This is the written version of our Hot Topic video presentation available at: MayoMedicalLaboratories.com/hot-topics

Welcome to Mayo Medical Laboratories Hot Topics. These presentations provide short discussion of current topics and may be helpful to you in your practice. Our speaker for this program is Dr. Bobbi Pritt, Director of the Clinical Parasitology Laboratory at Mayo Clinic in Rochester, Minnesota. Dr. Pritt discusses the testing and diagnosis of Trichomonas vaginalis.

Thank you Cara for that introduction. My topic today is Trichomonas vaginalis. I’ll discuss this underestimated pathogen, focusing on the different methods that are available for laboratory diagnosis.
Before I begin, I’d like to mention that I have no disclosures. However, I will be discussing the importance of testing males in addition to females for *Trichomonas vaginalis* infection and I’d therefore like to point out that the currently available commercial tests are not FDA approved for use with male specimens. Therefore, male specimens have to be validated independently by the testing laboratory before being accepted for clinical testing.
Also, because proper use of any test is essential for providing appropriate and cost effective patient care, I have a few points that may be helpful to you as you integrate *Trichomonas vaginalis* testing into your practice:

- First, testing is indicated for both men and women because both sexes can become infected and experience serious complications of infection.
- Second, there are a variety of tests available for *Trichomonas vaginalis*, and of these,
  - Wet mounts are the most widely used but have an overall poor sensitivity compared to other methods.
  - In comparison, nucleic acid amplification methods have the highest sensitivity and are now the preferred test for this organism.
- Finally, it is important to consider testing for other sexually transmitted diseases given the opportunity for coinfections in sexually active individuals.
So let’s begin now with a general introduction of *Trichomonas vaginalis*

- It is a flagellated protozoan pathogen
- Infection is called trichomoniasis
- Previously considered a minor “nuisance” pathogen
- The most common curable sexually transmitted infection (STI) in the United States and worldwide
- According to the Centers for Disease Control and Prevention (CDC), more than 3.7 million individuals in the United States are infected
This next slide shows a chart from the CDC that illustrates the estimated number of combined new and existing sexually transmitted infections in the US in 2008. Note that trichomoniasis is the third most common STI, and, of these, it is the most common curable infection. Actually, it is possible that trichomoniasis is even more common than shown here since it is not a nationally notifiable disease so these numbers are likely underestimated.
Epidemiology

- More women than men are infected
- Prevalence of infection *increases* with age
- The National Health and Nutrition Examination Survey (NHANES) study* found an overall prevalence of 3.1% (2.3 million) in reproductive age women
  - 1.3% in white women
  - 1.8% in Mexican American women
  - 13.3% in African American women


Regarding the epidemiology of infection, it is important to note that

- More women than men are infected and
- The prevalence of infection actually *increases* with age, with more individuals being infected above the age of 25. And this is in contrast to chlamydia and gonorrhea in which most infections occur in individuals under the age of 25.
- In the United States, the NHANES study found an overall prevalence of 3.1% (2.3 million) infections in reproductive age women. And when broken down by race, the investigators found prevalence rates of
  - 1.3% in white women
  - 1.8% in Mexican American women, and
  - 13.3% in African American women, clearly the highest risk group in this study.
This next slide shows the life cycle of *Trichomonas vaginalis*. Humans are the only known host and

- there is not thought to be an environmentally resistant cyst stage, unlike other protozoa that infect humans. Instead there are only trophozoites which are not capable of surviving in the environment for very long.
- Therefore, transmission requires direct contact with infected secretions, typically during sexual intercourse.
- After transmission, the trophozoites live in the lower genital tract of women and the prostate and urethra of men.
- You can see a photograph here of a Giemsa-stained trophozoite.
- The trophozoites multiply by longitudinal binary fission and then they
- Produce more trophozoites, thus perpetuating the infection in the host.
For pathogenesis, the organism causes damage when the
• Trophozoites attach to immune cells and epithelial cells
• And they secrete proteins that cause local inflammation and cellular destruction
  • Punctate hemorrhages
  • “Strawberry cervix” seen on colposcopic exam

For pathogenesis, the organism causes damage when the
• Trophozoites attach to immune cells and epithelial cells
• And they secrete proteins that cause local inflammation and cellular destruction
  • This results in punctate hemorrhages
  • And in women this is manifested as what is called the “strawberry cervix” which can be seen on colposcopic exam
The actual clinical presentation varies widely between hosts.

- In women, 70% of infections are asymptomatic.
- But when patients have symptoms, they can range from mild to severe and include:
  - Vaginal itching
  - Burning with urination
  - Production of a thin, frothy malodorous exudate that can be white, yellow, clear, or green and you see an example of an exudate here
  - And pain with intercourse (dyspareunia)
In men, the clinical presentation may include

- Urethral or penile pain and irritation
- Burning with urination or ejaculation
- Penile discharge
- Epididymitis
- Prostatitis
Clinical Presentation - Continued

• In men and women, symptoms can occur within days after infection or months to years later

• Complications:
  • Increased risk of acquiring and transmitting other STIs (eg HIV)
  • Preterm delivery
  • Low birth weight (<5.5 lbs)
  • Infertility (men and women)
  • Possibly cervical neoplasia/carcinoma

It’s important to note that
• In both men and women, symptoms can occur within days following infection or months to years later. Therefore, new onset of symptoms doesn't necessarily imply that the patient was newly infected.
• It is also important to be aware of the possible complications of trichomoniasis:
  • These include an increased risk of acquiring and transmitting other STIs such as HIV
  • And in pregnant women, infection may cause preterm delivery
  • And low birth weight
  • In both men and women, infection may cause infertility
  • And there is a possible link of infection to cervical neoplasia and carcinoma, although further studies are needed to really elucidate this risk.
For diagnosis, because

- The clinical presentation is nonspecific; infection can be confused with other STIs, yeast infections, and even bacterial urinary tract infections
- Therefore, you need laboratory testing for accurate diagnosis. There are multiple methods that can be used to diagnose infection
  - Traditionally, the microscopic examination of unstained or stained secretions has been used
  - *Trichomonas vaginalis* can also be cultured.
  - And more recently, antigen detection assays
  - And nucleic acid detection assays have become commercially available, and they represent an important step forward for sensitive detection of this organism.
Diagnosis – Microscopy

- The most commonly used method is microscopic examination of unfixed, unstained secretions or urine for motile trophozoites
  - “Wet mount”
- Fast and practical; can be performed in the physician’s office
- Also allows for identification of fungal elements and “clue cells” of bacterial vaginosis
- Suffers from low sensitivity (40%-70%)
- Must be performed within 2 hours

• The most commonly used method for diagnosis is microscopic examination of unfixed, unstained secretions or urine, looking for motile trophozoites. This method is commonly referred to as the “Wet mount”
• It’s fast and practical and it can be performed in the physician’s office, therefore allowing rapid diagnosis and treatment before the patient leaves,
• It also allows for identification of fungal elements or “clue cells” that may be present in vaginal secretions
• The problem is that it suffers from low sensitivity, the sensitivity ranges from 40% to 70%.

And it also has to be performed within 2 hours of collection so that the fragile trophozoites don’t break down and become unidentifiable microscopically.
Here is a movie of *Trichomonas* from a wet mount, and you can nicely see the anterior flagella that are responsible for the organisms’ movement.
This next slide shows a still image that I captured from the previous movie in which the anterior flagella and the posterior axostyle can be seen. These are key morphologic features for identification. Once the trophozoites die, these structures quickly break down, making identification nearly impossible.
Clinical specimens can also be permanently mounted and stained using a variety of stains such as Giemsa, Papanicolaou and acridine orange. The image on this slide is stained with Giemsa and shown at 1000 times magnification. With this preparation, you can easily see the nucleus, the axostyle, and the flagella of the *Trichomonas* trophozoites.
This next slide shows *Trichomonas* as it appears on Papanicolaou stain in both Preservcyt and SurePath media. The arrows point to the organisms in these 2 images. Note that the diagnostic features are not as easily seen in this type of preparation.
Another microscopic method that is available is
• Direct immunofluorescent antibody staining
  • More sensitive than wet mounts
  • More technically challenging
  • Requires a fluorescent microscope
• Microscopic methods are subjective and require significant training for correct interpretation

Now, before we move on to discuss other methods of detection, I’d like to note that all microscopic methods just by their nature, are relatively subjective and they require a significant amount of training before the reader can be competent in identifying organisms.
The first nonmicroscopic method that I will discuss is direct antigen detection.

- Specifically, the OSOM Trichomonas Rapid Test, which is a point-of-care test
- It’s rapid, easy to perform, and it is the only CLIA-waived test on the market
- Unlike microscopic examination, it doesn’t require live or intact organism, so that’s clearly an advantage to this method.
- Also, the sensitivity is close to that of culture when testing symptomatic patients; unfortunately the sensitivity is much lower in asymptomatic individuals and therefore it’s not a good test for general screening.
- It is also only approved for vaginal swabs, therefore limiting the type of specimens that a lab can accept without performing additional validation studies.
Diagnosis – Culture

- Diamond medium, commercial kits (e.g., InPouch TV, BioMed Diagnostics)
- “Gold standard”
- More sensitive than microscopy
- Study of vaginal discharge from 105 women using wet mount, culture and direct immunofluorescence assay (DFA)*:
  - Detected 31 cases by culture, 26 by DFA and 21 by wet mount


Culture is also available, using a variety of media such as
- Diamond medium and commercial kits like the InPouch TV
- Now, culture is considered the “gold standard” because it is
- More sensitive than microscopy.
- In an older study in which vaginal specimens from 105 women were tested using wet mount, culture and DFA:
  - The investigators found 31 cases detected by culture, 26 by DFA and 21 by wet mount, therefore showing that of these methods culture is the most sensitive.
Unfortunately culture is

• Technically challenging
• Material must be freshly inoculated into media for best success of growing and detecting the organism
• Time consuming: 3 to 7 days to detect growth; cultures must be examined daily
• Less sensitive than newer molecular methods

Unfortunately culture is
• Technically challenging
• and the material must be freshly inoculated into the media in order to achieve the best success of growing and detecting the organism
• It is also time-consuming, requiring 3 to 7 days to detect growth and the cultures need to be examined daily.
• Finally, it is less sensitive than newer molecular methods that are commercially available and that’s what we’ll talk about next.
The first molecular method that became available is the

- **BD Affirm VP III**
  - Hybridization probes for *Candida albicans*, *Gardnerella vaginalis*, and *T vaginalis*
  - Does not require live organisms
  - Subjective color endpoint
  - Only FDA approved for vaginal swabs
  - May be too sensitive for normal flora (*C albicans* and *G vaginalis*)
The most recently FDA-approved molecular method for *Trichomonas vaginalis* is the:

- Hologic/GenProbe APTIMA TV Assay which uses transcription mediated amplification for detection of *Trichomonas vaginalis* ribosomal RNA.
  - It is the most sensitive commercially available FDA-approved assay, detecting down to 0.1 organisms/mL of specimen.
  - A recent study compared the performance of the BD Affirm and Aptima assays and found that the:
    - Aptima assay detected 36.6% more positive patients than the BD Affirm assay
Diagnosis – Molecular Methods Continued

- FDA approved for female urine, vaginal swabs, and cervical swabs (including specimens in PreservCyt (ThinPrep) media).
- Male specimens are not FDA approved but may be validated by individual laboratories.
- Specimens placed in Aptima transport media.
- Same specimen can be used to test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

The APTIMA assay is

- FDA approved for female urine, vaginal swabs, and cervical swabs (including specimens in PreservCyt (ThinPrep) media). However, unfortunately,
- Male specimens are not FDA approved. Therefore male specimens such as male urine, urethral swabs, and prostatic secretions must be independently validated by individual labs prior to being accepted for clinical testing.
  - All specimens are placed in Aptima transport media and the
- Same specimen can be used to test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.
This next slide shows a chart comparing the different testing methods for *Trichomonas vaginalis*. The average sensitivity from various clinical studies is shown for each method. Note that the sensitivity varies significantly between the different methods, with the APTIMA test having the highest reported sensitivity.

<table>
<thead>
<tr>
<th>Test</th>
<th>Test Methodology</th>
<th>Average Reported Sensitivity</th>
<th>Average Reported Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet mount</td>
<td>Microscopy</td>
<td>61%</td>
<td>100%</td>
</tr>
<tr>
<td>Culture</td>
<td>Culture of live organisms</td>
<td>74%</td>
<td>100%</td>
</tr>
<tr>
<td>OSOM Rapid Test</td>
<td>Antigen detection</td>
<td>84%</td>
<td>100%</td>
</tr>
<tr>
<td>BD Affirm VP III</td>
<td>DNA hybridization probes</td>
<td>64%</td>
<td>100%</td>
</tr>
<tr>
<td>GenProbe APTIMA TV</td>
<td>Transcription mediated amplification</td>
<td>98%</td>
<td>99%</td>
</tr>
</tbody>
</table>

Treatment of trichomoniasis is generally accomplished using a

- Single dose of metronidazole or tinidazole. However, there are
  - Increasing reports of resistance to these agents and there may be an increased need for susceptibility testing in the future.
  - It’s also important to note that recurrent disease is common, with:
    - 1 in 5 individuals becoming reinfected after successful treatment within the first 3 months; and this is usually from repeated contact with the untreated sexual partner
      - A study by Hobbs et al found that 73% of male partners of infected women were also infected.
    - Therefore, it is important for all sexual partners of an infected individual to be treated to help prevent reinfection.
    - Condoms can also be used to prevent infection

In conclusion

- *Trichomonas vaginalis* is a common sexually transmitted pathogen of both men and women
- Most infected patients are asymptomatic but symptoms can be severe in some individuals and increases their risk of acquiring other serious sexually transmitted infections.
- Finally, nucleic acid amplification methods offer the highest sensitivity for detection of infection and should be considered the new “gold standard” for *Trichomonas vaginalis* testing.

Testing for *Trichomonas vaginalis* is now available at Mayo Medical Laboratories using the FDA approved Gen-Probe Aptima TV assay. The first test code listed here is for FDA-approved specimens, and the second test code is for non-FDA-approved specimens, specifically male specimens.
So with that I would like to conclude and thank you for your attention today.
Questions or requests…

Email to: MMLHotTopics@mayo.edu

For more information…

Visit MayoMedicalLaboratories.com or call Mayo Laboratory Inquiry at 800-533-1710