



Al-Mustaqbal University College
Dept. Medical Lab. Techniques
Diagnostic Microbiology 20/2021
By Prof. Dr. Habeeb S. Naher



LECTURE-18: Urinary tract infections (UTI) and sexually transmitted diseases (STD)

Classification of UTI:

Upper UTI:

Kidney infection (pyelonephritis, tubulointerstitial nephritis, glomerulonephritis, renal abscess).

Lower UTI:

- Urinary bladder infection (cystitis).
- Prostatic infection in men.
- Urethral infections (urethritis) classified as sexually transmitted diseases.

Prof. Dr. I.

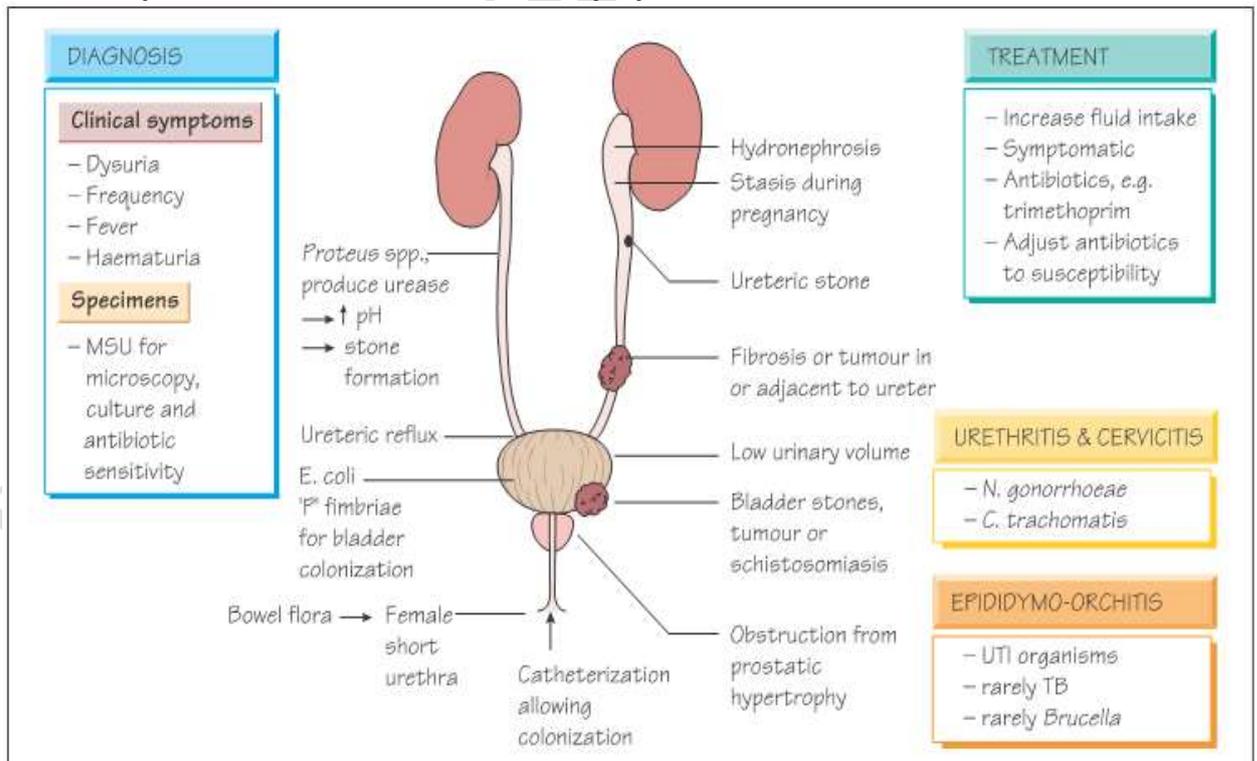
Symptoms of a Urinary Tract Infection



- Frequent urination, but very little urine may come out.
- Painful burning sensation before, during, and after urinating.
- Urinating blood.
- Urgent need to urinate, and in serious cases, unable to control bladder and leaks urine.
- Cloudy or foul smelling urine.
- Malaise or the general feeling of unwell.
- Severe pain in the lower abdomen region.
- Mild fever.

Symptoms of UTI

Anatomy of male and female Urinary system



Genitourinary infection syndromes and causative organisms.

| Syndrome | Organisms |
|-----------------------------|--|
| Genital ulcers | Herpes simplex <i>Chlamydia trachomatis</i> types L1–4 <i>Haemophilus ducreyi</i> <i>Treponema pallidum</i> () <i>Calymmatobacterium donovani</i> |
| Urethral discharge | <i>Neisseria gonorrhoeae</i> <i>C. trachomatis</i> |
| Pelvic inflammatory disease | <i>N. gonorrhoeae</i> <i>C. trachomatis</i> Mixed anaerobic infection |
| Vaginal discharge | <i>Candida albicans</i> <i>Trichomonas vaginalis</i> <i>Mobiluncus</i> spp. and others in non-specific vaginitis |

Diagnosis:

- 1) **Specimen:** Urethral and cervical swabs should be taken for both bacterial and viral diagnosis.
- 2) *N. gonorrhoeae*, *Chlamydia* and *herpes* simplex are optimally detected by nucleic acid amplification tests (PCR).
- 3) Samples positive for *N. gonorrhoeae* can be cultured for **susceptibility** testing.
- 4) **Syphilis** is diagnosed with enzyme immunoassay (EIA) together with traditional **treponemal tests**.
- 5) *Candida* or *Trichomonas* can be detected directly by microscopy examination.

Note: Only the lower part of the urethra is usually colonized by bacteria; the flushing action of urinary flow prevents ascending infection. The shorter female urethra makes urinary infection more common.

Laboratory diagnostic (considerations)

- 1) 'Dip-stick' test for **nitrite and leukocyte esterase** can identify patients with infection and the need for treatment without culture. It is rapid diagnostic test, widely used for proteinuria. The color changes from yellow to green= +ve result.

- 2) Culture using a midstream urine (MSU) specimen to reduce the risk of infection.
- 3) $>10^5$ colony-forming units(CFU)/mL of a single organism indicate infection, while $<10^5$ organisms/mL or a mixed growth suggests contamination.
- 4) Chronically catheterized patients usually have 'significant' numbers of organisms and multiple pathogens and may not have active infection.
- 5) All isolates are potentially significant from a suprapubic aspirate from an infant with suspected infection.
- 6) Susceptibility tests should be performed on all significant isolates.

Diagnosis:

Urethral and cervical swabs should be taken for both bacterial and viral diagnosis. *N. gonorrhoeae*, *Chlamydia* and herpes simplex are optimally detected by nucleic acid amplification tests (polymerase chain reaction-PCR). Samples positive for *N. gonorrhoeae* can be cultured for susceptibility testing. Syphilis is diagnosed with enzyme immunoassay (EIA) together with traditional treponemal tests. Direct microscopy may show evidence of *Candida* or *Trichomonas*.

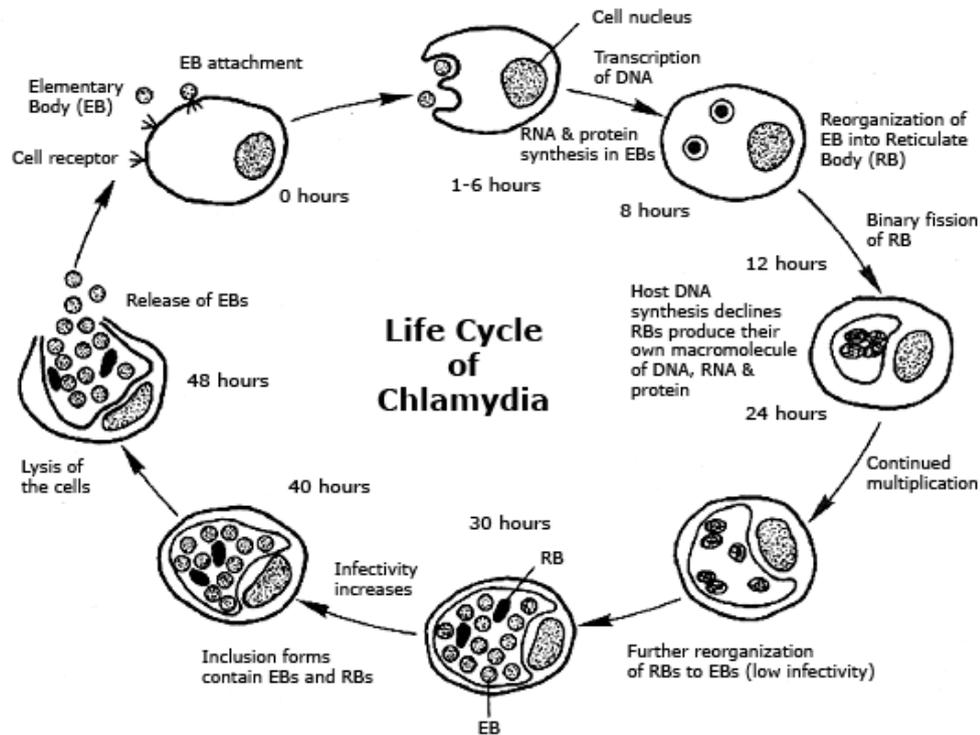
Chlamydiae:

Chlamydiaceae is a family of **obligate intracellular** bacterial parasites, **small, non-motile** and **gram-negative** with a tropism for columnar epithelial cells lining the mucous membranes. They are widely distributed in nature and are **responsible** for a variety of **ocular, genitourinary** and **respiratory diseases in man**. There is some evidence that they may be involved in some chronic diseases such as atherosclerosis.

Chlamydiae have the following properties of bacteria:

- ✓ They possess inner and outer membranes similar to gram-negative bacteria.
- ✓ They contain both DNA and RNA.
- ✓ They possess prokaryotic ribosomes.
- ✓ They synthesize their own proteins, nucleic acids, and lipids.
- ✓ They multiply by binary fission.
- ✓ They do not have 'eclipse phase' following cellular infection.
- ✓ They are susceptible to numerous antibacterial antibiotics.

Life cycle of chlamydia. It takes two forms of life cycle; 1-elementary body (EB) and 2-reticulate body (RB). (See the following figure, please)



Elementary body (metabolically inactive but infectious agent)

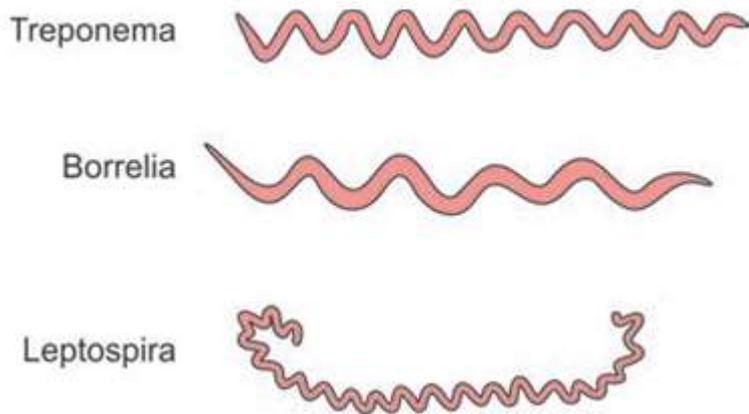
Reticulate body (metabolically active but non-infectious)

Laboratory Propagation:

Chlamydiae can be propagated in the **mouse, chick embryo or in cell culture**. The presence of chlamydial **inclusions** is determined by microscopy in conjunction with a suitable staining method, preferably fluorescence microscopy with labelled monoclonal antibody.

Spirochetes:

Description: Spirochetes are slender unicellular helical or spiral rods with a number of distinctive ultrastructural features used in the differentiation of the genera. They are gram- **negative** and are 0.1 to 3.0 μm wide and 5 to 250 μm in length. They are structurally more **complex** than other bacteria. The spirochetes have gram-negative type cell wall composed of an outer membrane, a peptidoglycan layer and a cytoplasmic membrane. **They differ from other bacteria that they have flexible cell wall around which several fibrils are wound.** These fibrils termed as **endo-flagella**. Their most distinctive morphologic property is the presence of varying number of endo-flagella. The spirochetes can be seen by dark ground microscopy, silver impregnation method and **immunofluorescence**.



Family Spirochetaceae has genera eight genera, the medically important three genera are: *Treponema*, *Borrelia* and *Leptospira*.

Treponema Pallidum Subspecies *Pallidum*: The name Treponema is derived from the Greek words trepo: to turn and nema, meaning thread; the name pallidum refers to its pale staining. they are **short slender spirochetes with fine spirals and pointed or rounded ends**. *Treponema pallidum* Subspecies *pallidum*

Treponemes Cause the following Diseases in Humans:

1. *T. pallidum* subspecies *pallidum* causes Venereal syphilis.
2. *T. pallidum*, subspecies *endemicum* (*T. endemicum*) causes endemic syphilis.
3. *T. pallidum* subspecies *pertenue* causes yaws *T. carateum* causes pinta.

Morphology:

It is a very delicate, spiral filament 6-14 μm (average 10 μm) by 0.2 μm , with 6-12 coils which are comparatively small, sharp and regular. The **ends are pointed and tapering**. Spirochetes show **rotary corkscrew-like motility and also movements of flexion; angulation**, with the organism bending almost to 90° near its center, is highly characteristic of *T. pallidum*. Its progression is relatively slow compared to that of many motile bacteria. During motion, secondary curves appear and disappear in succession but the primary spirals are unchanged. *See the figure*.



T. pallidum cannot be seen under the light microscope in wet films but **can be made out by negative staining with Indian ink**. It is feebly refractive. Its morphology and motility **can be seen under the dark ground or phase contrast microscope**. It does not take ordinary bacterial stains but stains **light rosered with prolonged Giemsa staining**. It can be stained by **silver impregnation** methods. Fontana's method is useful for staining films and Levaditi's method for tissue sections. **Immunofluorescence** methods can now be used to detect treponemes in tissues and body fluids.

Cultivation: There have been many claims of cultivation of *T. pallidum* in cultures but none has been proved. It is possible to maintain *T. pallidum* in motile and virulent form for 10-12 days in complex media under anaerobic conditions.

Laboratory Diagnosis

Laboratory diagnosis consists of demonstration of the spirochetes under the microscope and of antibodies in serum or CSF.

******See the following table for diagnosis of syphilis******

Table 48.2: Diagnostic tests for syphilis

| | |
|---|--|
| A. Demonstration of treponemes in the exudate | <ol style="list-style-type: none"> 1. Dark-ground microscopy 2. Direct fluorescent-antibody staining for <i>Treponema pallidum</i> (DFA- Tp) 3. Silver impregnation method (Levaditi's stain) 4. Enzyme immunoassay, Polymerase chain reaction (PCR). |
| B. Serological tests | <ol style="list-style-type: none"> a. Nontreponemal tests <p>Nonspecific (<i>reagin antibody</i>) tests using the cardiolipin antigen (<i>standard tests for syphilis or STS</i>).</p> <ol style="list-style-type: none"> 1. Wassermann complement fixation test 2. Kahn flocculation test 3. Venereal Disease Research Laboratory (VDRL) test 4. Rapid Plasma Reagin (RPR) test 5. Tolidine red unheated serum test (TRUST) b. Treponemal tests <ol style="list-style-type: none"> a. Group specific test using cultivable treponemal (Reiter strain) antigen <ol style="list-style-type: none"> I. Reiter Protein CF (RPCF) test (1953) b. Specific tests using pathogenic treponemes (<i>T. pallidum</i>) <ol style="list-style-type: none"> I. Test using live <i>T. pallidum</i> <p><i>Treponema pallidum</i> Immobilization (TPI) test</p> II. Tests using killed <i>T. pallidum</i> <ol style="list-style-type: none"> a. <i>Treponema pallidum</i> agglutination (TPA) test b. <i>Treponema pallidum</i> immune adherence (TPIA) test c. Fluorescent Treponemal Antibody Absorption (FTA-ABS) test III. Tests using <i>T. pallidum</i> extract <ol style="list-style-type: none"> a. <i>Treponema pallidum</i> Hemagglutination Assay (TPHA) <p>Microhemagglutination test for <i>Treponema pallidum</i> (MHA-TP)</p> b. <i>Treponema pallidum</i> Enzyme Immunoassays (TP-EIA): |

Prof. Dr. Habeeb S. N.