Sexually Transmitted Bacterial Infections

Lynda Britton, Ph.D., MLS(ASCP)^{CM}, SM

Professor of Medical Laboratory Science Ibritt1@Isuhsc.edu

Objectives

- Review sexually transmitted bacterial pathogens epidemiology, sequalae, and symptoms.
- 2. Describe laboratory testing of these pathogens.



Pathogenesis

Epidemiology

Diagnosis

Laboratory tests

Why STIs Are Important

- All communities impacted by STIs
- Individuals directly or indirectly pay for costs
- Difficult to control because people are reluctant to discuss sexual behavior
- Lack easy solutions because they are rooted in human behavior

Epidemiology

15 to 24 ages = 25%
of the sexually
experienced
population

Represent nearly half of all new infections

Disproportionate burden of disease among African Americans and Hispanics

Increasing STIs

Earlier initiation age for sexual relations

Longer delay to marriage

Multiple partners

Unprotected intercourse

Higher risk partners



Prevalence and Incidence in US





THE U.S. IS EXPERIENCING STEEP, SUSTAINED INCREASES IN SEXUALLY TRANSMITTED DISEASES

Combined diagnoses of chlamydia, gonorrhea, and syphilis increased sharply over the past five years





UNDIAGNOSED STDS CAN LEAD TO SEVERE HEALTH PROBLEMS

Diagnosed cases of chlamydia, gonorrhea, and syphilis represent just a small fraction of the true disease burden Left untreated, these STDs can produce severe, adverse effects

Pregnancy
increased

CONTINUED CONCERNS ABOUT ANTIBIOTIC RESISTANT GONORRHEA



Gonorrhea is expected to eventually wear down our last highly effective antibiotic



Lab tests show a small but growing fraction of gonorrhea samples have signs of emerging antibiotic resistance



CDC recommends a two-drug combination to preserve our last highly effective antibiotic

For more information, visit cdc.gov/nchhstp/newsroom



U.S. Department of Health and Human Services Centers for Disease Control and Prevention

11



Chronic pelvic pain

Infertility

Poor birth outcomes and death

Increases risk of HIV

Quality of life

Billions of \$\$\$

Pelvic Inflammatory Disease — Initial Visits to Physicians' Offices Among Females Aged 15–44 Years, United States, 2007–2016



* In thousands.

NOTE: The relative standard errors for these estimates are 23%–16%. See section A2.5 in the Appendix and Table 44. **SOURCE:** National Disease and Therapeutic Index, IMS Health, Integrated Promotional Services[™], IMS Health Report, 1966–2016.



Ectopic Pregnancy — Ratio* Among Commercially Insured Females with Live Births Aged 15–44 Years by Age Group, 2006–2017



* Ratios represent the number of ectopic pregnancy diagnoses per 100,000 live births. **SOURCE:** MarketScan Commercial Claims and Encounters Database, Truven Health Analytics, Ann Arbor, MI, 2006–2017.



Chlamydia and Gonorrhea — Rates of Reported Cases Among Infants <1 Year of Age by Year and Specimen Source, United States, 2014– 2018



Diseases that Produce Genital Ulcers

Syphilis

Chancroid



Lymphogranuloma venereum

Syphilis Epidemiology

- II5,000 cases of syphilis in 2018
- Increased 14.9% during 2017–2018
- MSM 54% of cases-42% HIV
- Congenital 40% increase in 2018

Syphilis — Rates of Reported Cases by Stage of Infection, United States, 1941–2018





* Per 100,000.

NOTE: See section A1.3 in the Appendix for more information on syphilis case reporting.

Primary and Secondary Syphilis — Distribution of Cases by Sex and Sex of Sex Partners, United States, 2018



Primary and Secondary Syphilis — Rates of Reported Cases by Sex and Male-to-Female Rate Ratios, United States, 1990–2018



Primary and Secondary Syphilis — Reported Cases by Sex and Sex of Sex Partners and HIV Status, United States, 2018





ACRONYMS: MSM = Gay, bisexual, and other men who have sex with men; MSW = Men who have sex with women only.

Syphilis: Treponema pallidum

- Darkfield wet mount
- Screen with a nontreponemal test
 - Rapid plasma reagin (RPR)
 - Venereal Disease Research Laboratory (VDRL)
- Confirm with treponemal

test

- FTA-ABS
- TPPA
- EIA



22 Public Health Image Library (PHIL) http://phil.cdc.gov/phil/details.asp

Primary Syphilis: Chancre



Public Health Image Library (PHIL) http://phil.cdc.gov/phil/details.asp

Secondary Syphilis Rash



Public Health Image Library (PHIL) http://phil.cdc.gov/phil/details.asp

Latent Syphilis

No signs or symptoms
 Detected by

serological tests



Tertiary Syphilis



Neurosyphilis

- Occurs 5-15 years after secondary syphilis
- Symptoms of pain, general paresis (incomplete paralysis), tabes dorsalis (staggering, wide-base gait), and optic atrophy and pupillary changes
- Diagnosis by increased WBCs in CSF and positive VDRL on CSF

Pregnant Women

69% of untreated infected pregnant women

Adverse pregnancy outcomes

- 25% stillbirth
- Serious birth defects
- Low birth weight
- Prematurity

Congenital Syphilis



Clinical course variable

Symptoms of secondary and tertiary syphilis

Rash

Damage to bones, mucous membranes, eyes, teeth, and auditory nerves

Neurosyphilis and gummas

Congenital Syphilis

- > 26% increase 2013-18-1,306
- 52.5% in South
- 33% liveborn & symptomatic
- 6% stillborn

Louisiana Department of Health's STD/HIV Program worked with policymakers in 2014 to pass a state law requiring syphilis screening in the first and third trimesters of pregnancy for all women, as well as at delivery for women at high risk. Congenital Syphilis — Reported Cases by Year of Birth and Rates of Reported Cases of Primary and Secondary Syphilis Among Females Aged 15–44 Years, United States, 2009–2018





Missed Preventative Opportunities

- No timely prenatal care and no timely syphilis testing 28%
- No timely syphilis testing despite receipt of timely prenatal care 9%
- No adequate maternal treatment despite a timely syphilis diagnosis 31%
- Late identification of seroconversion during pregnancy 11%

Laboratory Diagnosis

Primary and secondary syphilis:

- positive darkfield exam is definitive
- Presumptive diagnosis by a positive nontreponemal serology test (VDRL or RPR) ~80% Positive primary
- I00% secondary
- Confirmed by FTA-ABS or TPPA
- Nontreponemal tests become negative with successful treatment



U.S. Preventative Services Task Force

Recommendations for testing

- All pregnant women
- Persons at increased risk
 - MSM
 - Commercial sex workers
 - Exchange sex for drugs
 - Adult correctional institutions
 - Persons with other STIs

PPV falls when testing other populations

Direct Microscopy



Mount must be visualized immediately to see characteristic motility

DFA

Cannot be cultured

Timing of Serological Response



Neurology. Clinical Practice. 2014 Apr;4(2):114-122


Traditional Algorithm



RPR



Automated EIA

- More expensive, less labor
- Unable to distinguish between current and former infection



Reverse Algorithm



Tertiary Syphilis Diagnosis

- 70% positive by nontreponental tests
- ~100% positive by treponemal tests
- Negative nontreponemal tests followed by treponemal tests when symptoms indicate

Neurosyphilis Diagnosis



Congenital Syphilis

- Spirochetes visualized from rash or mucocutaneous lesions by darkfield
- Silver stain of placenta
- Molecular tests
- RPR or VDRL positive, confirmed by FTA-ABS or TPPA with 4-fold rise in titer over 3 weeks
- Drop in RPR or VDRL titer after successful antibiotic treatment

Curbing a Syphilis Outbreak in Alaska

2017-18 300% increase in Anchorage

88% men 87% MSM

71% of MSM reported using online dating apps

Only 60% reported being screened

Migration, travel, tourism, substance abuse and homelessness

Outreach: free syphilis screening, provider webinars, condom giveaways



Chancroid caused by Haemophilus ducreyi

Uncommon STD in U.S.

Asia, Africa, Caribbean

Occasionally causes outbreaks

Most common in uncircumcised males, HIV



Chancroid — Reported Cases by Year, United States, 1941–2018



NOTE: See section A1.3 in the Appendix for more information on chancroid case reporting.



Symptoms of Chancroid





- Painful genital ulcers, may be multiple
 - Necrotic base and irregular borders
- Enlarged, tender, inguinal lymph nodes
- Combination of symptoms is diagnostic with no evidence of syphilis or herpes

Laboratory Diagnosis

- Culture is difficult
- Gram stain of ulcer or lymph node material reveals pleomorphic gram negative coccobacilli that have been described as "school of fish" formations
- Poor sensitivity



Lymphogranuloma venereum

- Caused by serovars L1, L2, or L3 of Chlamydia trachomatis
- Tender unilateral inguinal and/or femoral lymphadenopathy
- Rare in U.S.
 - Primarily in MSM
- Common in Africa, Asia, and South America
- Prostitution, immigration, global travel

LGV Symptoms



- Rapidly healing, nonpainful, ulcerating papule at the site of inoculation
- Fever, headache, and myalgia
- Inguinal lymphadenopathy
- Proctitis
- Chronic colorectal fistulas and strictures

Transmission of LGV



Because lesion is painless and resolves quickly, infection may not be recognized



Seldom diagnosed before lymph node involvement is obvious



Therefore, transmitted to others when infection is unknown

Diagnosis

- Serology -- Four- fold rise in titer
- Rule out other causes of inguinal lymphadenopathy
- Cell culture, direct immunofluorescence, or nucleic acid detection



LGV 2015-16 MSM

- 38 cases in Michigan
- > 29 years (19-60)
- 6 newly diagnosed HIV
- ► 4 HCV
- 6 syphilis
- 8 gonorrhea
- 50% proctitis





STDs Characterized by Urethritis, Cervicitis, and Vaginitis

Gonorrhoea *Chlamydia* infections *Mycoplasma genitalium*



Neisseria gonorheae Epidemiology

- I.14 million/year
- 550,00 resistant to at least one antimicrobial
- >17,000 unique samples in specimen bank
- Second most common notifiable condition
- Rates among women increased 3.6% during 2017–2018 and 45.2% during 2014–2018

Rates among men increased 6.0%
 56 2017–2018 and 78.7% 2014–2018

Neisseria gonorrhoeae

- Gram negative diplococci
- Gram stain is useful for
 diagnosing symptomatic
 males if intracellular
 GNDs
- Fastidious



Gonorrhea — Rates of Reported Cases by Year, United States, 1941–2018



* Per 100,000.

NOTE: See section A1.3 in the Appendix for more information on gonorrhea case reporting.





Age Group

* Results are based on data obtained from unique patients with known sex of sex partners (n=77,314) attending SSuN STD clinics who were tested ≥1 time for gonorrhea in 2018.

NOTE: See section A2.2 in the Appendix for SSuN methods.

ACRONYMS: MSM = Gay, bisexual, and other men who have sex with men; MSW = Men who have sex with women only.



Symptoms



- Urethral discharge and dysuria in males
- Vaginal discharge or asymptomatic in females
- Pharyngitis
- Proctitis





Complications

Disseminated or invasive disease

- Septic arthritis
- Rash
- Heart
- Females: infection with GC or Chlamydia causes salpingitis— 30% of female infertility—1.5 million
- Blockage leads to pelvic inflammatory disease (PID)—10-20% from gonorrhea



Pelvic Inflammatory Disease

Pelvic inflammatory diseases



20-40% of infected
women develop PIDInfertility 20%Ectopic pregnancy
9%
• 20 case/1000 pregnancies
• Highest in 20 yearsChronic abdominal
pain 18%

of episodes = risk of TFI

Complications in Neonates



- Ophthalmia
 neonatorum
 - Eye infection in infants
 - If untreated, may cause blindness

Risk from Oral Sex

- Gonorrhea in the throat
- Disseminated infections
- More difficult cure than genital or rectal infections
- > Can be treated with recommended antibiotics



65

85% of sexually active adults aged 18-44 years reported having had oral sex at least once with a partner of the opposite sex

Diagnosis

- Males with discharge-gram stain
- Culture
- DNA probe
- Nucleic Acid Amplification



Male Specimen Collection





- If symptomatic, swab pus
- If asymptomatic, insert swab in urethra, rotate, and leave it in place
- Inoculate directly to medium in a "Z"
- Transport to the laboratory immediately
- Urine for NAAT



- Visualize the cervix with a warmed speculum w/out lubricant
- Remove mucus and/or secretions from cervix with a swab and discard
- Firmly, yet gently, sample endocervical canal by inserting the swab a few millimeters and rotate

Treatment

- Uncomplicated: single 500 mg IM dose of ceftriaxone and oral doxycycline 100 mg twice daily for 7 days
- Gonococcal expedited partner therapy 800 mg oral dose of cefixime and oral doxycycline 100 mg twice daily for 7 days
- No test of cure except oropharyngeal
- Retest at 3 months

Antimicrobial Resistance

- Azithromycin resistance increasing concern
- Results from multiple mechanisms
- Increased >7-fold over 5 years (from 0.6% in 2013 to 4.6% in 2018
- MSM-- 8.6%; 2.9% among men who have sex with women only

MMWR 2020;69:1911-1916.

Resistance



Year

Chlamydia trachomatis





- Small intracellular bacterium
- Gram negative cell wall
- Elementary bodies-infectious stage
- Reticulate bodies-inclusion in epithelial cells
Chlamydia Epidemiology

- 1,758,668 cases in 2018—most common
- I in 10 adolescents infected
- Exceeds all other notifiable infectious diseases in U.S
- 70% asymptomatic

Figure 5. Chlamydia — Rates of Reported Cases by Age Group and Sex, United States, 2018



Chlamydia — Rates of Reported Cases by State and Territory, United States, 2018



* Per 1 NOTE:



Chlamydia — Rates of Reported Cases by Sex, United States, 2000–2018



* Per 100,000.

NOTE: See sections A1.3 and A1.8 in the Appendix for more information on chlamydia case reporting and interpreting trends in chlamydia case reports.



Chlamydia — Positivity Among Females Aged 14–39 Years by Race/Hispanic Ethnicity and Age Group in Clinics* Providing Family Planning and Reproductive Health Services, STD Surveillance Network (SSuN), 2018



* Includes clinics (n=26) that tested >100 females for chlamydia in 2018 and testing coverage was >60%. **NOTE:** See section A2.2 in the Appendix for SSuN methods.



Chlamydia — National Estimates of Prevalence Among Persons Aged 14–39 Years by Sex, Race/Hispanic Ethnicity, or Age Group, National Health and Nutrition Examination Survey (NHANES), 2013–2016



Chlamydia — Proportion of STD Clinic Patients Testing Positive* by Age Group and Sex and Sex of Sex Partners, STD Surveillance Network (SSuN), 2018



Age Group



Chlamydia trachomatis

- I5-20% of men seen in STD clinics
- 3-5% of men seen in general medical practice
- Seroposivity asso. infertility & ectopic pregnancy
- No visible tubal pathology 33% lower pregnancy rate
- Increased risk of impaired development & implantation

Sequellae

- In pregnant women
 - Premature birth
 - Low birth weight
 - Can be spread to the baby during delivery
 - Can cause eye infection
 - Infection of respiratory tract that can develop into pneumonia



Sequellae





PID

- I0%--I5% of untreated chlamydial infections result in diagnosed clinical PID
- 7.4% of married females aged 15--44 years infertile
- > 750,000 cases diagnosed each year
- Infertility costs estimated at \$701 million annually

Chlamydia Screening

- Recommend annual screening for all sexually active females aged <25 years</p>
- Females aged ≥25 years if they are at increased risk for infection
- Rescreen after 3 mo.
- Targeted male screening in high prevalence settings

Diagnosis of Chlamydia

Culture

Enzyme immunoassay

Direct fluorescent antibody

Gene probe

Amplification of nucleic acid

FDA Approved Tests

NAAT	Producer	Detected Pathogen	Methods Used	Target	
Aptima Combo 2 assay	Hologic Inc.	CT/NG	TMA	NG, specific regions in 16S rRNA CT, specific regions in 23Sr RNA	
Aptima CT assay	Hologic Inc.	СТ	TMA	Specific regions in 16S rRNA	
Aptima NG assay	Hologic Inc.	NG	TMA	Specific regions in 16S rRNA	
Abbott RealTime CT/NG	Abbott	CT/NG	real-time PCR	CT, two specific regions in cryptic plasmid NG, specific sequence in OPA gene	
cobas 4800 CT/NG Test	Roche Diagnostics	CT/NG	PCR	CT, two targets, one in cryptic plasmid and one in chromosome NG, direct repeat (DR) 9 specific regions	
ProbeTec ET CT/GC Amplified DNA assay	Becton Dickinson	CT/NG	SDA	CT, specific region in cryptic plasmid (ORF) NG, pilin gene inverting protein homolog	
ProbeTec CT Q ^X Amplified DNA assay	Becton Dickinson	CT	SDA	Specific region in cryptic plasmid (ORF)	
ProbeTec NG Q ^X Amplified DNA assay	Becton Dickinson	NG	SDA	Specific region in pilin gene	
Xpert CT/NG	Cepheid	CT/NG	real-time PCR	CT, specific chromosomal DNA sequence NG, two specific chromosomal DNA sequences (both should be detected for a positive result)	
Aptima Mycoplasma genitalium Assay	Hologic Inc.	MG	TMA	Specific regions in 16s rRNA	

Advantages of Amplified Tests

37% more		25-30% more			Urine specimens:	
sensitive than		sensitive than			first catch, no	
probes		culture			cleansing	
	Vulva swabs can be collected by patient		Tan	nÞ	ons	

Mycoplasma genitalium

- Fastidious organism of *Mollicutes*, smallest prokaryote
- First isolated in 1981
- I5-20% acute and chronic non-gonococcal urethritis (NGU) in men
- 30% of persistent or recurrent urethritis
- Cervicitis—10-30% and 13-16% PID in women
- Adverse pregnancy outcomes

Disease	Level of disease association*
Urethritis	++++
Cervicitis	+++
Bacterial vaginosis	-
Endometritis and/or Pelvic Inflammatory Disease (PID)	+++
Preterm birth	+/-
Infertility (women)	+

* ++++ strong association, +++ association in most studies, + association from a few studies, +/- conflicting results (adapted from WHO 2013)

M. genitalium Epidemiology

- Majority remain asymptomatic and clear infection without developing disease
- Duration I-3 months
- Antibodies correlate to TFI in 9%
- ▶ 4% develop PID
- Asymptomatic screening not recommended

Prevalence



M. genitalium Epidemiology

- Similar incidence to Chlamydia trachomatis: 1% in sexually active heterosexuals
- Higher in MSM and STI clinics
- I,001 asymptomatic MSM, 95 had M. genitalium; 84.2% were macrolide resistant, and 17% were co-infected with Neisseria gonorrhoeae or Chlamydia trachomatis
- Rectal positivity 7%; 2.7% urine
- NGU but not proctitis

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 25, No. 4, April 2019

Diagnosis

- Difficult to culture
- NAAT preferred



- Hologic's Aptima Mycoplasma genitalium Assay
 - Transcription mediated amplification
 - FDA approved 2019
 - Urine, urethral, penile meatal, endocervical or vaginal swab samples
- Roche Cobas

Treatment

- Lacks cell wall
- Resistance to macrolides
- Doxycycline less effective
- Fluorinated quinolones



Bacterial Vaginosis

- Gardnerella vaginalis/ Atopobium vaginae and other bacteria
- Change in normal vaginal flora with increase pH



Causes of Dysbiosis

Hormonal changes

Age

Sexual practices

Antimicrobial drugs usage

Vaginal douching

Atopobium vaginae

Anaerobic GPR

Higher risk of preterm birth

Very rarely found without the presence of Gardnerella

 Gardnerella is scaffold

 R to metronidazole

97 Front. Cell. Infect. Microbiol., 04 March 2020

Prevalence

Most common vaginal condition in ages 15-44—29.2%

21.2 million

Asymptomatic

19% report no sex

25% pregnant

Increases based on lifetime sex partners

Adverse Outcomes



Arch Gynecol Obstet. 2019; 300(1): 1–6.

BV

- Synergistic interactions between BVAB such as G. vaginalis and Atopobium vaginae significantly enhanced the severity of BV by increasing bacterial burden
- Polymicrobial biofilm formation mainly by G. vaginalis thicker
- Dysbiosis associated with HPV
- Decreased Lactobacillus--increased incidence for all STIs
- Increased HIV shedding and acquisition

Diagnosis



- Homogenous, grey discharge
- Clue cells seen microscopically
- Fishy amine odor elicited by adding a drop of KOH to secretions
- ▶ pH greater than 4.5

Clue Cell Identification

• Wet mount:

- Sloughed epithelial cells
- Numerous tiny bacteria coating epithelial cells
- No WBCs



Clue Cells



Gram Stain of Clue Cells



Clue Cell Gram Stain



- Normal vaginal flora includes Lactobacillus, large, straight, gram positive bacilli
- Vaginosis has no Lactobacillus and numerous small, gram-variable coccobacilli

Vaginosis Discharge



Scoring Vaginal Smears

Organism Morphotype	Number/ oil field	Score	Organism Morphotype	Number/oil field	Score		
Lactobacillus	>30	0	Gardnerella	>30	4		
	5-30	1		5-30	3		
	1-4	2		1-4	2		
	<1	3		<1	1		
	0	4		0	0		
Add score and interpret:							
0-3	Normal						
4-6	Intermediate, retest						
7-10	Bacterial vaginosis						

Treatment

- Vaginal microbiota transplantation (VMT) could be effective in treating problematic vaginal infections
- Reconstitute Lactobacillus-dominated microbiota with no observable adverse effects in recurrent-BV patients
- Combined therapy metronidazole with Lactobacillus successfully treated 88% vs 40% recovery with only metronidazole
Case I



- 7-week-old with "snuffles," mucocutaneous lesions and slight fever
- Radiological studies revealed periostitis of bones
- Confirmed by serology with difficulty
- Parents also treated

- I5-year-old practicing gay male
- 24-hour history of dysuria, pus-like drainage from his penis, and tender left knee joint
- Gram stain revealed the causitive organism



- 30-year-old woman was the contact of man who tested positive at the STD clinic but was asymptomatic
- Pelvic examination demonstrated pus
- Two weeks later, she developed PID





- 40-year-old male had a painless ulcer on his penis two weeks after visiting a local prostitute
- She had no visible lesions
- No lymphadenopathy was noted

- > 24 y o pregnant woman
- Presented at PA with a foul smelling, gray, homogenous discharge
- No pain, itching, or other symptoms
- Gram stain revealed numerous epithelial cells covered by small gram variable rods



- ▶ 43 y. o. female
- Tender inguinal lymph nodes
- No lesions were found and her cervix normal
- Not had sex with anyone but her husband
- Husband frequently travels overseas
- Nothing seen on gram stain of the lymph fluid

