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Chlamydia in adolescent/adult reproductive management trial study (CHARM): Clinical core protocol

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ABSTRACT

Introduction: Chlamydia trachomatis (CT) is a common sexually transmitted pathogen with significant reproductive health implications. Data are mounting that the bacterial communities that reside within the vagina, collectively termed the vaginal microbiota, aid in defense against sexually transmitted infections. Disruptions in the vaginal microbiota, such as during episodes of bacterial vaginosis, may increase susceptibility to infection. Herein, we describe the clinical core protocol for a NIH NIAID Cooperative Research Center titled Eco-Pathogenomic of Chlamydial Reproductive Tract Infection. The primary goals of the project are to describe the interrelationships between the urogenital microenvironment, the properties of the pathogen and immunologic responses of the host in men and women, and their association with clinical outcomes of CT infection in women.
Methods: Men and women with confirmed genital CT infections were recruited to a number of study protocols, including cross-sectional and longitudinal sub-studies. Participants completed a demographic and sexual health questionnaire and underwent a physical exam at baseline. In the longitudinal study arms, biologic samples were collected daily, weekly, and monthly to determine the relationships between the vaginal microbiota, prevalent CT infection, re-infection and treatment.
Discussion: The biological samples and the demographic and history information collected throughout this study will be used for various analyses evaluating genomics, metabolomics and host immune responses in the context of CT infection.

1. Introduction

Chlamydia trachomatis (CT) is the leading bacterial sexually transmitted infection (STI) with over 1.7 million cases reported to the CDC in 2017, a case count rate of 528.8 cases per 100,000 population, making it the most common notifiable condition in the United States [1]. Moreover, many CT infections are asymptomatic or perceived by those infected as relatively mild and therefore, many are undiagnosed and unreported [2]. This is particularly true in men who often function as a reservoir for the infection [3]. The most serious sequelae of CT genitourinary infections in women are pelvic inflammatory disease (PID), ectopic pregnancy, and infertility [4]. Other complications from CT genital infection include ocular infection of newborns and dissemination to the joints of patients with reactive arthritis [5–8]. An estimated 90,000 women become infertile each year as a result of gonococcal or chlamydial PID [9].

The vaginal microbiota play a key role in preventing colonization by pathogenic organisms, including those responsible for STIs and urinary tract infections [10–17]. *Lactobacillus* spp. are considered keystone species of vaginal communities in reproductive-age women. These microorganisms produce lactic acid which lowers vaginal pH to less than 4.5 and provide protection from pathogens [18]. Significant alterations of the microbiological environment to low-*Lactobacillus* states, such as in the clinical syndrome bacterial vaginosis (BV), appear to be a biological risk factor for the acquisition and transmission of STIs/HIV [14–22], and therefore has tremendous public health implications. In a nationally representative sample, bacterial vaginosis was shown to have a prevalence of 29.2% with only 15.7% of women reporting vaginal

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symptoms [22]. Rates of bacterial vaginosis approach 50% in women attending clinics for screening for sexually transmitted infections [23].

These data support the importance of the vaginal microbiota as a first line defense against STI. The Chlamydia in Adolescents and Adults Reproductive Management (CHARM) study was designed to characterize the dynamics and the structure of the vaginal microbiota in association with CT infection. Our overarching hypothesis is that some states of the vaginal microbiota (symptomatic or asymptomatic) are associated with higher risk of chlamydial infection, and the goal of this study is to characterize the vaginal microbiota in participants with genital chlamydial infection. Here, we describe the clinical core, which is the portion of the study that collected biologic specimens and demographic and historical data regarding CT positive individuals.

2. Methods

This study was approved by the Institutional Review Board at the University of Maryland School of Medicine. A Certificate of Confidentiality was obtained from the National Institute of Health.

Sample size: Given the multifaceted nature of this study, each aim was powered separately. The goal was to ensure that each vaginal microbiota Community State Type (CST) was represented in each comparator group, which required over sampling to capture the rarer CST II and V. This methodology was adapted from that previously described by Abdo et al. [24].

CT and PID diagnosis: For the purpose of this study, detection of CT was determined by BD Probe Tech, the clinical test chosen by the University of Maryland Medical Center laboratory. For subjects treated for infections, treatment failure was defined as "persistent" infection as determined by a positive CT DNA test in an interval of 3 months in a patient who reported (a) no sexual exposure since receiving treatment, or (b) sex only with a treated partner after the partner completed treatment, or (c) used condoms for every sexual encounter since treatment.

Diagnosis of pelvic inflammatory disease (PID) was based on the CDC guidelines and was defined as a sexually active female experiencing pelvic pain with cervical motion, uterine, or adnexal tenderness with no other identifiable cause [24]. The diagnosis of PID by laparoscopy was not used as this would have likely been a major deterrent for potential participants and put them through unnecessary risks of surgery. These diagnostic criteria are clinically relevant as they represent clinical standard of care and have been used in other studies of PID [25,26].

Recruitment: Participants were recruited in one of two ways: either by community outreach screening or by provider referral. Community outreach screening was offered at several community sites including colleges, pharmacies and health fairs. The screening was free of charge. Those who requested free screening signed a consent form to be contacted by research staff with their test Results. Those who had positive tests were offered enrollment in the study. For provider-based referral, providers at the University of Maryland provided patients with positive chlamydia testing information regarding the study and the potential participants contacted the research staff if interested in enrollment. Those interested in the study would be offered an appointment for enrollment and treatment as soon as possible with same day appointments when possible in order to avoid any delays in treatment. For those who screened positive through outreach screening but declined enrollment in the study, free treatment was offered for those who qualified through the Title X funded practice in which the study enrolled, through the Baltimore City Health Department or by the person's primary care physician if they preferred.

All potential participants were then screened for enrollment using an eligibility checklist.

2.1. Inclusion/exclusion criteria

2.1.1. Inclusion criteria

1. Females and males between ages 12 and 41 years of age who have ever been sexually active.
2. Ability to understand and sign informed consent form.
3 Positive CT test.
4 Negative pregnancy test for females.

2.1.2. Exclusion criteria

1 Documented pregnancy.
2. Use of antibiotics or antifungal drugs within the past 30 days.
3. Severe chronic illnesses such as kidney failure, diabetes, HIV/AIDS or other chronic severe infections (with the exception of the HIV positive arms).
4. Currently participating in a drug clinical trial.
5. Received a vaccine within the last 30 days.

Enrollment: The subject consent and HIPAA forms were approved by the Institutional Review Board at the University of Maryland Baltimore. Participants were consented at their first study visit by a research staff member. As is consistent with Maryland State law [Md. Code Ann., Health-Gen. II § 20–102(c) [1]-(5)], which allows persons under 18 to consent for screening, treatment and counseling regarding sexually transmitted infection without parental consent or notification, minors provided their own consent for participation in the study.

Study Procedures: Participants were recruited for one of three arms of the study: cross sectional, longitudinal or self-sampling. All participants underwent a baseline study visit. The study visit included collection of demographic information, behavioral history, and medical history by Audio Computer Assisted Interview (ACASI) and during an in-person interview performed by a research team member. Participants then underwent a physical examination and specimen collection (vaginal smear slide, vagina/cervical/urethral swabs, anal swab, pharyngeal swab, saliva, urine, and blood) by a trained physician or nurse practitioner.

Cross Sectional Cohort: With the exception of HIV negative males, all other participants recruited into the study were offered enrollment into a longitudinal arm and, for women, enrollment into the self-sampling arm of the study. All HIV negative males and those others who declined enrollment in the longitudinal arms underwent a one-time study visit as outlined above.

Longitudinal Cohort: The longitudinal cohort included arms with CT positive women with and without PID as well as a smaller cohort of both males and females who were CT and HIV positive. As seen in Table 1, participants in the longitudinal cohort underwent study visits and pelvic examinations at baseline and then at 3 month intervals for 9 months. They each underwent a full physical exam at the baseline visit and at the conclusion of the 9 months.

Self-Sampling Cohort: All females that were enrolled in the study that did not have PID were offered enrollment in the self-sampling cohort. In addition to the longitudinal cohort, a second cohort of female participants was enrolled in the self-sampling arm of the trial. These participants underwent all of the same study visits and procedures as those enrolled in the longitudinal arm and additionally obtained self-collected samples at home. Participants obtained vaginal swabs by self-sampling at daily intervals for 4 weeks following visit 1 and antibiotic treatment, and then at weekly intervals for the first 3 months (or until visit 2).

The self-collected vaginal samples were obtained by inserting swabs 1–2 inches into the vagina, making several full circles to obtain sample on all sides of the swab, and then leaving swabs in the vagina for 20 s. Samples for vaginal pH were also obtained using a sterile glove. These self-collected samples were used to identify candidate CT persistent

Table 1
An overview of study procedures performed at each visit.

	Visit 1 (baseline)	Visit 2 3months	Visit3 6months	Visit4 9months
Registration	X	X	X	X
Informed consent	X	C	C	C
Vital signs	X	X	X	X
ACASI computerized demographic and behavior questionnaire	X	B	B	B
Urine for pregnancy test, blood for repository, HIV, STI screening	X	X	X	X
Medical History	X	B	B	B
General Physical Examination	X	–	–	–
Pelvic/urethral Examination	X	X	X	X

B = brief.
C= Continuous.
* = Comprehensive visit-more extended examination to comply with.

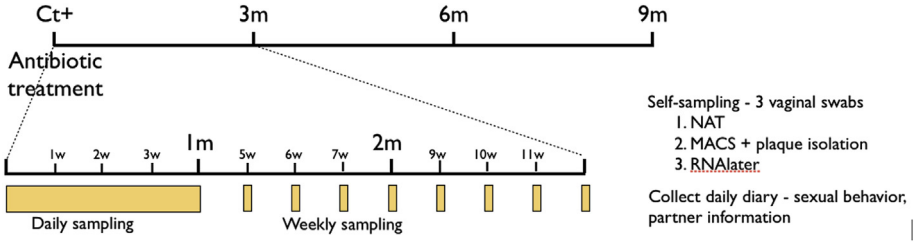

Fig. 1. Graphical representation of self-sampling cohort study design.

infections and the composition of the vaginal microbiota. Chlamydial genome sequencing was applied to selected samples representing persistent infection or cleared infections following antibiotic treatment. Participants in this cohort also kept a daily diary of symptoms and behaviors including personal activities such as antibiotic use, menstruation, and sexual activity (Fig. 1).

Baseline Demographics: Baseline Demographic and Behavioral Questionnaire was administered by ACASI and it covered demographics, lifetime substance use and sexual history including pregnancy and contraceptive method use. History of substance use was evaluated because substance use is frequently associated with high risk sexual behavior and may be an additional marker of risk. At each follow-up visit, participants were asked to complete a Follow-up Demographic and Behavioral Questionnaire, which included a shorter version of sexual history and substance use in the last 3 months by an ACASI and in-person interview with a study team member.

Medical history: The Baseline Medical History included a thorough history of past and current health problems and was administered by a practitioner. Detailed questions were included in routine health visits and covered reproductive, gynecologic and contraceptive history. The Follow-up Medical History during Brief Follow-up and Comprehensive Follow-up Visits included general health, hormonal contraceptive use (type and duration) and diagnosis and symptoms of STIs since the last visit.

Sexual history: Each survey (baseline medical history and follow-up medical history questionnaires) asked questions regarding sexual activity. The survey included queries on lifetime and current partners, gender of partners and frequency of condom use.

Clinical examination: Physical exams were performed every three months. At each study visit, the participants were asked about symptoms of vaginal or penile discharge, irritation, dysuria, or odor. A full physical exam was performed at baseline and 9 months. The full physical exam included skin, heart, abdomen, ocular, oropharynx, rectal and external genitalia and a complete pelvic examination. A brief physical examination was performed at 3 and 6 months. This included a urethral/pelvic exam to assess acquisition or resolution of STIs and collect additional research swabs.

Specimen collection and cervical cytology: *Urine Specimens*: At every visit, a first void urine was collected. A pregnancy test was performed as a routine on each urine specimen and was sent for CT/GC testing on males.

Blood Specimens: Blood was collected at Baseline and at each Comprehensive Follow-up Visit. HIV and syphilis rapid plasma regain (RPR) were obtained. HIV counseling was offered through our own State designated HIV Counseling and Testing unit with pre and post-test counseling and signed consent.

Pelvic Examination Specimens (female participants): Pelvic examinations included specimens for the detection of vaginal and cervical infections. This included: inspection of the external genitalia for the identification of lesions and collection of HSV and/or HPV specimens and collection of vaginal specimens for wet mount and KOH (BV, trichomonas and candida). Separate swabs were taken for gonorrhea and chlamydia (BD Probe Tech CT/GC) according to manufacturer's instructions. Bimanual examination was performed to assess for cervical motion or adnexal tenderness.

Treatment: Treatment was based on detection of CT by DNA test, detection of other sexually transmitted infections or clinical symptoms. Treatment regimens followed the CDC Treatment Guidelines and can be found in Table 4 [25]. For uncomplicated CT infection, a single dose of azithromycin was chosen rather than doxycycline to improve compliance. The efficacy of the two regimens is the same with azithromycin likely having a higher compliance due to one-time dosing as compared to twice daily dosing for seven days required for doxycycline [27]. Gonorrhea was treated with a single dose intramuscular ceftriaxone 250 mg injection, trichomonas vaginalis was treated with a one-time oral dose of 2 g of metronidazole, bacterial vaginosis was treated with a week of twice daily oral or vaginal metronidazole, yeast was treated with either one time oral fluconazole or three days of vaginal miconazole.

Data Collection: All demographic and history information was administered via ACASI, which directly adds all data points to a research database. The physical exam information was input by the clinician into an iPad that also directly inputs all data points into the database.

Results: Over the course of the study, 4943 people were screened through community screening, of which 480 tested positive for chlamydia and 311 agreed to participate in the study, 14 of whom were excluded (Figure 2). Of the patients who agreed to participate but were

Table 2
Demographic information by study arm.

	Female		Male		CSS		Female HIV		Male HIV		PID	
	N = 94		N = 118		N = 23		N = 10		N = 9		N = 30	
Age (years)	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
< 18	40	42.55	28	23.73	0	0	2	20	0	0	6	20.00
18–20.9	30	31.91	37	31.36	14	60.87	2	20	3	33.33	16	53.33
21–23	11	11.7	29	24.58	2	8.7	1	10	4	44.44	7	23.33
23+	13	13.83	24	20.34	7	30.43	5	50	2	22.22	1	3.33
Race/Ethnicity												
Asian	0	0	0	0	1	4.35	0	0	0	0	0	0
Black	85	90.43	108	91.53	18	78.26	9	90	9	100	30	100
Native American/Alaskan	1	1.06	0	0	0	0	0	0	0	0	0	0
White	3	3.19	3	2.54	3	13.04	1	10	0	0	0	0
Other/mixed	0	0	2	1.69	1	4.35	0	0	0	0	0	0
Unknown	5	5.32	5	4.24	0	0	0	0	0	0	0	0
Syphilis reactive	1	1.12	0	0	0	0	0	0	2	22.22	0	0
Neisseria gonorrhoeae	7	7.45	8	6.78	4	17.39	0	0	3	33.33	0	0
Marital status												
Married	2	2.17	7	5.93	3	13.04	1	10	0	0	2	6.67
Single	85	92.39	111	94.07	20	86.96	9	90	9	100	27	90.00
Separated/divorced/widowed	5	5.43	0	0	0	0	0	0	0	0	1	3.33
Currently in a relationship	51	54.26	65	55.08	11	47.83	6	60	4	44.44	15	50.00
Education												
None/no formal school	2	2.6	1	1.01	0	0	0	0	0	0	0	0
8th grade or less	7	9.09	13	13.13	0	0	1	10	0	0	2	8.00
> 8 and < 12	33	42.86	33	33.33	2	12.5	4	40	1	16.67	10	40.00
12/high school grad	31	40.26	47	47.47	13	81.25	5	50	5	83.33	13	52.00
Some college	3	3.9	3	3.03	0	0	0	0	0	0	0	0
Technical school graduate	1	1.3	2	2.02	1	6.25	0	0	0	0	0	0
Alcohol consumption												
Every day	0	0	2	2.27	0	0	0	0	0	0	0	0
About once a week	8	13.79	28	31.82	2	11.76	3	42.86	2	25	5	21.74
2-3 times per week	7	12.07	7	7.95	4	23.53	0	0	2	25	2	8.70
Once per month or less	40	68.97	48	54.55	10	58.82	3	42.86	2	25	12	52.17
Several times a month	3	5.17	3	3.41	1	5.88	1	14.29	2	25	4	17.39
Current smoker	19	20.21	52	44.07	4	17.39	2	20	3	33.33	7	23.33

Missing data were excluded from analysis.
CSS chlamydia self-sampling cohort.
PID pelvic inflVammatory disease.

excluded before enrollment, reasons included severe illness, recent treatment with antibiotics, duplicate enrollments and failure to provide informed consent. Additionally, 13 participants who were initially enrolled were found to have a negative CT test at the time of enrollment and were therefore excluded from the study (for a total of 27 excluded participants). Those excluded for testing negative for CT at enrollment

Table 3
Sexual practices in each cohort.

	Female		Male		CSS		Female HIV		Male HIV		PID	
Total (N)	94		118		23		10		9		30	
	N	%	N	%	N	%	N	%	N	%	N	%
Sexual orientation												
Heterosexual	83	91.21	109	94.78	18	78.26	6	66.67	0	0	25	83.33
Homosexual	1	1.1	2	1.74	0	0	0	0	7	77.78	0	0
Bisexual	7	7.69	4	3.48	5	21.74	3	33.33	2	22.22	5	16.67
Condom use												
Always	10	10.64	17	14.41	1	4.35	3	30	4	44.4	0	0
Usually	26	27.66	36	30.51	5	21.74	2	20	3	33.33	12	40.00
Sometimes	29	30.85	33	27.97	9	39.13	2	20	2	22.22	9	30.00
Rarely	14	14.89	13	11.02	2	8.7	0	0	0	0	5	16.67
Never	15	15.96	19	16.10	6	26.09	3	30	0	0	4	13.33
Lifetime number of sex partners												
< =5	50	53.76	25	22.12	6	27.27	4	40	1	11.11	10	33.33
6–8	17	18.28	17	15.04	7	31.82	0	0	2	22.22	8	26.67
9–15	13	13.98	39	34.51	4	18.18	2	20	2	22.22	8	26.27
> 15	13	13.98	32	28.32	5	22.73	4	40	4	44.44	5	16.66
Ever used contraception	82	87.23	NA	NA	21	91.3	8	80	NA	NA	27	90.00

Missing data were excluded from analysis.
CSS chlamydia self-sampling cohort.
PID pelvic inflammatory disease.

Table 4
Treatment protocol.

PID	Ceftriaxone 250 mg 1 dose IM injection + Doxycycline 100 mg PO twice daily for 14 days + Metronidazole 500 mg PO twice daily for 14 days
Cervicitis	Ceftriaxone 125 mg 1 dose IM injection + Azithromycin 1 gm PO single dose
Urethritis	Azithromycin 1 gm PO single dose
Chlamydia (uncomplicated)	Azithromycin 1 gm PO single dose
Gonorrhea	Ceftriaxone 250 mg 1 dose IM injection + Azithromycin 1 gm PO single dose

included three females in the cross sectional arm, one in the CSS arm, six females in the PID arm, two in the cross sectional male arm and one in the male HIV arm. Retention and completion rates as well as subsequent positive CT testing are outlined in Figure 2. Throughout the study, 11 participants were dropped prior to completion. Ten of these participants were dropped when they became pregnant and one participant died during the course of the study of an unrelated cause. Of the 157 females enrolled in the study, 23 were enrolled in the self-sampling longitudinal cohort, 15 of which completed visit 5 at 9 months.

Demographic information is outlined in Table 2. Most participants were African American. Participant age, education range and substance use varied throughout the study arms. Sexual practices including gender of sexual partners, lifetime partners, condom use and contraception are reported in Table 3. Notably, consistent condom use was low across all cohorts, but was the highest in men living with HIV.

Discussion: The samples collected from this protocol have been and continue to be used for studies performed under the NIH-NIAID STI Cooperative Research Center (CRC) titled Eco-Pathogenomics of Chlamydial Reproductive Tract Infection (EPCRTI). More recently, CHARM samples are being used in the follow-up CRC "Eco-Pathogenomics of Sexually Transmitted Infections (EPSTI)."

The overarching objective of EPCRTI is the discovery of the essential correlates of chlamydial infection of the human reproductive tract in the human reproductive tract. The EPCRTI project was conceived to prime effective translational research through two related objectives: the identification and characterization of chlamydial antigens of relevance to vaccine development in the naturally infected host and the characterization of chlamydial metabolic, physiologic or pathogenic mechanisms that are at play in the complex, natural environment of the female genital tract in order to provide targets for possible intervention. To achieve these objectives, project EPCRTI leverages some of the most advanced and innovative methodologies in the study of bacterial infectious diseases. These include characterizing the vaginal microbiota using high-throughput 16S rRNA sequencing, *meta*-transcriptomics to identify responses of the vaginal microbiota to chlamydial infection, the isolation of isogenic antibiotic-resistant mutants and the predictive power of biomathematical modeling of chlamydial infection and disease. The composition of the genital microbiota, the occurrence of secondary infection, the application of antibiotic treatment and the diversity of *Chlamydia*'s physiologic attributes and genome sequence are among the common variables being evaluated both longitudinally and cross-sectionally. Some of the analyses which have utilized CHARM data and/or samples under projects EPCRTI and EPSTI projects have been previously reported [28–32].

Conflicts of interest

All authors report no conflicts of interest.

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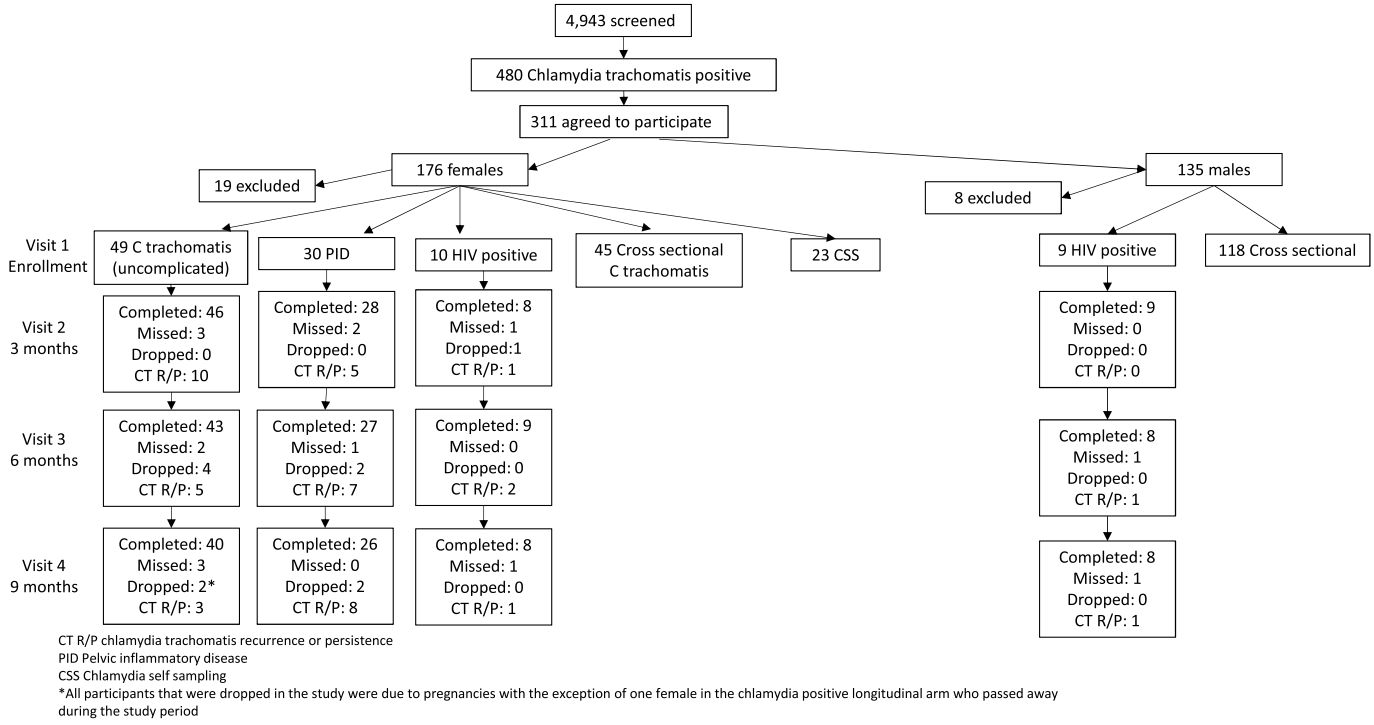

Fig 2. Screening, enrollment, study completion and chlamydia trachomatis positivity rates

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