasma's and "A" corresponds to association and "C" corresponds to causation. So most of the data that's out there is from studies in Mycoplasma Genitalium and as such, which in itself is a lot of studies are in progress. But when it comes to some of the other mycoplasma, there are that he's out there. But there are some some interesting associations than causations. So, for example, Mycoplasma Hominis is strongly associated bacterial vaginosis and it's there's moderate evidence or association, or actually causation, of pelvic inflammation disease. The Ureaplasma species there's actually strong evidence for causation of acute NGU, especially in young
patients. And there's some interesting associations with neonatal lung disease. So, what about prevalence of Mycoplasma Genitalium? So there is very limited data available regarding this but from this studies it seems like in the general populations the number ranges from 1 to 3 percent. And which is higher than the prevalence rate of Neisseria Gonorrhoeae and lower than the prevalence rate of chlamydia and trichomoniasis. In the high risk population in the U.S., this was among the center of clinical cohort study. The rates were as high as 16 percent for females and 17 percent for males, and a large percentage of these subjects were asymptomatic. I wanted to mention this because I think these numbers are actually underestimated. And this will become clear as we move through the presentation and you will see we have more sensitive, more sophisticated methods for detecting this, and as more sensitive methods are available now, this prevalence rate seems that initially identified prevalence rates seem to be lower. This will become clear in a little bit. A major issue with emergence of drug resistance Mycoplasma Genitalium is the syndrome management of non-gonococcal urethritis. So, this particular tree of these treatment regimens are primarily focused on eradication of Chlamydia Trachomatis. So, the three levels of syndromic treatments include "A" which is 7-day doxy regimen, and this is actually very ineffective against Mycoplasma Genitalium with very high failure rates. Mycoplasma Genitalium, there's a lot of, there's a number of studies showing the correlation between resistance failure and resistance with tetracycling. But the exact determinants of resistance are not really well understood. The second option is a single dose azithromycin, one gram. This is the first line therapy against Mycoplasma Genitalium in the past this was affective but this is not sufficient for Mycoplasma Genitalium and can actually lead to drug resistance. And the increased prevalence of drug resistant strains, the numbers can be as high as 40 percent, This treatment can also be rendered ineffective. There's a lot more information about the determinants of resistance and for Azithromycin. And these ones are mutations in the 23s ribosomal RNA gene. Positions 2058 and 2059. The second line treatment for all Mycoplasma Genitalium is Moxiflozacin. And this one is a multi-day regimen. Of course this is more expensive and requires more patient compliance but the report but the cure rates are fairly high. Nevertheless, there is concern about using this [inaudible]. There's talk in the literature but using this regimen very judiciously, due to emergence of drug resistance that has been reported in Japan, Australia, Europe. And for Moxiflozacin, the resistant determinants are primarily mutations in the parC gene, that encode Toposiomerase 4, and this corresponds S83 and D87. So, in the ideal world while we would not do syndromic management, we would do perception management. And so you would have specific FDA approved diagnostic tests for Mycoplasma Genitalium, like we have for Chlamydia and Neisseria Gonorrhoeae. This will be followed by detection of drug releases. As I mentioned, this was allowed for precision based treatment but that is not the case. So they're certain challenges associated with clinical testing especially identification of Mycoplasma Genitalium. Mycoplasma Genitalium is unculturable axenically. You can grow it but it's very fastidious, it needs cell culture, very few specialized labs in the world, a handful of them can actually do it. It takes about six months to grow, so in a clinical setting that's actually useless. Again, this organism is very fragile, it doesn't have a cell wall so you actually need very special attention to media and transport conditions. So, for our purposes this is not really an option. Again, screening methods do not apply here because it doesn't have a cell wall. There are serology tests that have been developed; these are immunofluorescent immunoblotting methods. But, to me they are not available in the clinical setting, primarily because they show poor sensitivity. You have to remember that mycoplasma's can enable antigenic variation, this also contributes to decreased sensitivity of some of these tests. The right course for Mycoplasma Genitalium diagnosis has been
nucleic acid amplification test or the NAAT test. And these are available but they generally not offered by typical clinical labs they’re offered mostly by big reference labs. And there is a whole slew, there is not one standardized test there is a whole slew of tests; some of them are Singleplex PCR tests others are Multiplex PCR tests. And as I mentioned many of them are developed in the house or home-grew tests. But now, there are some more commercial options that are also available. So just to compare and contrast what’s available there, there is a lot of options available for Chlamydia Trachomatis, Neisseria Gonorrhoeae, even Trichomonas testing has the approved platforms, so that is not the case for Mycoplasma Genitalium. Now kind of looking into what, why that was. I think for the longest period of time, people were really debating the role of of this organism and morbidities specially in woman. And I think now there is more data, convincing data, regarding disease and reproductive and sexual health in women. And I think we we should have more platforms approved in the coming years. So now we are left with, at this moment in time, you are left with this number of tests are not FDA approved. So how and in many instances, although that reference labs provide these tests, the actual performance characteristics of these tests are not really described as specified. So when the clinicians actually order these tests, how do they make sense of the accuracy of the test? What if the test actually has very low sensitivity? Would that mean that if you get a negative result is it a false negative? So what do we do here? So, the University of Rochester Medical Center we offer genital mycoplasma testing, as a send out list the ARUP reference lab. We do molecular testing and we have a bundle. So we have a multiplexed qualitative PCR test. It’s when as I mentioned there is no test that is FDA approved and this is a lab developed test. And it detects before clinically relevant. Mycoplasmas and innocuous issues. They acceptable specimens for these include gentle swabs, urine or cervical and vaginal specimens. And the turnaround time for this test is about three to five days, and this one is New York State Department of health approved test. My animations have been a bit messed up but they're different targets for each of these organisms in the molecular test. We also offer culture, but this is restricted to Mycoplasma Hominis and Ureaplasma species And there is more choice for acceptable specimens there, there's body fluids, CSF, respiratory, semen samples, et cetera. Mayo Clinic also offers a Mycoplasma Genitalium test, but currently there is no test offered for detection of resistance. So cultured, the turnaround time is about eight days and again this one is also a New York State department of health approved test. This is how, this is a quick overview on how the culturable mycoplasma’s look like. So on the top panel, you see Ureaplasma Urealyticum and Mycoplasma Hominis, and this is not a very specialized media known as the A7 agar. These come up in about a week. The Ureaplasa Urealyticum colonies look black and the Mycoplasma Hominis have this fried egg kind of appearance. But only the genital mycoplasma that we sometimes get on our routine clinical microbiology media is Mycoplasma Hominis. But this one is actually, this one has poor sensitivity on the routine media that we use. But, again, sometimes we observe when the plates are kept for a longer time, and they have, they’re very tiny and have this characteristic dewdrop like appearance. So coming back to NAAT testing options for Mycoplasma Genitalium. As I mentioned, most of these tests are laboratory developed tests and commercial tests. None of them are FDA approved, but one of them is CE marked. So, I'm not going to go through this entire chart and this is actually just a select few available options that I want to show you, but, this list can be much much longer. And as you can see the red arrows corresponds to CE marks, so, CE mark corresponds to conformity European and these ones are marketed in Europe and Australia. The only test that both identifies Mycoplasma Genitalium and detects Azthromycin resistance, is this new test out of Australia; the resistance plus MG by speeDX but this one is only marketed in Europe and in Australia.
The one test that has generated a lot of interest and this is from kind of going into the literature is this new test developed by Hologic, APTIMA. And this one is, again, a CE approved test. We have this particular test uses a different kind of amplification methodology, which is known as Transcription mediated Amplification so it's a little bit different from the conventional PCR. And they have commercialized this particular testing for this platform known as Panther, this is a new platform coming out of Hologic that permits automated specimen processing in a high throughput manner. And in the U.S., analyze high quality, analyze specific reagents available. To ease our reagents that are very useful for clinical laboratories to meet the quality requirements of the CLIA standards. So this actually is a very promising test and based on the manufacturer claims that they are on their way towards [inaudible] purpose test. You will be hearing more and more about this test. This is just a little bit, quickly, about Transcription Mediated Amplification. It's based on instead of DNA amplification and DNA amplicons, were here you're relying on RNA transcription and amplification and uses two enzymes known as RNA polymerase and reverse transcriptase. It's very rapid, isothermal, it generates billion fold amplification in minutes. It's very sensitive. So there is, there is a number of studies following this particular platform coming into the market. The one study that I thought was particularly well-designed and very useful is the study out of Denmark and Norway and Sweden. So this is the Nordic APTIMA Mycoplasma Genitalium evaluation study or the NAME study, and this paper just came out in May 2018. This really the first large scale prospective evaluation of this particular platform. And so the goals of this study were to obtain clinical to, to do clinical and analytical evaluation of this platform and also to obtain data on Mycoplasma Genitalium prevalence and antimicrobial resistance in the three Nordic countries in 2016. So their methods involved, basically their timeline was was a year February 2016 to 2017. They had about 5269 patients, both male and female, for consecutive attendees in three STD clinics each in each of the countries. Over a wide variety of sample types urine, vaginal, urethral, and rectal swabs. And comparing this test, they used another transcription mediated amplification test, which is researching only, which uses a different target. And a lab developed test. This is a PCR test and this is the one that's routinely used in many of the epidemiological studies. So a lot of studies that are out there or reference labs use this particular real-time qPCR test Out getting the mgpB gene. They defined the true positives in their study as "Identified as positive in at least two out of the three tests that are compared with each other. So what were their results? So out of these 5269 patients, they found 382 to be positive. So The prevalence rate was about 7.2%. And this was consistent with what's reported in the literature, lower than Chlamydia Trachomatis and higher than Neisseria Gonorrhoeae. In the pooled data, the platform did very very well. The sensitivity was about 99 percent, greater than 99 percent, and so was the specificity. But other research use, only APTIMA has also performed quite well. When you compare and contrast it with existing PCR, the PCR test was actually had much more sensitivity compared to these tests. The sensitivity range from about 73 to 81 percent but the specificity is even higher. And so their findings were actually consistent with some of the other studies. There was a study from France, and the study that I’m showing here is a study from Australia. And again, that the numbers were a very high sensitivity as compared to PCR methods. This is so, in their paper, and if somebody is really invested in this topic I highly recommend looking at the different tables because it will break it down by different countries, Male versus Female, and they break it down in many many different ways. There's one particular table that I wanted to show. So, over here the red boxes correspond to the different samples. So, on the top red box, you see urethral samples from females. And for these, if you compare the sensitivity of the PCR verses the sensitivity of the TMA, you can see that the sensitivity of the PCR was
particularly bad about 50 percent. In the next lower red box you see rectal samples from male and you see a similar trend. So I think this is this was a very interesting data set. So the conventional PCR actually missed greater than 20 percent on average across all the different specimens of positive samples. So this has a lot of implications for previous studies which use PCR, you know, for determining their prevalence rates. Suggesting that Mycoplasma Genitalium in the previous studies, was likely underestimated. So these were the conclusions of the study. Again, it was a very large study with about greater than 5000 patients. The transcription-mediated amplification was much better than the PCR assay. I didn’t talk about this much, but they also looked at the prevalence of resistance. And among these positives, the rate of Azithromycin resistance was high, about 41 percent and Moxifloxacin was about 6 percent. And the big, sort of broad message or conclusions from this study were, validation and quality assurance studies are required for different kind of testing platforms. Really large scale studies are required in different parts of the world, to truly understand the real prevalence rate, and surveillance is crucial to identify local trends. This is something that I put in there, I think that this particular platform offered by Hologic is a very sensitive method and it represents a true new gold standard. So as more and more products come out in the market one should look out for comparisons with this methodology, as compared to the older methodology to really establish the actual sensitivity and efficacy of the testing methods. Just very quickly, this is the only platform that's out there that does simultaneous ID and Azithromycin. This is again, resistance plus MG, offered by an Australian company. They did some testing of this, but again over here they compared their test with a conventional PCR test and they found a 99 percent concordance, what I would have liked as comparison with the TMA test. Currently, it's only carried in European and Australian markets an currently this company is seeking FDA approval for the US market. So you might hear about this in the future. So the general take home points from this presentation are: there are high prevalence rates of Mycoplasma Genitalium and antibiotic resistance is increasing. There is an increased move to create an awareness about this particular pathogen within the clinical community and the public at large. The syndromic treatment regimens for NGU are not necessarily efficacious against Mycoplasma Genitalium and in fact are commuting to the emergence drug resistance. This makes for an argument that an accurate diagnosis of Mycoplasma Genitalium will help you tailor your regimen and prevent the emergence of drug resistance. In terms of diagnostic options this organism is not culturable in most labs, even if it is there's very few labs in the world that can actually do it. So the option that's available is nucleic acid testing. There's no current FDA approved methods as yet, but you might hear of at least a few in the near future. The most promising platform seems to be this Hologic APTIMA MG assay, this is CE marked. And the Nordic NAME study suggests that the previous prevalence association and causation data might have been hampered by the lower sensitivity of some of the PCR methods that were used in the past. In terms of detection of antibiotic resistance for most labs, this is still done in the classical way, this is using sequencing which is very tedious and labor intensive. And it is primarily done for research and surveillance purposes. I've really not seen it being offered by many many clinical labs because of the clinical nature of the test. there's a single platform that's out there that's being marketed in Australia and Europe. And Lastly, these are some points that I came across through reading in the literature when covering this presentation. But for Mycoplasma Genitalium also, again, while Moxifloxacin is promising, there is emergence of drug resistance. To be Bowsher. The community should start looking into the efficacy of other novel antimicrobials like Lefamulin, Gapotidacin, and also other dual antimicrobial therapy. Regular national and international surveillance tests are needed. And as new platforms and methods emerge large
comparative studies are needed. With that, I have come to the end of my presentation. I have left my email if anybody has any specific questions regarding, so please feel free to write to me and I’ll take any.

[00:32:32] questions.

[end]