

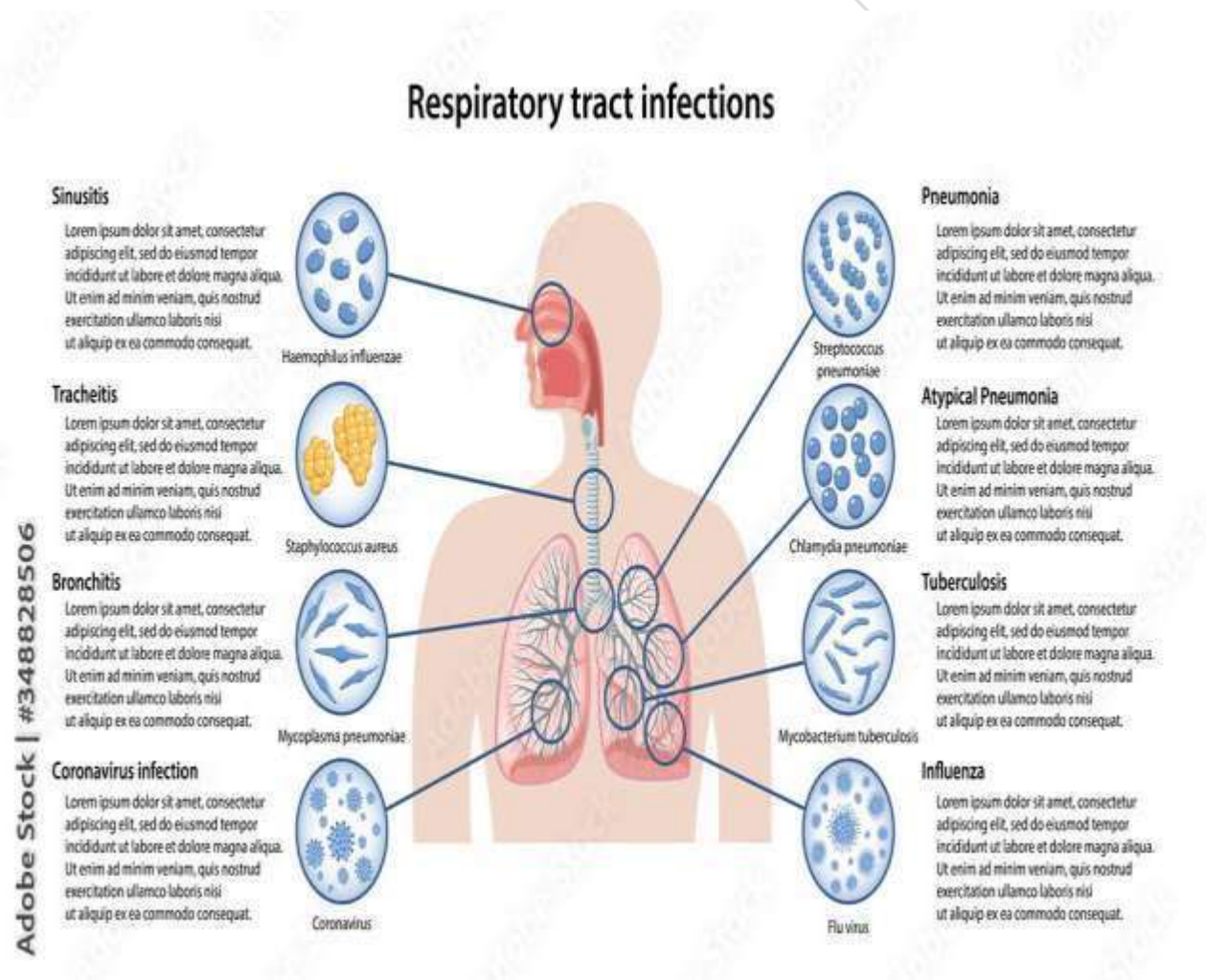


## Lecture-18, 19: Diagnosis of bacterial respiratory tract infections

### Bacterial infections of respiratory tract.

Respiratory system has divided into two major parts:

- ✓ Upper respiratory tract includes (nose and pharynx).
- ✓ Lower respiratory tract includes (larynx, trachea, bronchial tube and alveoli).



**Fig. (1): Summary of bacterial respiratory tract infection**

Each part or organ of this system has **own resident microflora**. Many factors play a vital role in challenging and limitation of **number and type of microflora colonizing**. Also each parts of respiratory tract **having physical factors** such as **hair, mucus membrane** lining the tract, **cilia** movement, **sneezing, coughing** besides **oxygen tension** in lung, which act all collectively as **unbreakable defense line**.

In addition, **innate immunity** and **circulating antibodies stabilize natural balance**, which represents equilibrium state between **host immunity** and **action of pathogens**. Ear, eye and nose are all share common canal, so any infection of one of these parts may cause infection to others. **Nasal cavity** for example consider as a reservoir for genus **Staphylococcus** along with other **gram-positive bacteria**. Nasal cavity is the pathway for deeper parts of respiratory tract for example resident bacteria of **nasal cavity** may and **will find its way** to the system causing problems here location and **to nervous system** such as **meningitis**. **Ear infection**, on other hand may be the way for **enteric bacteria** to **reach to un-limited area in respiratory or nervous systems**. *E. coli meningitis* is one example among many of such cases. **Tonsils** are the major front line of defense, yet, it is frequently had infected with so many species of bacteria, **Gram-negative** as well as **Gram-positive** bacteria.

**Infection of respiratory tract** sometimes classified as **adult or childhood infections** in this regard, *Bordetella Pertussis* the causative agents of whooping cough is the **example of childhood infections**. **Respiratory infections** may have classified as **accidental or seasonal infections**. The latter has associated with possible changes in the weather, from winter to summer and vice versa, bacterial infection may come second to viral infection in this aspect. **Accidental infection** is the infection that man acquired during daily life.

**No limitation** for the types of bacteria that may **cause** infection to **respiratory system** regardless the way that bacteria inter the system. Most of **normal flora of upper respiratory tract** play an important role in **causing opportunistic disease**.

*Staphylococcus, Streptococcus, Haemophilus, Corynebacterium, Neisseria, Bacteroides, Fusobacterium, and Actinomyces*, are typical examples for these bacteria.

Nearly any type of **gram-positive** or **negative** bacteria **Pneumonia**, **Mycoplasma** and **Chlamydia spp.**, can cause respiratory infection. On other hand, may cause **non-specific pneumonia**, while **Tuberculosis** caused by **Mycobacterium tuberculosis complex**, both of these diseases involved **lower** respiratory tract. **Sore throat** is a common infection of upper respiratory tract caused specially by **hemolytic Streptococci**, besides other **gram-positive cocci** or **gram-negative bacilli** (*Haemophilus spp.*).

The middle and inner ear are normally sterile, while outer ear and auditory canal contain the **normal flora of mouth and nose**. When a person coughs, sneezes or blow the nose these microorganisms may reach middle or inner ear and causing infection. **Tears in eyes decreases the number of microorganisms** that may find its way to eye because it's content of **lysozyme that destroys bacterial cells**. (fig.1).

## **Bronchitis**

**1. Acute bronchitis:** It is an acute inflammation of the tracheobronchial tree generally self-limited and with eventual (final) complete healing and return of function. Causative agent: *Mycobacterium pneumoniae*; *Bordetella pertussis*

**Laboratory diagnosis:**

Specimen: **Sputum**

Procedure: **Gram staining, culture, biochemical and serological test** for microbe identification.

## **2. Chronic bronchitis**

It has defined as chronic productive cough for at least three months in each of two successive years.

**Causative factors:** **Cigarette smoking; Air pollution;** Exposure to harmful stimuli  
**Bacteria that improve chronic bronchitis are:** *Streptococcus pneumoniae*; *Haemophilus influenzae*; *Mycoplasma pneumoniae* *Branhamella catarrhalis*.

**Laboratory diagnosis:**

Specimen: **Sputum**

Procedure: **Gram staining, culture, biochemical and serological test** for microbe identification.

**Pneumonia:** It is infection of the lung parenchyma.

**Causative agents:** *Strep. pneumoniae*, *Staph. aureus*, *Hemophilus influenzae* and *Mycoplasma pneumoniae*.

**Route of entry of microbes to the lung:**

- Aspiration of oral and gastric secretion
- Haematogenous spread from distant foci
- Direct inoculation and local spread from surrounding tissue
- Inhalation

**Laboratory diagnosis:**

Specimen: **Lower respiratory secretion** which indicated by **greater than 25 Neutrophils and less than 10 squamous epithelial cells per high power field.**

**Procedure:** Gram staining, culture, biochemical and serological test for microbe identification.

## **Bacterial Diagnosis of TB infection**

**Tuberculosis:** It is a disease caused by group of *Mycobacterium spp.*, namely *Mycobacterium tuberculosis* complex. *M. tuberculosis* is of human origin, *M. bovis* is of cattle origin, *M. avium* is of bird origin.

**The main problem of these bacteria is:**

1. Their high resistance to environmental stress such as dryness.
2. Survive in dry sputum for months.
3. Members of genus mycobacterium are very resistant to chemical and antibiotic treatment. All these features are because of their highly **contents of cell wall of lipids**. Cell wall lipid content makes these bacteria **difficult to stain** with ordinary stains. Therefore, special stain is required (Acid Fast Stain: AFS). **AFS** depends on **penetration of Carbol-fuchsin dye to cell wall with aid of heat**, once it is in there, a complex of

stain and lipid of cell wall is formed, this complex is **not removed** by normal **decolorizing agent (alcohol)**, it **resists even the decolorizing** with acid-alcohol from which it takes its name (Acid Fast Bacteria). Air born **droplets, milk**, or even **prolonged contact** with sick peoples consist collectively the major pathways for **transmission of disease**, yet, **air born rout** is the **important rout of entry**, fine particles containing one or two TB. **Cells travels** from patient for a distance of one meter **to another person** (air born) will enough to cause a disease in susceptible individual; normally these bacteria are overcoming by **host defense**. If bacteria succeeded to penetrate host defense, then **alveoli** will the **battlefield (area)** of the disease.

**Bacilli multiply in macrophages protect themselves against killing process**, in a self-protection process host try to limit the drastic (severe) effect of the pathogen by forming a **tubercle**, which is a **matrix tissues, exudates, WBCs**, and other materials. *M. tuberculosis* tend to arrange in cord formation, which increase the immune response of host resulting in what is called hypersensitivity reaction which lead ultimately to tissue damage.

### **Lab. diagnosis:**

Mycobacterium may come from a wide range of samples, these include; **sputum, lung wash, urine, wound, CSF, lymph secretion, bone, gastro-intestinal material**. The prime diagnostic parameter is **culturing of materials** (regardless the origin of it) on suitable culture medium, the medium commonly used is (**L-J M**), enriched media with **high contents of nutrition** to aid the **long period of incubation**. TB bacilli appear as **hydrophobic colonies with wrinkled (crumpled) surface**. Because of long time of incubation, **alternative diagnostic methods** have employed such as **PCR** or other methods.

**Blood film** might of little help in diagnosis of TB. Since **WBCs**, count may **still normal** with marked **elevation in number of monocytes**. **ESR** on the other hand might more evident In this regard, **ESR is shooting up reaching levels of 100 mm/h** or higher.

Commercial kits for diagnosis of **IgM and IgG for TB**. Are available now in local markets.

**AFB** serves as a **screening test** in diagnosis of TB., the existence of **even a single bacilli/** many microscopic fields is **enough to consider it " AFB positive"**, yet the **absence** of AFB from the investigated sample **does not mean that " patient has no TB**. And vice-versa the existence of AFB does not mean that patient is a TB. Patient. Since may other bacteria such as **Nocardia** may show a similar appearance of TB.

**Diphtheria disease:** the causative agent of this disease is *Corynebacterium diphtheriae*

**Diphtheria** is most commonly an infection of the upper respiratory tract and causes fever, sore throat, **hypoxia** due to airway obstruction by the **pseudomembrane**. And malaise. The **pseudomembrane** is a thick, gray-green fibrin membrane, forms over the site(s) of infection as a result of the combined effects of bacterial growth, toxin production, necrosis of underlying tissue, and the host immune response. The involvement of **cervical lymph nodes may cause profound swelling of the neck (bull neck diphtheria)** as shown in **figure-2**, causing Life-threatening systemic complications as a result of the action of **diphtheria toxin**.

*Corynebacterium diphtheriae* is a **Gram-positive, nonmotile, clubshaped bacilli**. Older cultures often contain **metachromatic Granules (polymetaphosphate)** which stain **bluish-purple** with methylene blue.

Figure-2

typical Diphtheria bull neck

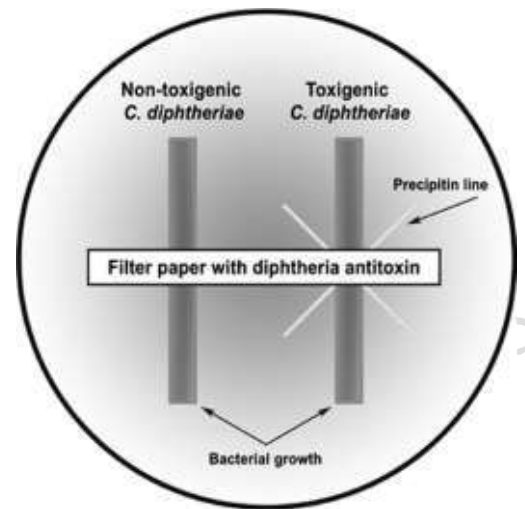


## Diagnosis

Culture media: **Loeffler agar** or **Mueller-Miller tellurite agar**.

Specimen: **Pharyngeal tonsils** swab. The most common assay for **toxigenicity** is the **Elek immunodiffusion test (Figure-3)**

**Figure-3: Procedure of Elek immunodiffusion test.** A Sterile filter paper impregnated with diphtheria antitoxin is imbedded in agar culture medium. Isolates of *C. diphtheriae* are then streaked across the plate at an angle of 90° to the antitoxin strip. Toxigenic *C. diphtheriae*.



This test is based on the **double diffusion of diphtheria toxin and antitoxin in an agar medium**. A sterile, antitoxin-saturated filter paper strip is embedded in the culture medium, and *C. diphtheriae* isolates are streak-inoculated at a 90° angle to the filter paper. The production of diphtheria toxin can be detected within 18 to 48 hours by the formation of a toxin-antitoxin precipitin band in the agar.

**Whooping cough disease: the causative agent of this disease is *Bordetella pertussis*.**

*B. pertussis* is a small Gram-negative rod-shaped, encapsulated, non-motile, obligate aerobes, catalase and oxidase positive. Numerous antigens and virulence factors are produced by *B. pertussis*.

**Symptoms and signs whooping cough:**

- blocked or runny nose.
- sneezing.
- raised temperature.
- uncontrolled bouts of coughing that sounds like a 'whoop' or are followed by a 'whooping' noise.
- vomiting after coughing.

**Diagnosis:**

**Specimen: nasopharyngeal secretions nasopharyngeal swabs.** These specimens should be immediately plated onto Regan-Lowe medium or Bordet-Gengou agar which is the most widely used.



**Figure-4: *B. pertussis* on Bordet-Gengou Agar with blood**



*Bordetella pertussis* usually grows after 3 to 4 days of incubation at 37° C. (Also, it can be identified by API-NE, PCR and ELISA).

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