VAGINAL MICROBIOTA AMONG ADOLESCENT AND YOUNG ADULT WOMEN WITH PELVIC INFLAMMATORY DISEASE

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Background Pelvic Inflammatory Disease (PID) is a polymicrobial infection currently treated using syndromic management with broad-spectrum antibiotics. There are limited data describing the vaginal microbiota among adolescent and young adult women with PID, and how the post-PID microbial state may predispose to subsequent infection due to ongoing infection and shifts in vaginal microbiota. The purpose of this pilot research is to examine the microbial environment among adolescent and young adult women with acute PID.

Methods This analysis utilizes stored samples from 13–25-year-old patients (n=26) diagnosed with acute PID and enrolled in the Technology Enhanced Community Health Nursing (TECHN) study, a large randomized controlled clinical trial designed to test a multi-faceted intervention for prevention of PID. Vaginal microbiota was characterized by 16S rRNA gene sequencing (V3-V4 regions) and clustered into community state types (CSTs).

Results At baseline, the majority of patients with acute PID were in a low-Lactobacillus or L. iners-dominated state (CST I (L. crispatus dominated (N=3, 11.54%), CST III L. iners-dominated (N=7, 26.9%), CST IV Low-Lactobacillus (N=15, 57.69%), CST V L. jensenii-dominated (N=1, 3.85%). The single CST V case had a relatively low abundance (55%) of L. jensenii.

Conclusion Preliminary vaginal microbiota testing among AYA with PID revealed over 1/2 of participants had a low abundance of Lactobacillus spp indicative of bacterial vaginosis and risk to STI. Over 1/4 had L. iners-dominated microbiotas, which are also often associated with BV Lactobacillus spp and are thought to protect against pathogens; however, the level of protection may vary by strain. Additional research should examine these findings in larger samples, including PID-negative patients for comparison, and assess the changes in the vaginal microbiota associated with successful clearance of pathogens. Such work may improve understanding of the vaginal microenvironment during PID and elucidate a path to shift from syndromic management to precision treatment among affected patients.

Disclosure No significant relationships.

DETECTION OF CHLAMYDIA, GONORRHOEA AND MYCOPLASMA GENITALIUM IN SEMEN AND IN ESWAB MEDIUM USING HOLOGIC APTIMA ASSAYS

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Background The objectives of this study were to determine the limits of detection (LODs) of Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG) and Mycoplasma genitalium (MG), using Aptima Combo 2 for CT/NG and Aptima Mycoplasma genitalium assays in presence of different volumes of semen and ESwab medium (bioMérieux, France) used for specimen collection. Furthermore, the preservation conditions of these bacteria in ESwab medium were assessed.

Methods Semen specimens were collected anonymously from patients consulting at the reproductive biology department of Bordeaux University Hospital. To determine LOD, quantified cultures of each bacterium were spiked in semen or ESwab medium. Different volumes added to APTIMA® Specimen transfer tubes were tested with Aptima Combo 2 CT/NG and Aptima Mycoplasma genitalium assays. Storage conditions (5 days at room temperature and 7 days at 4°C) of each bacterium in ESwab medium were evaluated. All the experiments were performed in triplicate on the Hologic® Panther system.

Results The semen was a slight source of amplification inhibition for CT and MG. However, using a volume of 50 μL of semen, the LOD of CT, NG and MG in semen remained low, at 10^3 IFU/mL, 10 CFU/mL and 10^2 CCU/mL, respectively. A detected is a wild-type (WT) or has any of 5 macrolide resistant mutations (MRM) in the 23S rRNA gene. The STD6 ACE assay (Seegene Canada Inc.) which is Health Canada approved and Aptima require further testing for MRM by 23S rRNA sequencing. The objectives were to enroll 300 women to self-collect 3 vaginal swabs (VS) and first-void urine (FVU) to be tested for MG in the 3 assays and for MRM.

Methods Aptima was performed on a Panther instrument. STD6 ACE used EasyMag extraction and gel electrophoresis. SpecDx was extracted on an m2000sp and amplification was on an Abi 7500. Positives from Aptima and STD6 were tested for MRM by sequence typing. Extra positives by Aptima were blindly tested with negatives by alternate assays, detecting distinctly different 16S rRNA and 23S rRNA. Agreements and Kappa values were calculated for MG detection and MRM.

Results For 190 women, MG-positives by SpecDx were 24 VS and 17 FVU, compared to 17 VS and 9 FVU by STD6. Aptima detected 34 VS and 29 FVU as positive. Overall agreements and Kappa values were very good K 0.82 (VS) to good K 0.69 (FVU) between Aptima and SpecDx, but moderate to fair for Aptima or SpecDx with STD6. Confirmatory testing of extra positives confirmed 79% (11/14) as positive for Aptima and 0% (0/6) for STD6. Patient MRM rates from each assay were 60% (15/25) SpecDx, 63.6% (7/11) Aptima and 57.9% (11/19) STD6.

Conclusion Aptima was more sensitive on both VS and FVU. VS was a better sample than FVU for SpecDx. STD6 lacked sensitivity and specificity for the identification of MG. MRM rates were similar for the three identification and typing systems.

Disclosure No significant relationships.

COMPARISON OF ASSAYS AND SPECIMEN TYPES FOR THE DIAGNOSIS OF MYCOPLASMA GENITALIUM AND MACROLIDE RESISTANT MUTATIONS

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Background The Aptima MG assay (Hologic Inc.) detects Mycoplasma genitalium (MG). SpecDx Resistance Plus MG (SpecDx Pty Ltd) simultaneously determines whether the MG
volume of 50 µL of semen could be chosen for the diagnostic of these bacteria with Aptima assays. In ESwab medium, the LODs of CT, NG and MG were equivalent (between 1 and 10 IU, CFU or CCU/mL) whatever the volume of ESwab added in the APTIMA® specimen transfer tubes. A volume of 200 µL of ESwab allowed performing several different Aptima assays and the LOD of bacteria remained low whatever the storage conditions.

Conclusion Aptima Combo 2 for CT/NG and Aptima Mycoplasma genitalium assays can be used to detect these three sexually transmitted pathogens in semen and in clinical specimens preserved in ESwab medium.

Disclosure No significant relationships.

Background Mycoplasma genitalium (MG) has been associated with nongonococcal urethritis among men and cervicitis among women. Infection with MG has been linked to increased risk of HIV infection and potentially with adverse reproductive health outcomes. We currently have limited data regarding the positivity rates for this organism in different locations in the U.S. Typically, chlamydia, gonorrhea and trichomiasis rates are highest in the Deep South compared to other regions of the country, but we do know if this is the case for MG. We took advantage of a multi-site, MG-focused clinical study being conducted in the US to assess the positivity rates, a reflection of prevalence from a convenience sample, at different collection sites.

Methods Symptomatic men and women were recruited from 8 sites in the US. Sites were located in the Deep South (Alabama, Louisiana, Mississippi, and Texas) and other regions (California, Connecticut, Indiana, and Maryland). Participants reporting dysuria, abnormal discharge, genital itching/pain, pelvic pain, or pain/bleeding during intercourse were considered symptomatic. MG status was determined by a combination of results from MG assays since.

Results 24/173 (13.9%) men and 21/219 (11.0%) women were MG-infected. The positivity rates were 13/129 (10.1%) and 11/44 (25.0%) for men recruited in the Deep South and other regions, respectively (p=0.013). Among women the rates were 21/184 (11.4%) and 3/35 (8.5%) (p=0.624).

Conclusion While the sample size is small since the study is ongoing, it is interesting to note that the majority of participants have been enrolled in Deep South and these positivity estimates are likely fairly robust. This is an important lesson given the disparity in described MG rates around the world. Rates have been reported to be high among symptomatic men in Western Europe and Australia, but lower in other settings. Investigation into the causes for differential distribution may be important to designing appropriate control strategies.

Disclosure No significant relationships.

References
1. Dorothy Machakos, 1Yusha Tao, 1Hannah Shilling, 1Jorgen Jensen, 1Magnus Unemo, 1Gerald Murray, 2Eric Chow, 3Nicola Low, 3Suzanne Garland, 3Christopher Fairley, 2Lenka Vodstrcil, 6Jane Hocking, 2Lei Zhang, 2Catriona Bradshaw, 1Gerald Murray, 2Eric Chow, 5Nicola Low, 1Suzanne Garland, 2Christopher Fairley, 1Dorothy Machakos*, 6Jane Hocking, 2Lei Zhang, 2Catriona Bradshaw.

Background Treatment for Mycoplasma genitalium is becoming increasingly complicated by antimicrobial resistance. We summarised published global data on the prevalence of macrolide and fluoroquinolone resistance-associated mutations in M. genitalium and examined trends over time.

Methods We searched PubMed, EMBASE and Medline until December 31, 2017. We included studies that reported the percentage of key mutations associated with macrolide resistance (235 RNA gene: A2071C/G/T; A2072C/G/T) and/or fluoroquinolone failure (parC gene: S83R/I; D87N/Y) among M. genitalium positive specimens. Data were extracted by geographic region, collection year, sex, and risk group (men who have sex with men [MSM] or heterosexual). Summary estimates (95% confidence intervals [CI]) were calculated using random-effects meta-analyses. Subgroup and meta-regression analyses were conducted to assess heterogeneity.

Results 47 studies met the inclusion criteria reporting resistance-associated mutations for macrolides (n=45) and fluoroquinolones (n=18). Global prevalence of macrolide resistance mutations increased from 4.9% [95% CI 0.0–15.7%] before 2009, to 46.3% [30.7–62.2%] in 2016–17 (p-trend=0.001). This increase was greatest in the Western Pacific region (Australia in particular) where prevalence increased from 12.6% [2.6–26.9%] to 69.2% [60.7–77.1%] (p-trend<0.001). Prevalence of macrolide resistance-associated mutations was also higher among MSM (72.3% [58.6–84.5%]) than heterosexual men (37.3% [25.8–49.6%]) (p<0.001). Global prevalence of fluoroquinolone resistance mutations was 6.3% [4.2–8.9%] with no changes over time or by risk group, but regional variations were present with highest prevalence in the Western Pacific region (14.9% [9.7–20.9%]) and North America (11.2% [2.9–23.3%]), and lowest in Europe (2.8% [1.7–4.1%]). Dual class resistance mutation prevalence was 2.5% [1.1–4.2%] with no change over time or by risk group. Regional variations were similar to those for fluoroquinolone resistance mutations.

Conclusion Resistance to recommended first and second line treatments for M. genitalium is a growing public health problem. Global surveillance and antimicrobial resistance-guided therapies are needed to inform more effective regional strategies for the control and treatment of M. genitalium.

Disclosure No significant relationships.

References
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