Trichomonas – Time to Know the Facts

Conflicts of Interest

- Research support and/or participation in clinical trials from the following companies and sources that have to do with today’s subject:
  - NIH, DOH RI, APHL
  - Gen-Probe, BD, Cepheid

The Rodney Dangerfield of STIs

Trichomonas vaginalis gets no respect

Case

- 55 yo man presents to MD for routine physical exam and minimal urogenital complaints
- Previous exam, including prostate WNL
- Social history: Divorced for 3 years and currently “dating”…..alot
- HPI/PE: no specific complaints other than occasional urinary frequency and difficulty or pain urinating, and sometimes pain during or after ejaculation

Case

- 45 yo woman presents to MD for GYN exam
- Previous exam, including PAP WNL
- Social history: Divorced for 3 years and currently “dating”…..alot
- HPI/PE: no specific complaints except occasional itching in vaginal area, GYN exam shows only some thin watery discharge
- Wet mount negative

Case

- Urine Culture negative
- HIV negative
- Nucleic Amplification urine test (NAATs) negative for CT/GC
- Urine NAAT positive for TV
Case 3A

- Urine Culture negative
- HIV negative
- NAAT Endocervical swab negative for CT/GC
- Endocervical swab NAAT positive for TV

Presentation Outline

- Disease background in context of other STIs
- Pathogenesis
- Clinical Sequelae
- Current TV detection methods
- Comparison of methods with NAAT
- Trichomonas epidemiology clarified
- Treatment
- Considerations for future action and Public Health

Genital Infections – Basic Tenants

- Asymptomatic partners transmit infections
- Many infections with overlapping in signs and symptoms so clinical diagnosis not specific
- Diagnostic Testing is recommended:
  - Appropriate RX can be focused
  - Specific diagnosis increases therapeutic compliance
  - By both patient and partner
  - Complies with state PH reporting
- Alas….current available test diagnostics lacking
  - 25-40% of patients the entity is not clear

Satterwhite, Torrone et al, 2013 STD, 40(3) 187-193

<table>
<thead>
<tr>
<th>STI</th>
<th>New Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td>19,000</td>
</tr>
<tr>
<td>HIV</td>
<td>49,000</td>
</tr>
<tr>
<td>Syphilis</td>
<td>55,400</td>
</tr>
<tr>
<td>HSV-1 Genital Infections</td>
<td>776,000</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>820,000</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>1,090,000</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>2,860,000</td>
</tr>
<tr>
<td>HPV</td>
<td>14,100,000</td>
</tr>
</tbody>
</table>

20 million new infections/year (incidence); 110 million total infections (prevalence)
COST in US dollars $16 BILLION

Trichomonas vaginalis – Healthcare Associated Costs

- Direct medical costs for TV infection alone:
  - estimated at $34.2 million
- Sequelae related to increased risk for HIV transmission:
  - $167 million
- Misdiagnosis and inappropriate RX of common entity: $$?
  - Vaginosis, vaginitis that continues to come back over and over
  - No routine testing for TV unless symptomatic or high risk
  - Partners NOT empirically tested or treated
- NOT a reportable disease
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Life-cycle of *T. vaginalis* is simple

- Single-celled protozoan
- Divides by binary fission
- Only infects humans
- Infection transmitted sexually
- Natural infection exists on the mucosa of the genital lumen

**Trichomonas vaginalis**

- Single-celled protozoan
- Divides by binary fission
- Only infects humans
- Infection transmitted sexually
- Natural infection exists on the mucosa of the genital lumen

**Pathogenesis of Trichomonas – Much to Learn**

- Attaches to epithelial cells
  - Releases proteins which by direct contact result in:
    - Micro-ulcerations
    - Subsequent intense local immune response mainly with:
      - Neutrophils but also
      - Lymphocyte recruitment including CD4+ cells
    - Recruitment of inflammation-related factors:
      - Cytokines and chronic stimulation that has implications for pathology and cancer
  - Phagocytosis
    - Vaginal bacteria and host cells which alters normal flora
    - Vector for spread of other organisms
      - Carrying pathogens attached to their surface into fallopian tubes, into prostate

**Clinical Features**

- Incubation period 4-28 days
- Symptoms and signs in women
  - Vaginal discharge, pruritus, and irritation, dysuria
  - Vaginal discharge, odor, edema or erythema, pH > 4.5
  - Painful in only 10%
  - Vulvovaginal syndrome (strawberry cervix) specific for TV but only on colposcopy and rarely on routine examination
- Extent of inflammatory response my determine severity of SXS
- 50% of women are asymptomatic
- Most men are asymptomatic (>$75%$
- Recent studies suggest TV is more common cause of NGU than previously thought
- TV should be considered in male that fails therapy of NGU

**Pathology of Trichomonas vaginalis**

- TV prefers an anaerobic environment and pH 6.0
- Can deal with pH 4.5 – which may be why some infections with low organism burden
- Healthy vagina pH 3.8-4.5
- TV phagocytosing vaginal bacteria creates a more alkaline environment (> 4.5 pH)
- TV contains structures called Hydrogenosomes (no mitochondria)
- Accomplish fermentative carbohydrate metabolism and
- Produce H2 gas
- Inflammatory response to the parasite may determine severity of symptoms
- Factors that influence the host response:
  - Hormone levels
  - Coexisting vaginal flora
  - Strain of TV
  - Relative concentration of organism present
Associated risks and sequelae associated with trichomoniasis

<table>
<thead>
<tr>
<th>Prenatal</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature rupture of membranes</td>
<td>Vaginitis, cervicitis, endometritis, PID</td>
</tr>
<tr>
<td>Pre-term Delivery</td>
<td>Cervical erosion</td>
</tr>
<tr>
<td>Low Birth Weight</td>
<td>Cervical dysplasia</td>
</tr>
<tr>
<td>Intellectual Disability of Baby</td>
<td>Prolonged HPV Infection</td>
</tr>
<tr>
<td></td>
<td>Infertility/fallopian tube damage</td>
</tr>
<tr>
<td></td>
<td>Bacterial vaginosis</td>
</tr>
<tr>
<td></td>
<td>Increased transmission/acquisition of HIV/HSV</td>
</tr>
<tr>
<td></td>
<td>Presence of other STIs *</td>
</tr>
<tr>
<td></td>
<td>Increased risk of post-surgical gynecological infections</td>
</tr>
</tbody>
</table>

*Neisseria gonorrhoeae, Chlamydia trachomatis, human immunodeficiency virus, herpes simplex virus, and human papillomavirus.

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- Disease background
- Pathogenesis
- Clinical Signs and Symptoms, Sequelae
- Current TV detection methods
  - Comparison of methods with NAAT
  - Trichomonas epidemiology clarified
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Current Estimation of Diagnostic TV Testing Performed

- Wet mount 48%
- BD Affirm 13%
- NAAT 10%
- OSOM 8%
- InPouch 29%
- Pap Smear %?

Wet Mount

- Vaginal swab in symptomatic patients
- Place in saline tube and then onto slide
- Look for motile trichomonads
- Needs to be done within few minutes – labs up to 2 hours.....
- Experienced reader…
- Requires live organisms and motility
- 45-80% sensitive

PAP Smear

- Fixed preparation
- May find TV in association with Leptothrix bacteria
- Poor sensitivity and poor specificity
- ?? If automated reading instruments pick this up since scanning for unusual cells
- 50% sensitive??
PAP Smear

- Fixed preparation
- May find TV in association with Leptothrix bacteria
- Poor sensitivity and poor specificity
- ?? If automated reading instruments pick this up since scanning for unusual cells
- 50% sensitive!!

Spaghetti and meatballs

Rapid Antigen Detection

Rapid Antigen Test

- Point of care test - minutes
- Benefits:
  - Relatively inexpensive
  - No live organism needed
  - Transit time not an issue
  - Acceptable sensitivity in symptomatic patients
- Disadvantages:
  - Vaginal swab only
  - Symptomatic patients only
  - Single test format
  - No BV or candida
  - No other STIs
  - Poor sensitivity for a screening test/asymptomatic

InPouch TV by BioMed – Wet mount/Culture

Reference Standard (2009)
- Slide/culture media pouch
- Incubation 37°C
- Reviewed days 1, 3, 5, 7
- Advantage
  - Specificity and Sensitivity?
- Disadvantage
  - Inconvenient for collector/lab
  - Expensive 2° to tech time
  - Time to detection long
  - Single Test system
  - Not paired with other common STIs

APTIMA TV Assay (FDA-cleared April, 2011)

- Hologic APTIMA technology (Combo GC/CT)
  - Nucleic Acid Amplification testing (NAA)
  - Single specimen collection (same tube as for other STIs GC/CT)
  - FDA-cleared for Symptomatic and Asymptomatic women
  - Screening and Diagnostics
  - Urine, vaginal swab, endocervical swab in Gen-Probe transport collection device, Thin Prep liquid cytology specimen
  - Tigris Instrument – fully automated
- Disadvantages
  - No men, self-collection or alternate sites
  - Pharyngeal, rectal
  - Validation has been done by labs for these purposes
  - Tigris instrument
  - Large space and volume, cost

HOLOGIC
FDA-Cleared 2013 for TV

- A small footprint with a width of 48", a depth of 32", and a height of 69"
- 275-1000 samples per 8 hour shift – multiple assays planned
The BD Viper™ System:
- FDA Cleared for TV in 2012
- Strand displacement Amplification
- Efficiency
  - Up to 736 results per 8.5 hour shift
  - 20 minutes of hands on time per run
  - On-board sample DNA extraction
- Flexibility
  - Runs up to five STI assays simultaneously
  - GC, CT, TV, HSV 1 and 2
- Accuracy
  - Automated sample processing and ready to use reagents
  - Automatic system checks

BD Viper XTR

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Comparison of commercially available diagnostic tests for TV detection in women:

<table>
<thead>
<tr>
<th>Test</th>
<th>TV Target</th>
<th>Assay methodology</th>
<th>Time to Result (range)</th>
<th>Near Patient Testing</th>
<th>Cost</th>
<th>Analytical sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSOM Rapid Test</td>
<td>TV antigen</td>
<td>Immunological (antibodies)</td>
<td>&lt;1 hour</td>
<td>Yes</td>
<td>$12.94/ test</td>
<td>&gt;2,500 org/mL of sample</td>
</tr>
<tr>
<td>BD Affirm VPIII</td>
<td>TV DNA</td>
<td>Hybridization Probe</td>
<td>1 hour – batched daily</td>
<td>Possible; Typically Batched</td>
<td>350/000 org/mL of sample</td>
<td></td>
</tr>
<tr>
<td>APTIMA BD Viper</td>
<td>TV rRNA</td>
<td>TMA SDA</td>
<td>3.5 hours – daily</td>
<td>No</td>
<td>0.1 org/mL of sample†</td>
<td></td>
</tr>
</tbody>
</table>

*Data are from respective manufacturers.
†An analytical sensitivity of less than 1 organism per mL is due to the abundance of rRNA target per organism (>1 million copies) and the TMA amplification of the target rRNA molecule to 100,000 analytical sensitivity.

Comparison of Wet Mount, Culture, Antigen OSOM and APTIMA for TV:

Wet Mount: 50.8%
Culture: 79.6%
OSOM: 87.0%
APTIMA: 98.4%


Comparison of % of Positive TV Tests by Wet Mount vs. APTIMA TV (n=1,086):


Diagnostic Test Performance Side by Side – Same Results:

Huppert: 50.5%
Nye: 54.1%
Differences in test sensitivity stratified by the presence or absence of vaginal symptoms

<table>
<thead>
<tr>
<th>Test Method</th>
<th>All Patients (n=330)</th>
<th>Vaginal Symptoms Present (n=210)</th>
<th>Vaginal Symptoms Absent (n=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Mount</td>
<td>50.8 (37.7-63.9)</td>
<td>57.5 (40.8-72.9)</td>
<td>38.1 (18.1-61.5)</td>
</tr>
<tr>
<td>Culture</td>
<td>75.4 (62.7-85.5)</td>
<td>77.5 (61.5-89.1)</td>
<td>71.4 (47.8-88.7)</td>
</tr>
<tr>
<td>OSOM</td>
<td>82.0 (70.0-90.6)</td>
<td>92.5 (79.6-98.4)</td>
<td>61.9 (38.4-81.9)</td>
</tr>
<tr>
<td>ATV</td>
<td>98.4 (91.2-99.9)</td>
<td>97.5 (86.9-99.9)</td>
<td>100 (83.8-100)</td>
</tr>
</tbody>
</table>


Comparison of TV Detection Methods
Low Prevalence - RI

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Affirm (n=781)</th>
<th>Cx (n=446)</th>
<th>PCR (n=766)</th>
<th>TMA (n=1993)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (range)</td>
<td>63.4% (44.0-83.3)</td>
<td>68.5% (57.3-94.5)</td>
<td>88.5% (61.9-94.7)</td>
<td>100.0% (96.6-100)</td>
</tr>
<tr>
<td>Specificity (range)</td>
<td>99.9% (97.1-100)</td>
<td>100% (96.3-100)</td>
<td>100% (96.4-100)</td>
<td>100% (96.4-100)</td>
</tr>
</tbody>
</table>

Ranges are broad due to the different ‘gold standard’ tests used in the literature to interpret true positive infections.

Overall reported sensitivity and specificity averages (ranges)* of TV detection methods in vaginal specimens

<table>
<thead>
<tr>
<th>Test</th>
<th>Avg Sensitivity (range)</th>
<th>Avg Specificity (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet mount</td>
<td>66.7 (43.0-83.3)</td>
<td>100</td>
</tr>
<tr>
<td>Culture</td>
<td>74.3 (57.3-94.5)</td>
<td>100</td>
</tr>
<tr>
<td>OSOM</td>
<td>84.6 (61.8-94.7)</td>
<td>99.8 (97.1-100)</td>
</tr>
<tr>
<td>BD Affirm VPIII</td>
<td>63.8 (44.0-83.3)</td>
<td>100 (99.9-100)</td>
</tr>
<tr>
<td>PCR</td>
<td>89.1 (83.0-100)</td>
<td>98.6 (96.3-100)</td>
</tr>
<tr>
<td>APTIMA TV</td>
<td>98.1 (96.6-100)</td>
<td>98.9 (96.4-100)</td>
</tr>
</tbody>
</table>

Ranges are broad due to the different ‘gold standard’ tests used in the literature to interpret true positive infections.

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Trichomonas in Men

- Infection common in men
- Missed diagnosis in men due to lack of testing and/or symptoms
- Non-reportable disease
- Revolving infection in partners
  - 72% of male partners of women with TV were also positive for the infection
  - 75% were asymptomatic

Hobbs et al, JCM, 2006

Age Breakdown of TV Infections in Women presenting with vaginitis/vaginosis

- Hobbs et al, JCM, 2006
APTIMA Amp versus Affirm VPIII Hybridization for TV

Figure 1:
Infections in 705 Symptomatic Females

290(40.4%) patient specimens were determined as positive for bacterial vaginosis (BV) by the Affirm™ VPIII. Of concern related to these BV results however, is that 144 specimens considered true positive TV cases, 6 (42.9%) were negative for TV by Affirm but positive for BV. Issues related to recollection of symptoms, continued transmission of TV and development of resistance are potential concerns if an incorrect diagnosis of BV is made in lieu of a correct diagnosis of TV.

Table 1: Specimen Type Sensitivity % (95% CI)* Specificity % (95% CI)*

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinician-collected vaginal swab</td>
<td>100.0 (96.7 – 100.0)</td>
<td>99.3 (97.9 – 99.5)</td>
</tr>
<tr>
<td>Endocervical swab</td>
<td>100.0 (96.7 – 100.0)</td>
<td>99.4 (98.6 – 99.7)</td>
</tr>
<tr>
<td>PreservCyt solution</td>
<td>100.0 (96.5 – 100.0)</td>
<td>99.6 (98.8 – 99.9)</td>
</tr>
<tr>
<td>Urine</td>
<td>98.2 (98.4 – 98.7)</td>
<td>98.0 (97.8 – 98.2)</td>
</tr>
</tbody>
</table>

* Sensitivity and Specificity measured at 95% CI

**Conclusions from Validation Study**

- Study provided clinical validation of the ATV assay for the intended uses in the United States:
  - Asymptomatic Screening and Diagnostic Testing in women for Trichomonas
  - FDA cleared highly accurate and fully automated amplified test system with ease of use specimens such as urine and vaginal swab should enhance testing for this pathogen
  - Testing for men FDA-cleared as well would be optimal
  - Any Amplification method is likely to have similar performance compared to other TV detection methods (Viper)

**Prevalence of Trichomonas vaginalis and Co-Infection with Chlamydia trachomatis and Neisseria gonorrhoeae in the United States as Determined by the APTIMA® Trichomonas vaginalis Nucleic Acid Amplification Assay**

1 North Shore LIJ Health System Laboratories, Lake Success, NY, USA; 2 Rhode Island Hospital, Providence, RI, USA; 3 University of North Carolina, Chapel Hill, NC, USA; 4 Hartford Hospital and Clinical Laboratory Partners, Hartford, CT, USA; 5 Gen-Probe Incorporated, San Diego, CA, USA; 6 Johns Hopkins University School of Medicine, Baltimore, MD, USA

- Determine the prevalence of Trichomonas vaginalis (TV) using the APTIMA Trichomonas vaginalis (ATV) assay among women being screened for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) in the United States
- Determine the co-infection rates of TV, CT and NG

**Methods**

- Samples were obtained from 7,593 women aged 18 to 89 years undergoing routine screening for CT and GC by the APTIMA Combo 2™ Assay
- Samples were obtained from 30 laboratories in 21 states
- Clinic types included:
  - OB/Gyn clinics
  - Emergency room
  - Hospital in-patient
  - Family practice
  - Family planning
  - Internal medicine
  - Jail
  - STD clinics
Prevalence of TV, CT, and GC Infections by Age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>TV+</th>
<th>CT+</th>
<th>GC+</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-19</td>
<td>907</td>
<td>8.50%</td>
<td>14.40%</td>
</tr>
<tr>
<td>20-29</td>
<td>3972</td>
<td>8.30%</td>
<td>8.00%</td>
</tr>
<tr>
<td>30-39</td>
<td>1667</td>
<td>7.90%</td>
<td>2.50%</td>
</tr>
<tr>
<td>40-49</td>
<td>720</td>
<td>11.30%</td>
<td>1.00%</td>
</tr>
<tr>
<td>50+</td>
<td>324</td>
<td>13.00%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

*In the calculations of prevalence, the denominator may be less than that the N shown due to missing or invalid assay data.

Prevalence of TV, CT, and GC Co-infections by Age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>TV+ + CT+ + GC+</th>
<th>CT+ + TV+ + GC+</th>
<th>CT+ + TV+ + GC+</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-19</td>
<td>907 0.20%</td>
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</table>

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Prevalence of TV, CT, and GC Infections by Race

<table>
<thead>
<tr>
<th>Race</th>
<th>TV+</th>
<th>CT+</th>
<th>GC+</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Indian/Alaskan Native</td>
<td>19</td>
<td>10.50%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Asian</td>
<td>131</td>
<td>3.50%</td>
<td>5.50%</td>
</tr>
<tr>
<td>Black/African-American</td>
<td>1352</td>
<td>20.30%</td>
<td>12.10%</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>715</td>
<td>5.00%</td>
<td>5.00%</td>
</tr>
<tr>
<td>Native Hawaiian/Other Pacific</td>
<td>14</td>
<td>7.10%</td>
<td>7.10%</td>
</tr>
<tr>
<td>White</td>
<td>1668</td>
<td>5.70%</td>
<td>5.70%</td>
</tr>
<tr>
<td>Other/Unknown</td>
<td>3658</td>
<td>8.70%</td>
<td>5.20%</td>
</tr>
</tbody>
</table>

Prevalence of TV, CT, and GC Infections by Collection Site

<table>
<thead>
<tr>
<th>Site</th>
<th>TV+</th>
<th>CT+</th>
<th>GC+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Planning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family Practice</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Internal Medicine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palliative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palliative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STD Clinic</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Conclusions from Prevalence Study

Ginnochio et al

- TV prevalence (8.7%) is higher than CT (6.7%) and GC (1.7%)
- TV prevalence is highest in women >40 years (>11%), while CT and GC prevalence is lowest in that age group (<2%)
- TV prevalence is highest among black women (20.2%)
- Co-infection of TV with CT and/or GC was relatively low and highest in women <20 years
- TV prevalence was highest in jail (22.3%) and emergency room (17.0%) collection sites
- The high TV prevalence in all age groups suggests that all women being screened for CT/GC should also be screened for TV

Treatment of TV

- Single 2 g oral metronidazole
- Vaginal gel not as effective (most commonly used for BV)
- Tinidazole
  - Twice the ½ life of metro (12-14 hrs)
  - Better tolerated
  - True resistance < 10%; can be overcome with higher dosing
  - Therapy is ineffective if partner is not treated!
- Treating for BV is not the same as treating for TV

Diagnostic Problem with TV...
What is it? A vaginal nuisance or an STI?
Getting TV on the Map

- Educate the healthcare providers
- Don’t know the prevalence or the sequelae
- Trichomoniasis is not always associated with symptoms, age or obvious sexual risk behaviors
- Educate the public/patient (Chapin’s PR campaign?)
  - Did you know the most common treatable STI is one that your physician may not be testing for??
  - What STD is a college student’s MOM more likely to have than their college student?
- Educate the Laboratory
  - Know the benefits/disadvantages of current tests methods
  - Many labs/providers do wet mount and think it is OK

Implementation of Trichomonas Testing
Combined Concerted Effort

PUBLIC HEALTH
HEALTHCARE PROVIDERS
LABS
PATIENT
Insurance

The STD you have heard of...and are going to do something about it.....
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