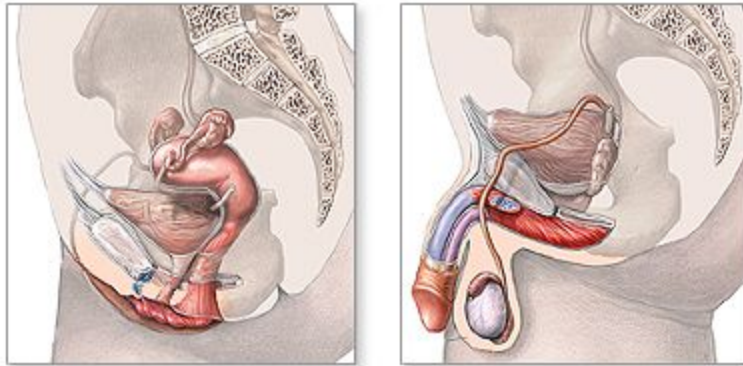


3- Chlamydia, syphilis & gonorrhoea (STD)

Microbiology 435's Teamwork
Reproductive Block



إِنَّا كُلُّ شَيْءٍ خَلَقْتَهُ بِقَدْرِ ٤٩

Learning Objectives:

- Know the causative agents of syphilis, gonorrhoea and Chlamydia infections.
- Realize that these three infections are acquired through sexual intercourse.
- Know the pathogenesis of syphilis, gonorrhoea and Chlamydia infection.
- Describe the clinical feature of the primary, secondary tertiary syphilis and complications.
- Recall the different diagnostic methods for the different stages of syphilis.
- Describe the clinical features of gonorrhoea that affect only men, only women and those ones which affect both sexes.
- Describe the different laboratory tests for the diagnosis of gonorrhoea
- Describe the morphology and the distinct life cycle of the Chlamydia.
- Realize what are the different genera, species and serotypes of the family Chlamydia.
- Recognize that Chlamydia cause different diseases that affect the eye (causing trachoma) and the respiratory system (mainly cause a typical pneumonia).
- Know the different urogenital clinical syndromes caused by Chlamydia trachomatis that affect men, women and both sex.
- Realize that these urogenital syndromes are difficult to differentiate clinically from the similar ones caused by N.gonorrhoeae.
- Know the treatment of syphilis, gonorrhoea and Chlamydia infections.
- Realize that there are no effective vaccines against all these three diseases.

- Important
- Males notes
- Females notes
- Extra

Revised by
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Resources: 435 females & males slides and notes, wikipedia,
Lippincott's Illustrated Reviews: Microbiology-
Third Edition

Editing file: [Here](#)

Credit: [Team members](#)

Introduction (take-home message)

- **Syphilis, Chlamydia and Gonorrhoea** are main STDs, caused by **delicate** organisms that cannot survive outside the body. Infection **may not** be localized.
- Clinical presentation may be similar (urethral or genital discharge, ulcers).
- One or more organisms (Bacteria, virus, parasite) may be transmitted by sexual contact.
- Screening for HIV is required.
- If not treated early may end up in serious complications.

1. Chlamydia

Chlamydia is an **obligate intracellular bacteria** with elements of bacteria but **no** rigid cell wall¹. **Chlamydia belongs to Chlamydiae phylum species and has different serotype**



- **Fail** to grow on artificial media
- Use host cell metabolism for growth and replication.
- Form **inclusion bodies**.

Life cycle: The extracellular infectious form, the elementary body, is taken up by phagocytosis into susceptible host cells. Inside the cell, the particle reorganizes over the next 8 hours into a larger, noninfectious reticulate body, which as it divides, it fills the endosome with its progeny, forming an inclusion body. After 48 hours, multiplication ceases, and reticulate bodies condense to become new infectious elementary bodies. The elementary bodies are then released from the cell by cytolysis, ending in host cell death. **In short: elementary body enters (infects), reticulate body replicates.** [Picture](#)

Infective form is elementary body (but inclusion body by itself is not infective)

Species	Disease
<p><i>C. trachomatis:</i> *Don't confuse with trichomonas</p> <p>Details (this picture was at the end of the males' slides)</p>	<ul style="list-style-type: none"> ● A,B,C → Trachoma (most common cause of blindness in the past). contagious eye infection يسمنونها تراخوما أو الرمد الحبيبي وتسبب ● D - K (D,E,F,G,H,I,j,k) → <ul style="list-style-type: none"> ○ Inclusion conjunctivitis² (in children): 50% of infants born to mothers excreting <i>C. trachomatis</i> during labor show evidence of infection during the first year of life. Most develop inclusion conjunctivitis, 5-10% develop infant pneumonia syndrome. <ul style="list-style-type: none"> ○ Genital infection. <i>Next page</i> ● L1, L2, L3 → Lymphogranuloma venereum (LGV): <ul style="list-style-type: none"> ○ Can enter through skin or mucosal breaks ○ Common in South America and Africa. ○ Papules, and inguinal lymphadenopathy.³ ○ Chronic infection leads to abscesses, strictures and fistulas.
<i>C.psittaci</i>	<ul style="list-style-type: none"> ● Psittacosis (birds related) اسمه داء البيغاء ينتقل عبر البيغاء causes TB and respiratory infection
<i>C.pneumoniae</i>	<ul style="list-style-type: none"> ● Respiratory infections also cause atherosclerosis and cardiac infection

¹ The cell envelope has two lipid bilayers (similar to gram negative cells), but no peptidoglycan or muramic acid is present.

² Inclusion conjunctivitis is an inflammation of the conjunctiva by **Chlamydia trachomatis**.

³ LGV is characterized by transient papules on the external genitalia, followed in 1 to 2 months by painful swelling of inguinal and perirectal lymph nodes.

Chlamydia genital infections

Epidemiology	<ul style="list-style-type: none"> Spread by genital secretions, anal or oral sex. Common in homosexual Wide spread, 5-20 % among STD clinic in USA. very common in CALIFORNIA Human are the sole reservoir. 1/3 of male sexual contacts of women with <i>C.trachomatis</i> cervicitis develop urethritis after 2-6 weeks incubation period. Women contacts of men who is having urethritis will develop cervicitis (Urethritis in male & cervicitis in female)
Pathogenesis	<ul style="list-style-type: none"> Chlamydia have tropism (It likes) for epithelial cells of endocervix and upper genital tract of women, urethra, rectum and conjunctiva of both sexes. Release of proinflammatory cytokines, leads to tissue infiltration by inflammatory cells, progress to necrosis, fibrosis and scarring.
Genital infections	<p>In men:</p> <ul style="list-style-type: none"> Urethritis (non gonococcal urethritis (NGU)⁴, which can present as dysuria and thin urethral discharge in 50 % of men). Discharge is usually thin. Often confused with UTI. Urethritis in men is most commonly caused by neisseria gonorrhoeae, thus, other causes of urethritis are called NGU.⁵ Epididymitis & proctitis⁶. <p>In women:</p> <ul style="list-style-type: none"> Cervicitis (Uterine cervix infection may produce vaginal discharge but is asymptomatic in 50-70% of women) Salpingitis (Salpingitis and pelvic inflammatory disease can cause sterility and ectopic pregnancy) It is one of the causes of infertility in women Urethral syndrome⁷, Endometritis & proctitis.
Diagnosis	<ul style="list-style-type: none"> Polymerase chain reaction (PCR) or Ligase chain reaction (LCR) are the most sensitive methods of diagnosis. Performed on vaginal, cervical, urethral swabs, or urine. It does not stain by gram stain. Isolation on tissue culture (McCoy cell line) but it is <i>rarely done</i>: <i>C.trachomatis</i> inclusions can be seen by <u>iodine</u> or <u>Giemsa</u> stained smear.⁸
Treatment	<ul style="list-style-type: none"> Azithromycin single dose for non-LGV infection. Erythromycin for pregnant women. Doxycycline for LGV.⁹
Prevention and control	through early detection of asymptomatic cases, screening women under 25 years to reduce transmission to the sexual partner. Important

⁴ currently the most common reportable infectious disease in the United States

⁵ Chlamydial NGU is symptomatically similar to infections caused by *Neisseria gonorrhoeae*, although the average incubation time is longer (2 to 3 weeks), and the discharge tends to be more mucoid and contains fewer pus cells. In addition, the two infections often occur simultaneously.

⁶ **Proctitis** is an inflammation of the lining of the rectum.

⁷ **Urethral syndrome**, also known as symptomatic abacteriuria, has many of the same symptoms as urethritis, including abdominal pain and frequent, painful urination. often has no clear cause. most common in women.

⁸ Isolation of chlamydia in McCoy cell line has been the **GOLD SLANDERED** but it's rarely done because it needs time and special conditions. Iodine will stain glycogen and the inclusion bodies that are specific to chlamydia trachomatis .

⁹ Erythromycin should be used in small children and pregnant women because of the effects of tetracyclines on teeth and bones

2. Gonorrhoea السيان

Definition:

A STD disease acquired by direct genital contact. It is **localized** to mucosal surfaces (but leads to intense inflammation) with **infrequent spread** to blood or deep tissues. Caused by *N.gonorrhoeae*.

Clinical manifestations:

IP (incubation period): **2-5 days** IP in chlamydia and syphilis is longer (2-6 weeks)

Symptoms are similar to *Chlamydia* infection:

Men ¹⁰	Women Might be asymptomatic	both
<ul style="list-style-type: none"> ● acute urethritis and ● acute profuse purulent urethral discharge. <p>Discharge in chlamydia is thin, while in gonorrhoea it is profuse, purulent and painful</p>	<ul style="list-style-type: none"> ● mucopurulent cervicitis¹¹ ● urethritis with discharge. ● Pelvic inflammatory disease (PID) 	<ul style="list-style-type: none"> ● Urethritis ● Proctitis¹² ● Pharyngitis may occur¹³

Pelvic Inflammatory Disease (PID):

- PID occurs in 10-20% of cases, includes **fever, lower abdominal pain, adnexal tenderness, leukocytosis** with or without signs of local infection.
- **Salpingitis** and pelvic **peritonitis** cause **scarring** and **infertility**.
- **if untreated**, it spread to the bloodstream causes **disseminated Gonococcal Infection (DGI)**:
 - Clinically: **Fever**, migratory **arthralgia** and **arthritis**, purulent arthritis involving large joints, petechial, maculopapular rash. Thus sometimes called the "arthritis-dermatitis syndrome"
 - **Metastatic infection such as Endocarditis, Meningitis & Perihepatitis may develop**

¹⁰ Symptoms of gonococcal infection are more acute and easier to diagnose in males. (yellow discharge and dysuria).

¹¹ A greenish-yellow cervical discharge is most common, often accompanied by intermenstrual bleeding.

¹² Rectal infections: Prevalent in men who have sex with men, rectal infections are characterized by constipation, painful defecation, and purulent discharge.

¹³ Pharyngitis is contracted by oral-genital contact. Infected individuals may show a purulent pharyngeal exudate, and the condition may mimic a mild viral or a streptococcal sore throat

Gonorrhoea (cont.)

Epidemiology	<ul style="list-style-type: none"> Rates among adolescents are high, about 10% increase per year in USA . Inability to detect asymptomatic cases such as women and patient fail to seek medical care hampers control . Major reservoir for continued spread are asymptomatic cases. Non-sexual transmission is rare.
Pathogenesis	<ul style="list-style-type: none"> Neisseria gonorrhoeae is a Gram negative diplococci. It grows on chocolate agar and on selective enriched media and CO2 required. It is Not a normal flora. It posses pili and outer membrane proteins (IA and Opa proteins) that mediate attachment to non-ciliated epithelium. <p>Neisseria meningitidis and Neisseria gonorrhoeae are Gram negative diplococci, but neisseria meningitidis (CSF sample) grows easily in blood and chocolate agars, while Neisseria gonorrhoeae requires selective media because there is a lot of normal flora in the vagina that may interfere with its growth. Thus when we take a specimen from genital tract we culture it in selective media.</p>
Diagnosis	<p>→ Transport media¹⁴ is required unless transfer to the lab is immediate.</p> <ul style="list-style-type: none"> Direct smear for Gram stain of urethral and cervical specimens to see Gram negative diplococci within a neutrophil (intracellular), more sensitive in men. If the gram negative diplococci is extracellular (outside WBC) it is normal flora not N. gonorrhoeae Culture on Thayer-Martin or other selective medium. Thayer-Martin medium is a chocolate agar supplemented with several antibiotics (Vancomycin, Colistin, Nystatin)¹⁵ Confirmation: Isolates identified by sugar fermentation¹⁶ of glucose only (does not ferment maltose or sucrose) or Coagglutination test. <p>Gram negative diplococci within neutrophil (intracellular), how to confirm it is N. Gonorrhoeae?</p> <ol style="list-style-type: none"> Sugar fermentation: Gonorrhoea=ferment Glucose. Neisseria Meningitidis= ferment Maltose and glucose Coagglutination test: If there is agglutination with Neisseria it is positive and the test is accurate for diagnosis of gonorrhoea.
Treatment	<ul style="list-style-type: none"> Treatment is guided by local resistance pattern and susceptibility testing. <ul style="list-style-type: none"> Ceftriaxone IM (or oral Cefixime recommended). Ciprofloxacin or Ofloxacin Azithromycin, Doxycycline (orally for 7 days), both cover C.trachomatis infection as well Partner should be treated as well. Counseling. After treatment if discharge comes back, there is resistance or co-infection usually C.trachomatis

¹⁴ **Transport media** are special **media** formulated to preserve a specimen and minimize bacterial overgrowth from the time of collection to the time it is received at the laboratory to be processed.

¹⁵ To suppress the growth of nonpathogenic Neisseriae and other normal and abnormal flora. (GOLD STANDARD)

¹⁶ All Neisseria species are oxidase-positive. To differentiate between species, sugar utilization tests are used.

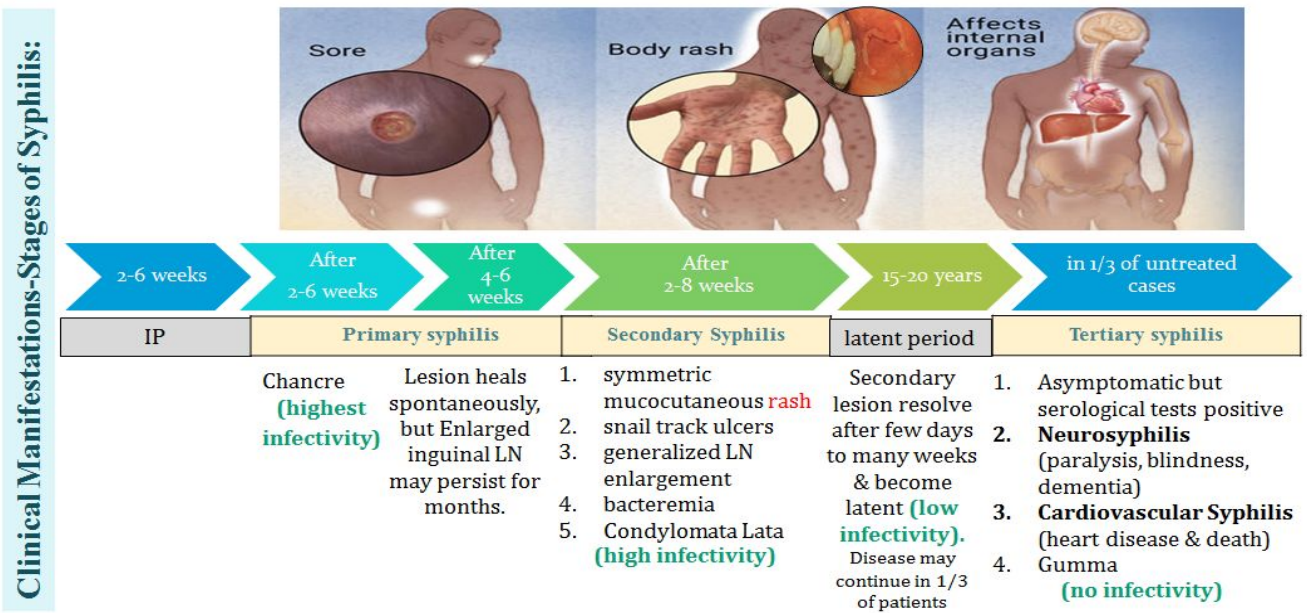
3. Syphilis الزهري

Definition: A chronic systemic infection, sexually transmitted, caused by a spiral organism called *Treponema pallidum* subsp. *pallidum*¹⁷ (Phylum: Spirochaetes)

Features:

- The organism grow on cultured mammalian cells **only**. They inject it in animals, takes days to month for the etiology to appear. It is very sensitive and difficult to grow.
- NOT stained by Gram stain.¹⁸
- Readily seen only by **immunofluorescence (IF)**, **dark field** microscopy or **silver impregnation histology technique** (tiny thin spiral organisms visualized using heavy metals. [Picture](#)).

Syphilis	
Epidemiology	<ul style="list-style-type: none"> • An exclusively human pathogen. • Transmission by contact with mucosal surfaces or blood (blood donors should be screened for HIV and syphilis), less commonly by non-genital contacts with a lesion, sharing needles by IV drug users, or transplacental transmission to fetus. • Early disease is infectious, Late disease is not.
Pathogenesis	<ul style="list-style-type: none"> • Bacteria access through inapparent skin or mucosal breaks. It is very infectious, if the patient has the lesion and the doctor has a very tiny wound he can get the infection. • Slow multiplication produces endarteritis & granulomas. • Start by small ulcer. Ulcer heals but spirochete disseminate. • Latent periods may be due to surface binding of host components. • Injury is due to delayed hypersensitivity responses to the persistence of the spirochetes.



¹⁷ Other subspecies: 1) T. p. endemicum, which causes **bejel** or endemic syphilis, 2) T. p. carateum, which causes **pinta**, 3) T. p. pertenue, which causes **yaws**

¹⁸ Spirochetes tend to be gram negative, however, because of how thin *Treponema pallidum* is, it is difficult to classify its gram stain.

1. **Primary syphilis:** It appear after an IP of about 2-6 weeks .

- **Chancre** is a **painless** (HSV will be painful/Chancre caused by herpes is painful but syphilis typically is **painless**), indurated ulcer with firm base and raised margins on external genitalia or cervix, anal or oral site (**appears on the place of the sexual contact**).
- Lesion heals spontaneously¹⁹ after 4-6 weeks. Enlarged inguinal lymph nodes may persist for months.

2. **Secondary Syphilis:** Develops 2-8 weeks after primary lesion healed. Characterized by:

- Symmetric mucocutaneous rash (distributed on trunk and extremities often **palms, soles and face**).
- Mouth lesions (**snail track ulcers**)
- Generalized **non-tender** lymph nodes enlargement (**full of spirochete**)
- Bacteremia causing **fever**, malaise and other systemic Manifestations.
- 1/3 develop **Condylomata Lata**: which are **painless** mucosal **warty** erosions on genital area and perineum²⁰. **ورمات صغيرة مثل الثآليل warty erosions**

3. **Latent syphilis**

- Secondary lesion resolve after few days to many weeks but disease continue in 1/3 of patients.
- **Disease enter into a latent state**, where there is no clinical manifestations but **infection evident by serologic tests**. Relapse cease...
- **Risk of blood-borne transmission from relapsing infection or mother to fetus continue.**

4. **Tertiary (late) syphilis:** in 1/3 of untreated cases.

Manifestations may appear after **15-20 years** or may be **asymptomatic** but serological tests positive.

- **Neurosyphilis:**
 - Chronic meningitis, with increased cells and protein in CSF, leads to degenerative changes and psychosis.
 - Demyelination causes peripheral neuropathies.
 - Most advanced cases result in **paresis** (personality, **affect**, **reflexes**, **eyes**, **sensorium**, **intellect**, **speech**) due to the effect on the brain parenchyma and **posterior columns of spinal cord and dorsal roots**²¹.
- **Cardiovascular Syphilis:**
 - **Arteritis**, which leads to aneurysm of aorta and aortic valve ring.
- **Localized granulomatous reaction** called **gumma** on skin, bones, joints or other organs leads to local destruction .

Congenital syphilis²²: if the mother is not treated, fetus is susceptible to acquire syphilis **after 4th month** of gestation. Leading to:

- **Fetal loss or Congenital syphilis:** Rhinitis, rash, bone changes (**saddle nose**²³, **saber shine**²⁴), anemia, thrombocytopenia, and liver failure.

¹⁹ but the organism continues to spread throughout the body via the lymph and blood

²⁰ Condyloma (plural: "Condylomata", from Greek "knuckle") refers to an infection of the genitals. The two subtypes are:

1- Condyloma **acuminata**, or genital warts, caused by human papilloma virus subtypes 6, 11, and others (*next lecture*)

2- Condylomata **lata**, white lesions associated with secondary syphilis.

²¹ **Tabes dorsalis**, CNS block, u remember?

²² for congenital syphilis (the baby gets the infection through the placenta), the clinical presentation is divided into two categories:

1- presenting during the first 2 years (early syphilis).

2- presenting after two years of age (although he got the infection transplacentally) it's called late syphilis .

²³ **Saddle nose** is a condition associated with nasal trauma (most common), congenital syphilis, among other conditions.

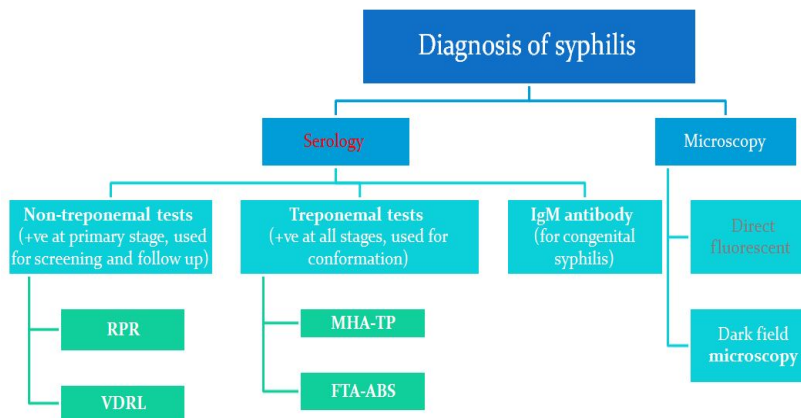
²⁴ malformation of the tibia.

Syphilis (cont.)

Diagnosis

*It mainly depends on serology

1. Dark field **microscopy** of smear from primary or secondary lesions. May be negative.
2. **Silver impregnation is diagnostic to 1ry stage**
3. **Serologic tests: more commonly used** as they are easier to perform. Include:
 - 1-Nontreponemal tests (nonspecific):** antibody to **cardiolipin** (lipin = a lipid complex, cardio= extracted from beef heart), this Ab. (cardiolipin) is called "**reagin**". **The tests are:**
 - **rapid plasma reagin (RPR)** ²⁵
 - venereal disease research laboratory (**VDRL**).
 - Become positive during the **primary stage** "**The chancre**" (possible exception: patients with HIV), antibody peak in **secondary syphilis**, then slowly wane in **later stages**.²⁶
 - **Positive in patients who have other diseases and in pregnant and in hepatitis and other infection but mainly Treponema pallidum syphilis**
 - Used for **screening**²⁷ and **follow up**.
 - We use it in screening of primary and secondary syphilis or we use it to check if the patient is responding to treatment, but it is not specific to syphilis.
 - If the patient is positive to RPR we have to do confirmatory test. If it is negative he doesn't have syphilis.
- 2- Treponemal tests:** using **treponemal antigen to detect specific antibody to T.pallidum** by:
 - A- **Fluorescent Treponemal Antibody (FTA-ABS)** specific & + in early stages
 - B- Microhemagglutination test (**MHA-TP**) (antigen attached to erythrocytes)
 - I put buffer so i can delete the non treponemal pallidum.
 - This is positive at all stages حتى لو المريض تعالج
 - **Positive results of treponemal test confirm RPR and VDRL.**
- 3- IgM** used to diagnose **congenital syphilis**.



Interpretation of serological tests for syphilis (picture):

- Positive nontreponemal tests and specific treponemal test is positive= the patient has syphilis
- If the nontreponemal tests positive and treponemal test is negative= no syphilis
- Nontreponemal tests Negative and treponemal tests positive= previously treated
- Both negative? no syphilis

Treatment

- **Treponema is sensitive to Penicillin**, but hypersensitive patients are treated with *Tetracycline, Erythromycin or Cephalosporin*.

Prevention

- **Counseling**

²⁵ The rapid plasma reagin (RPR) test uses the same antigen as the VDRL, but without the need of a microscope.

²⁶ This test is very useful as the trend of titres are correlated to disease activity (i.e. falling titres indicate successful treatment). It has a very good sensitivity for syphilis, except in late tertiary form.

²⁷ used to screen for syphilis (it has high sensitivity), but more specific tests (Treponemal tests) are used to diagnose the disease.

L3: SUMMARY OF STDs (Chlamydia, syphilis & gonorrhea)

	Chlamydia	Gonorrhea	Syphilis
• Definition	Intracellular replication, forming inclusion bodies Genital infection is caused by <i>C. trachomatis</i> (D-K)	Caused by <i>N. gonorrhoeae</i>. Acquired by direct genital contact. It is localized to mucosal surfaces.	chronic systemic infection, caused by <i>Treponema pallidum</i> subsp. pallidum
Epidemiology	Spread by genital secretions, anal or oral sex Wide spread	Inability to detect asymptomatic cases & patient fail to seek medical care hampers control .	Transmission by contact with mucosal surfaces or blood , or transplacental
• Pathogenesis	proinflammatory cytokines → infiltration by inflammatory cells → necrosis, fibrosis	localized in epithelium → inflammation. Posses pili and outer membrane proteins that mediate attachment to non ciliated epithelium.	Bacteria access → multiplication → endarteritis & granulomas → Ulcer heals but spirochete disseminate → Latent periods due to surface binding of host components. Injury due DTH & spirochetes.
Clinical manifestations	Men: NGU & epididymitis Women: Cervicitis (asymptomatic 50-70%), salpingitis, urethral syndrome & endometritis Both: proctitis. Infants: inclusion conjunctivitis , 5-10% infant pneumonia syndrome.	Men: acute urethritis with profuse purulent urethral discharge. Women: mucopurulent cervicitis, urethral discharge, PID (pain and fever), if it spreads by blood= DGI (fever, rash and arthritis) Both: urethritis, Proctitis, Pharyngitis may occur	Primary: Chancre (painless) Secondary: rash, nail track ulcers , Bacteremia, Condylomata Lata *Primary and secondary are infectious* Latent: no symptom but infection evident by serologic tests Tertiary: (not infectious) <u>1-Neurosyphilis:</u> chronic meningitis, demyelinating & PARESIS <u>2-Cardiovascular Syphilis:</u> Arteritis → aneurysm <u>3- others:</u> "gumma" local destruction
Diagnosis	1- PCR or LCR: the most sensitive methods 2- culture (McCoy cell line) but it is <i>rarely done</i> : <i>C.trachomatis</i> inclusions can be seen by <u>iodine</u> or <u>Giemsa</u> stained smear	1-Gram stain → G-ve diplococci (intracellular) 2-Culture on Thayer-Martin 3-fermentation of glucose only or Coagglutination test.	1. Dark field microscopy 2. Serological (mainly): A-Nontreponemal tests: RPR & VDRL (screening & follow up) B- Treponemal tests: FTA-ABS & MHA-TP (confirmation) C- IgM: used in congenital syphilis.
• Treatment	1-Azithromycin non-LGV 2- Erythromycin for pregnant 3-Doxycycline for LGV.	1-Ceftriaxone or Cefixime 2-Ciprofloxacin or Ofloxacin 3-Azithromycin or Doxycycline if co-infected with <i>C.trachomatis</i>	- Penicillin -if allergic: <i>Tetracycline, Erythromycin or Cephalosporin.</i>

MCQs

1: A 35 year-old male presented to the clinic with dysuria & thin discharge for 5 days. Urine analysis revealed high white blood cells count but culture was negative. Which one of the following bacteria is the most likely cause of his complains?

- A. Chlamydia trachomatis
- B. E.coli
- C. Neisseria gonorrhoeae
- D. Treponema pallidum

2: Which ONE of the following is the most infectious phase of syphilis disease?

- A. Latent phase
- B. Late primary phase.
- C. Early Primary phase
- D. Tertiary phase

3: which ONE of the following is the most common cause of infertility in both sexes?

- A. Chlamydia
- B. Gonorrhoea
- C. Syphilis
- D. HPV

4: Which ONE of the following has an incubation period less than 5 days?

- A. Gonorrhoea
- B. HSV
- C. Chlamydia
- D. Trichomonas

5: Patient with dysuria reported having extramarital sexual relationship. The gram stain revealed intracellular gram-negative diplococci. Which ONE of the following is the causative agent?

- A. Gonorrhoea
- B. Chlamydia
- C. E.coli
- D. Cyanobacterium

6: A young man presented with purulent painless lesion with raised edges on his external genital organ. He was diagnosed to have primary syphilis. Which of the following is the most applicable diagnostic method for this condition?

- A. Dark field microscopy
- B. Exudate cellular
- C. Serology using non-treponemal antigen
- D. Serology using treponemal antigen

ANS : A, C, A, A, A, C