SHEET NO. 6

Microbiology

Respiratory system

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* anything [written in gray and between square brackets] is mentioned in the slides but not by the doctor during our lecture.

* anything highlighted with yellow is an important information that the doctor focused on and repeated it many times.

* anything highlighted with blue is an additional information that wasn't mentioned in 2019 sheet.

good luck :)

Miscellaneous respiratory tract infections

Atypical Pneumonia

- Atypical pneumonia implicated in these 3 bacterial causes:

1- Mycoplasma; Mycoplasma *pneumoniae.* → lack cell wall

2- Chlamydia; Chlamydia *pneumoniae* and Chlamydia *psittaci.* → gram negative

3- Legionella; Legionella *pneumophila.* → gram negative

- [These related to Gram-ve bacteria.. Attached to respiratory mucosa..Not common part of Respiratory flora..Opportunistic pathogens]

- Atypical pneumonia is different from pneumonia caused by Streptococcus which is the typical pneumonia. These 3 bacteria mentioned earlier cause mostly milder forms of pneumonia, characterized by slow and gradual development of symptoms unlike other forms of pneumonia which can develop more quickly,

that's why it's also called (Walking Pneumonia); patients are still able to walk, function, and do daily life activities without knowing they have pneumonia, hence the name "Walking Pneumonia". Another characteristic to distinguish it from the typical pneumonia is that even though it's generally less severe but early symptoms are more severe than those of typical pneumonia. In addition to that these bacteria do not respond to antibiotics prescribed for streptococcal pneumonia which are mainly penicillins and cephalosporins.

- Hard to diagnose them so it's unknown how much percentage do they make from all cases of pneumonia but in books it's stated that 10% - 20% of community acquired pneumonia caused by Chlamydia *pneumonia,* Mycoplasma *pneumonia* and Legionella *pneumophila*.

* Mycoplasma . *pneumoniae* and Chlamydia . *pneumoniae* specifically can be transmitted from person to person through droplet nuclei but they're not highly contagious moreover Mycoplasma and Chlamydia could be part of normal flora but there's no high percentage of healthy carriers. On the other hand in infections caused by Legionella there's no person to person transmission, an individual acquire it from breathing aerosols containing Legionella which usually contaminate water systems and air conditioning systems, they live there by forming biofilms or inside amoeba.

❖ Mycoplasma pneumoniae

- The smallest size Bacteria, their most distinguished feature is that they lack cell wall, so diagnosis using gram stain is invalid, also they don't response to penicillins and cephalosporins.

- Mycoplasma are extracellular pathogens although there are intracellular events in their life cycle but it's known that they're extracellular bacteria.

* Members of Mycoplasma that cause human diseases :

1. Mycoplasma *pneumoniae*
2. Mycoplasma *genitalium* which is subdivided into

a) Mycoplasma *hominis* (genitourinary tract infection)

b) Ureaplasma *parvum* (genitourinary tract infection) and Ureaplasma *urealyticum*

- [Lipid bi-layer Membrane], Aerobic Growth, if they were part of normal flora they colonize respiratory and genitourinary mucosa, [various Mycoplasma spp. Associated with disease.. Human, Animals, Birds]

- M. *pneumoniae* spread from person to person by droplets but they're not highly infectious.

- Often develop Low fever & dry cough symptoms ..few days-weeks.. anemia, rashes, neurological syndromes..meningitis, encephalitis.

- Infection caused by M. *pneumoniae* could be as simple as Acute/ Subacute pharyngitis (mainly subclinical), bronchitis or a milder form of pneumonia.

- Common syndromes associated with M. *pneumoniae* -usually develop after 2-3 weeks of infection of Mycoplasma- include anaemia (hemolytic anaemia) where there will be production of cold agglutinins which are auto-antibodies against antigens on RBCs, and neurological syndromes such as meningitis, encephalitis and brain abscesses. It's unknown whether it's direct extension of mycoplasma to the CNS or autoimmune response towards M. *pneumoniae*.

- [Common Infection in Fall-Winter], mostly affect older children and young adults (unlike Chlamydia which affect school age children)

- [Severe forms of M pneumonia have been described in all age groups.]

- Lab diagnosis of Mycoplasma is difficult because they're common contaminants, but we can uses special culture medium (FRIIS Medium), PCR recently developed to detect Mycoplasma from pleural fluid or blood, serological Cold-Agglutination test was used previously but not anymore because it could be found in other infections not only Mycoplasma *pneumoniae* and serological tests to look for antibodies against Mycoplasma.

Treatment: Fluoroquinolones like levofloxacin and moxifloxacin and Macrolides like Azithromycin.. No Vaccine

❖ Chlamydia Species

- Chlamydia attach to human mucosal membrane so they can be part of normal flora, person to person transmission is possible but to a less extent than Mycoplasma one of the reasons is that Chlamydia have two forms in their life cycle:

1) elementary body -infectious form- and 2) reticulate body -metabolically active form- , elementary body doesn't resist dryness outside the body, so there should be prolonged close contact to transmit Chlamydia *pneumoniae* specifically because Chlamydia *psittaci* are zoonosis transmitted from Psittacine birds (like parrots).

- obligate intracellular.. [intracytoplasmic inclusions..Rapidly killed outside body, dryness & high temperature > 4 C.]

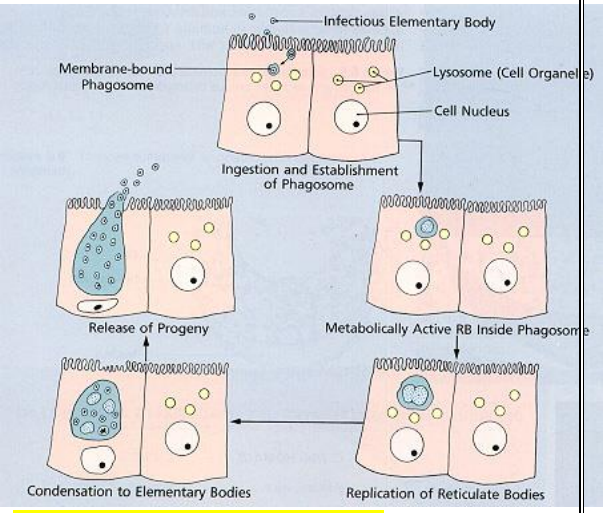
- Chlamydia *trachomatis*, Serotypes C ,K : Common cause of sexually transmitted disease (STD) [Nonspecific urethritis.. mother to newborn babies..maternal fluid..] Atypical pneumonia, [Eye infection..Opthalmia neonatorum]

- A & C serotypes of endemic Ch. *trachomatis* cause Trachoma (leading infectious cause of preventable blindness in developing countries) [conjunctival scarring, damage eyelids & Cornea.. blindness.]

[About half of all newborns with Chlamydial pneumonia develop inclusion conjunctivitis.. 1-2 weeks starts mild - severe eyes redness, swollen eyelids, inflammation & yellow thick discharge eyes.]

* So just put in your mind that atypical pneumonia of Chlamydia mainly caused by *pneumoniae* and *psittaci* but *trachomatis* could also cause atypical pneumonia although they mainly cause STDS and trachoma.

➢ Chlamydia Life Cycle



- Infectious elementary bodies attached to the host mucosa and promoting its entry →Cytoplasm phagosome→the elementary bodies differentiate into reticulate bodies, which are metabolically active but noninfectious→ reticulate bodies divide by binary fission → the reticulate bodies reorganize themselves to form new elementary bodies → these newly formed reticulate bodies leave the cell to infect adjacent cells or can get out of the body with droplets nuclei and are infectious if there was close prolonged contact, but they don't remain infectious for a long period of time because as stated previously elementary bodies can't resist environmental conditions and that's why Chlamydia is not highly contagious.

➢ Chlamydophila Pneumonia

- C. pneumoniae: droplets infection, targeted age groups are Infants/children "school age children" (younger than those infected by Mycoplasma) often develops gradually.. [several weeks mild respiratory symptoms, dry irritating prolonged cough..nasal congestion.. with/without fever..Few weeks..No blood sepsis.]

- C. pneumoniae infections in adults often asymptomatic, mild, May include sore throat (pharyngitis), headache, fever, dry cough.

- **Diagnosis:** Sputum, throat-nasal swab and apply gram staining method (they're gram negative), ELISA Specific antibodies, MaCoy Cell Culture, and Microimmunofluorescence MIF which is the most sensitive test for Chlamydia nowadays.
- **Treatment:** Tetracyclines (drug of choice) but they have many contraindications and in this case we can use Macrolides, and Fluoroquinolones such as levofloxacin and moxifloxacin .. No Vaccine

➢ Chlamydophila Psittaci

- C. psittaci causes Zoonotic diseases.. Human infection followed contact with birds (parrots, pigeons, turkeys, and ducks).. A rare human disease called psittacosis (ornithosis).

- people who keep birds like parrots are more prone to get Chlamydia *psittaci.*

- [Humans respiratory tract can be infected via inhalation bacteria shed from feathers, secretions, and droppings.]

- [localized inflammation in Bronchi & lung tissues.]

Signs Symptoms: Starts mild..flu-like illness that can develop into atypical pneumonia and usually resolves spontaneously without any complications, [ended with severe disease including fatal pneumonia, associated high fever, dry cough, headache.]

Diagnosis &Treatment: similar to other Chlamydia.

❖ Legionella pneumonphila

- Leginonella Gram negative , [Pathogenic-Nonpahogenic spp.] often found in water systems , air conditioning and bath tubs as biofilms or inside amoeba, [found in natural aquatic bodies and wet soil]. Facultative Anaerobes Growth in Cold/Hot (4- 80C) Water..] Transmitted through Inhalation via Air Condition, Wet Soil.. Cause outbreak of disease.

- No person to person transmission; so if someone infected with Legionella kissed someone else will he/she transmit the bacteria to the other one?

The answer is no.

* They're obligate intracellular, once they enter the body they get picked up by alveolar macrophages, it's said that Legionella mistaken macrophages for amoeba so they get inside macrophages and start replicating there.

- Lung Mucosa→ multiply intracellular within the macrophages.. [High Fever .. Incub. period 2-10 days .. Nonproductive /Productive dry cough.. Shortness of breath, Chest pain, Muscle aches, Joint pain, Diarrhea, Renal Failure, higher mortality rate]

Two clinical forms of Legionella (collectively known as legionellosis):

1) Legionnaire diseases: *most severe form; pneumonia, gastrointestinal symptoms (hepatosplenomegaly) and delirium. Involvement of more than one organ (including kidneys) because they live inside macrophages they get access to the whole reticuloendothelial system.

2) Pontiac fever: *most common form; fever, throat pain, flu like illness and there's no pneumonia.

- Legionnaires' disease and Pontiac fever are not contagious.

Risk factors: include heavy cigarette smoking, old age underlying diseases such as renal failure, cancer, diabetes, or chronic obstructive pulmonary, suppressed immune systems, corticosteroid.

Diagnosis: Special Culture Media, blood/urine specimen or throat swabs for detection Specific antibodies or Antigens by PCR, or ELISA

Treatment: Macrolides (azithromycin), levofloxacin, moxifloxacin .. No Vaccine.

OPPORTUNISTIC MYCOSES

- There are opportunistic fungal infections and endemic systemic mycotic infections,* the difference between them is that opportunistic fungal infections

have very low value of pathogenesis, they usually develop diseases in Immunocompromised individuals not the immunocompetent that's why they're called "opportunistic" whilst systemic endemic mycotic infections can affect healthy individuals(90~95% resolve spontaneously) and immunocompromised individuals where they experience serious complications.

• Opportunistic mycoses are caused by globally distributed fungi that are either members of the human microbiota, such a Candida species (indigenous), or environmental yeasts and molds(exogenous) .

• [They can produce disease ranging from superficial skin or mucous membrane infections to systemic involvement of multiple organs.]

• Patients at risk include those with hematologic dyscrasias (eg, leukemia, neutropenia) , patients with HIV/AIDS with CD4 counts less than 100 cells/µ L, as well as those treated with immunosuppressive (eg, corticosteroid), cytotoxic drugs, post organ transplantation, cancer therapy and even those who take broad spectrum antibiotics.

❖ Cryptococcus neoformans

• Cryptococcus neoformans causes cryptococcosis.

• A widespread encapsulated yeast that inhabits soil around pigeon roosts; capsule is anti-phagocytic.

• Transmission through inhalation of spores from birds or pigeon's droppings that are in the soil.

• Common infection of AIDS, cancer or diabetes patients.

• Infection of lungs leads to cough, fever, and lung nodules.

• Dissemination to meninges and brain (Cryptococcus is neurotropic) can cause severe neurological disturbance and death.

↳ most recognized form of cryptococcosis is meningitis, although initially the infection starts in the lungs.

➢ Diagnosis:

***Microscopic:** India Ink for capsule stain (50-80% + CSF); it shows the capsule.

↳ disadvantage of India ink: it miss up to 50% of cases.

***Culture:**

• Bird seed agar

• A new test is used nowadays in Europe and USA but not in Jordan called Latex Particulate Agglutination test (LPA) is considered diagnostic for Cryptococcus.

• Routine blood culture

***PCR**

❖ Aspergillosis: Diseases of the Genus Aspergillus

-Very common airborne soil fungus

* 2 types of Aspergillosis: 1) allergic aspergillosis and 2) invasive aspergillosis.

- Aspergillosis most probably caused by Aspergillus *fumigatus*.

* Other two members (Aspergillus *parasiticus* and Aspergillus *flavus)* mainly implicated in production of aflatoxins.

- 600 species, 8 involved in human disease; A. fumigatus most commonly

- Serious opportunistic threat to AIDS, leukemia, and transplant patients

- Infection (through inhalation of spores) usually occurs in lungs – spores germinate in lungs and form fungal balls "aspergilloma" ; can colonize sinuses, ear canals, eyelids, and conjunctiva, this is in the invasive form.

* mildest form of aspergillosis is rhinal allergy and bronchopulmonary allergy which are clinically defined as asthma and patients would have high IgE levels.

- Bronchopulmonary allergy or Invasive aspergillosis in preformed cavities can produce necrotic pneumonia, and infection of brain (meningitis) , heart (endocarditis) , and other organs.

- Surgery (debridement of aspergilloma and especially if they have cavitary lesions) , Amphotericin B (Intravenous) and nystatin.

❖ Zygomycosis (Mucormycosis or rhinocerebral Mucormycosis)

- Zygomycota are extremely abundant saprophytic fungi found in soil, water, organic debris, and food.

- Genera most often involved are Rhizopus, Absidia, and Mucor.

↳ Infection through inhalation of sporangiospores of these 3 species,

they germinate in the nostrils; they're very invasive and tend to go to the brain hence the name " Rhinocerebral Mucormycosis "

- Usually harmless air contaminants invade the membranes of the nose, eyes, heart, and brain of people (Rhinocerebral mucormycosis) with diabetes and malnutrition, with severe consequences.

* Diabetic patients infected with Rhinocerebral mucormycosis usually present with DKA (diabetic ketoacidosis).

-[main host defense is phagocytosis]

Diagnosis: is made by direct smear and by isolation of molds from respiratory secretions or biopsy specimens.

Treatment: Control Diabetes ,surgery & amphotericin B

Prognosis: very poor

❖ PNEUMOCYSTIS

• Pneumocystis *jirovecii* (Pneumocystis *carinii*) is the cause of a lethal pneumonia in immunocompromised persons, particularly those with AIDS.

↳ very commoninfection in AIDS patients causes them pneumocystis pneumonia (PCP).

* Also cause interstitial plasma pneumonitis.

* Previously was considered as protozoa but by ribotyping it has proven that it's a fungus.

• Definite diagnosis of pneumocystosis depends on finding organisms of typical morphology in appropriate specimens (Sputum, BAL)

• The organism has not been grown in culture; can not be cultured ex vivo

* Isolated from tissue biopsies and stained with silver stain.

• TMP-SMX (Trimethoprim / Sulfamethoxazole) is treatment of choice

ENDEMIC MYCOSES

• Endemic mycosis is caused by a thermally dimorphic fungus, they claim both morphological states of fungi; filamentous form in the environment (room temperature) while in the body (at body temperature) they turn into yeast -single cell- .

- Infections are initiated in the lungs following inhalation of the respective conidia (usually asexual) or spores (could be sexual or asexual).

↳conidia and spores are the production elements of fungi.

• Each of the four primary systemic mycoses—coccidioidomycosis, histoplasmosis, blastomycosis, and paracoccidioidomycosis—is geographically restricted to specific areas of endemicity.

• Most infections are asymptomatic or mild and resolve without treatment. However, a small but significant number of patients (Immunocompromised patients or immunocompetent individuals with other health issues) develop pulmonary disease,

❖ Histoplasmosis-1

Histoplasma *capsulatum*.. Dimorphic fungus with conidia and yeast forms at body temperature and hyphae & marcoconidia in vitro culture.. Common in soil enriched with excreta of birds, so people who deal with birds and poultry are more prone to histoplasmosis.

- The most prevalent fungal/mycotic infection worldwide.

- The name "*capsulatum*" is a misnomer, it has no capsule; while performing staining the cytoplasm shrinks leaving a hollow between it and the cell wall so they thought it a capsule previously.

Endemic in southern U.S.A, Australia.. Less other countries like Africa. In Africa,there is Histoplasma *duboisii* not *Capsulatum.*

• The primary site of infection is usually pulmonary→ inhalation dust with microconidia→ Phagocytosed by macrophages, obligate intracellular parasites→Causing slight inflammatory reaction.

* Firstly there will be inhalation of **macroconidia** "tuberculate macroconidia" then inside the body they convert into **microconidia** (which are diagnostic).

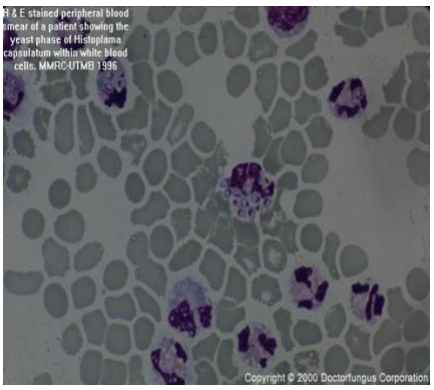
- Most cases of histoplasmosis are asymptomatic /subclinical, benign.. Flulike syndrome, spontaneous resolution .

• Few (especially Immunocompromised individuals) may develop chronic progressive lung disease manifested by Granuloma formation & fibrosis, chronic cutaneous or systemic disease involve any internal organ.. Fatal systemic disease.

• All infected persons become positive by histoplasmin skin test.

↳ histoplasmin is one of the antigens of Histoplasma can be detected in skin or lungs; this test and other similar tests of endemic mycoses are not specific there are alot of cross-reactivity, the patient may be infected with other endemic mycoses and the test would give false positive result for Histoplasma. They're used nowadays only for epidemiological studies.

- Since Histoplasma *capsulatum* targets phagocytes it gains access to the whole reticuloendothelial system so one of the manifestations is hepatosplenomegaly.



➢ Histoplasma capsulatum in infected White Blood cells

❖ Coccidioidomycosis & Blastomycosis

• Coccidioides *immitis* causes coccidioidomycosis *also called "Valley Fever" mainly in California & Blastomyces *dermatitidis* causes blastomycosis, soil inhabiting Dimorphic Fungus.. Endemic in south-western U.S.A., northern Mexico and various parts South America.

* Spores of Coccidioides *immitis* exist in desert sand; that's why the're called "Valley fever". Infectious spores of Coccidioides *immitis* are called "arthrospores" but in diagnosis what we look for in tissue called "spherules" where the spores would be inside a sac (كيس) and these spores called "endospores".

- So whenever you hear these 3 terms (Arthrospores, Spherules and Endospores) when talking about fungi you should always think of Coccidioides *immitis*

* Spores of Blastomyces *dermatitidis* usually exist in decaying organic matters (decaying woods).

- A feature of these two mycotic infections is the presence of mucocutaneous/skin manifestations in the form of subcutaneous nodules >> erythema nodosum or erythema multiforme.

• Respiratory infection, resulting from the inhalation of microconidia, often resolves rapidly leaving the patient with a strong specific immunity to re-infection.

• [Some individuals the disease may progress to a chronic pulmonary condition or a systemic disease involving the meninges, bones, joints, subcutaneous, cutaneous tissues.. Antigen Skin test positive.. Not significant in diagnosis.]

❖ Paracoccidioidomycosis

• Paracoccidioides *brasiliensis* is the thermally dimorphic fungal agent of paracoccidioidomycosis (South American blastomycosis), which is confined to endemic regions of Central and South America.

* To differentiate it from Blastomyces *dermatitidis* (which is endemic in North America) what we look for in Blastomyces *dermatitidis* is a single cell with broad based single bud, while in Paracoccidioides *brasiliensis* it would be a single cell with broad based multiple buds. This is in contrast to Cryptococcus *neoformans* which has narrow based buds.

* Route of transmission is through inhalation of the corresponding spores; most people infected are asymptomatic but some of them may develop chronic progressive pulmonary disease as well as systemic disease involving multiple organs.

• P brasiliensis is inhaled, [and initial lesions occur in the lung. After a period of dormancy that may last for decades, the pulmonary granulomas may become active, leading to chronic, progressive pulmonary disease or dissemination.]

❖ Laboratory Diagnosis

* The same approach is used in all fungal infections.

• Direct microscopy and culture should be performed on all specimens (sputum, bronchial washings, CSF, pleural fluid tissue biopsies from various visceral organs).

• wet mounts in 10% KOH with india ink.. Ovoid-budding yeast cells (b) Gram-stain smear..

- Microscopic smears processed with KOH depending on the type of organism and type of specimen then observe the morphology; it is the most used test but it requires expertise, other diagnostic techniques require time.

• Cultures on Sabouraud dextrose agar should be maintained for one month at 25C.... fungal growths & Wet Mount.. Identification ..produces hyphae-like conidio-phores & Spores.. Color of fungal growth

• Serological tests are of limited value.. not significant

* We can look for antibodies to observe the prevalence and there are 2 antibodies test used in endemic mycotic diseases: 1) immune diffusion and

2) complement fixation; you can't use them to diagnose acute mycotic infections as it requires the patient 2 weeks to develop antibodies.

• Detection of Histoplasm antigen in blood & urine is significant

- Skin tests that are similar in principle to tuberculosis skin test were used previously but not anymore because they're not significant due to cross-reactivity and because some people are anergic; their T cells are functionally inactive so they give negative results in skin tests even in the presence of the endemic mycotic infection>> (very poor prognostic sign)>> they might develop fulminant disease.