LABORATORY DIAGNOSIS OF PULMONARY MICROBIAL INFECTIONS OTHER THAN PULMONARY TUBERCULOSIS

A Presentation

By

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OBJECTIVES

The objectives of this lecture are to

- Define pulmonary infections.
- Enlist pulmonary infection causing microbes.
- Discuss various types of laboratory diagnosis for the identification of pulmonary microbial infections.

INTRODUCTION

- **Pulmonary infections:** A type of infection that affects the lungs and other parts of the respiratory tract system.

- Pulmonary infections and diseases may be caused by smoking tobacco, breathing in tobacco smoke or other forms of air pollution.

- It can also be mainly caused by a variety of microbes including bacteria, fungi, viruses and parasites.

Bacterial pulmonary infections

- **The following bacteria are causing pulmonary infections in humans:**

- *Mycobacterium tuberculosis*
- *Streptococcus pneumoniae*
- *Klebsiella pneumoniae*
- *Haemophilus influenza*
- *Streptococcus pyogenes*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*

Streptococcus pneumoniae

- ***Streptococcus pneumoniae*** is a **Gram positive**, capsulated **diplococcus**.

- They are non-motile, non-spore forming, aerobic and facultative anaerobes. **Initial colonization** in the **nasopharynx** (URTI) **spread** to the **lungs**.

- ***S. pneumoniae*** was recognized as a **major cause** of **pneumonia**.

- ***Streptococcus* pneumonia** is the **most common** form of **community acquired pneumonia**.

Laboratory diagnosis

- **Specimens:** Sputum.

- **Gram staining:** In the Gram staining method, ***Streptococcus pneumoniae*** shows **Gram positive diplococci**.

- **Blood agar media:** Alpha hemolysis.

- **Optochin Test:** Sensitive.

- **Bile Solubility Test:** Positive.

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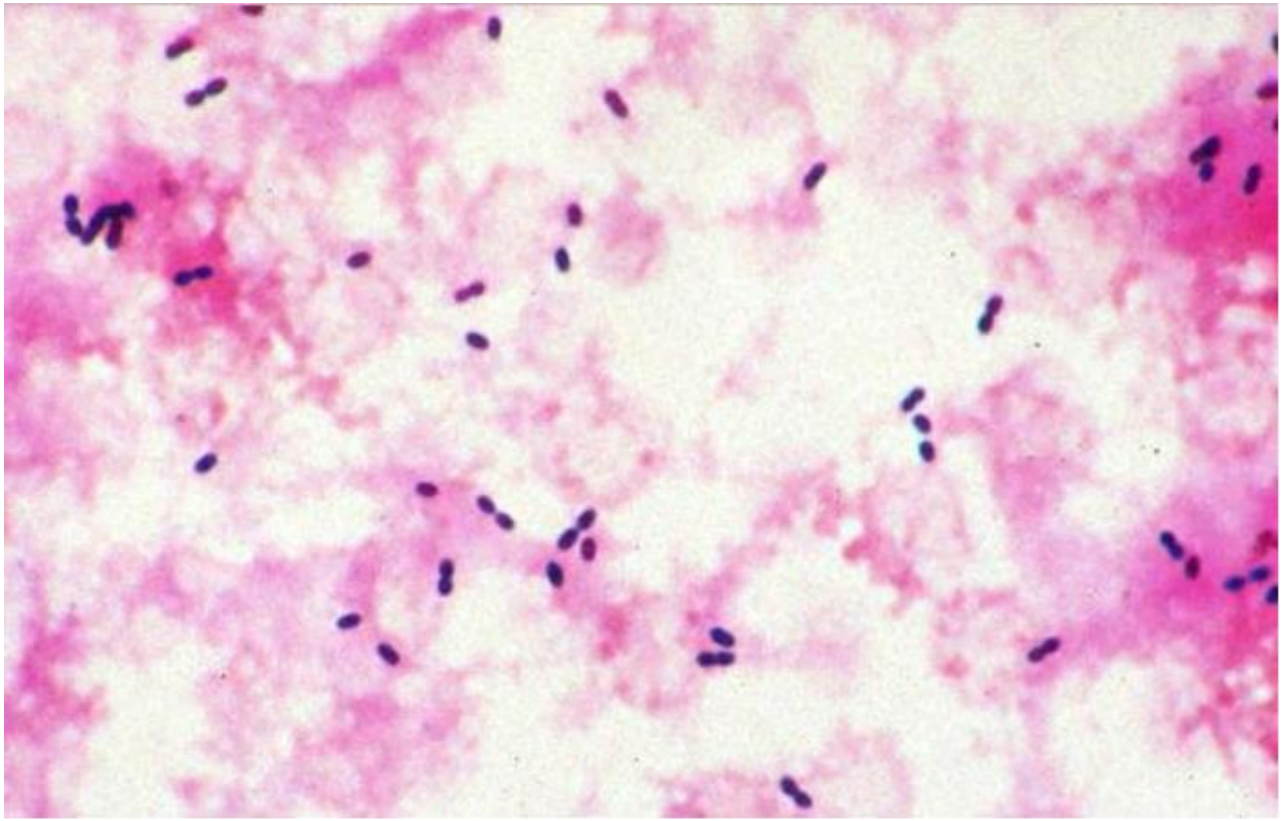
Gram stain of *S.pneumoniae*



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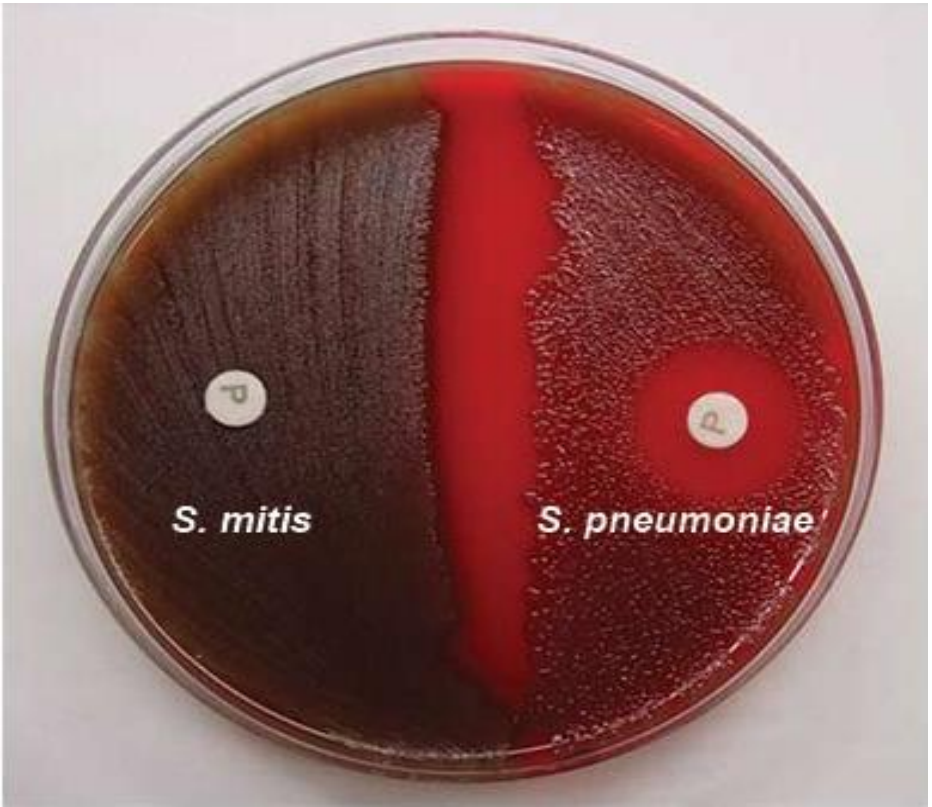
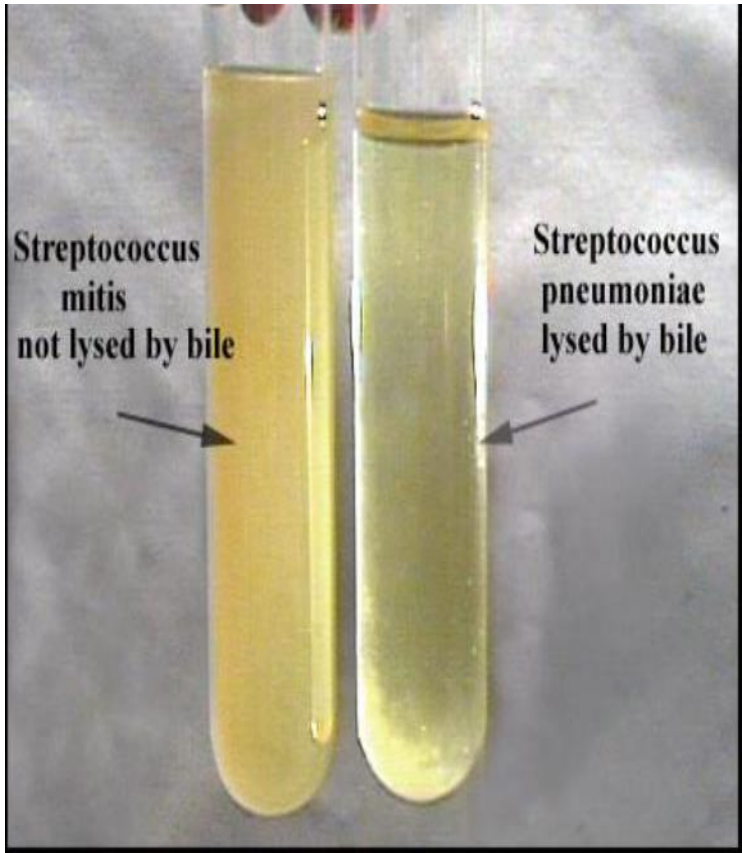
S. pneumoniae

Optochin test



Bile solubility test



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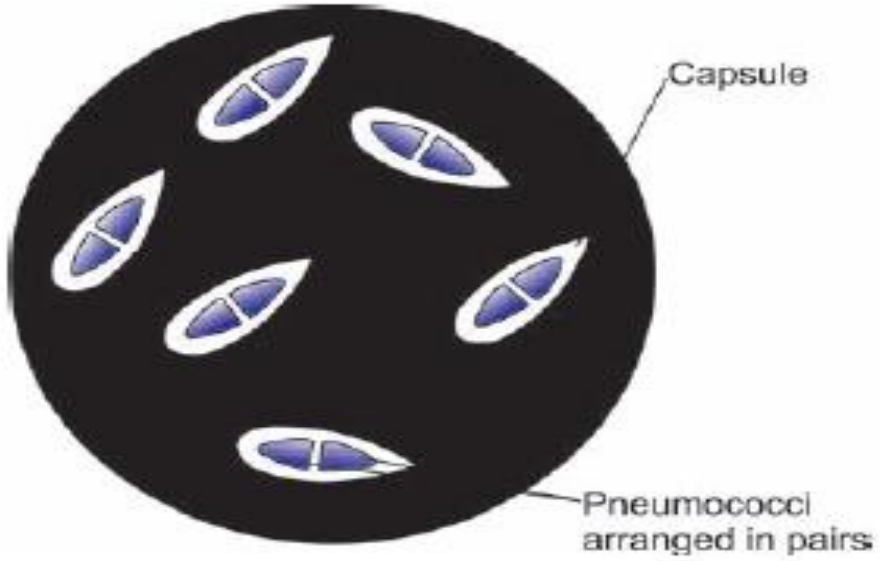
Capsule staining

- In India ink capsule staining method, ***Streptococcus pneumoniae*** shows **white capsules** surrounding **purple bacterial cells**.

Capsule staining of *S.pneumoniae*



Fungal pulmonary infections

- **The following fungi are causing pulmonary infections in humans:**

- *Cryptococcus neoformans*
- *Candida albicans*
- *Aspergillus species*

Cryptococcosis neoformans

- ***Cryptococcus neoformans*** is a capsulated, oval, **yeast-like fungus**.

- This fungus has a worldwide distribution. It is found in soil and bird droppings.

- The **capsule** is made up of **polysaccharides**. The **capsule** acts as a **virulence factor**.

- **Pathogenesis:** Inhalation of yeasts causes pulmonary infections.

Laboratory diagnosis

- **Specimen:** Sputum

Direct microscopic observation

10% potassium hydroxide wet mount method

- 10% KOH wet mount is useful for the identification of this fungus.

- **Observation**: ***C.neoformans*** appears as **colorless budding yeast cells** in **KOH wet mount**.

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Lactophenol Cotton blue wet mount method

- ***C.neoformans*** appears as **blue colored** budding **yeast cells** in the **LPCB wet mount**.
- **India ink stain:** The India ink stain reveals the capsule surrounding the *Cryptococcus neoformans* yeast cells.

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Fungal culture

- **Sabouraud dextrose agar medium**: This SDA medium is primarily used for fungal culture.

- ***C.neoformans*** produces **smooth**, **cream colored** and **mucoid colonies** on **SDA medium**.

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- **Bird Seed Agar (BSA) medium:** It is used for **selective** and **differential** isolation of ***C.neoformans*** from **clinical specimens**.

- ***C.neoformans*** produces **golden brown** to **black colored**, smooth colonies on **BSA medium**.

- **Serology diagnosis:**

  - Detection of **capsule antigen** by **agglutination** test.
  - **Urease test**: Positive.
  - Direct immunofluorescence test.

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C.neoformans

SDA media

BSA media

Viral pulmonary infections

- **The following viruses are causing pulmonary infections in humans:**

- Adenovirus
- Coronavirus
- Influenza viruses
- Parainfluenza viruses

Adenoviruses

- **Adenoviruses** are **double stranded DNA** and **non-enveloped viruses**.
- Adenoviruses primarily infect children, but adults are also infected.
- Adeoviruses are spread in aerosols, in fecal matter and by close contact.
- Fingers spread the virus to the eyes.

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- **Viruses** infect **mucoepithelial cells** in the **respiratory tract**.
- Over **50 serotypes** of **adenoviruses** have been **isolated** from **human sources**.
- **Disease** is determined by the **tissue** of the **specific serotype**.
- Respiratory infections are caused by low-numbered serotypes (1, 2, 3, 5, and 7) and gastrointestinal infections by high-numbered serotypes (40, 41, 42).

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- For example, serotypes **1-3, 7** cause **pneumonia** and serotypes **40 and 41** are responsible for **diarrhea**.

- Adenovirus causes pharyngitis, pneumonia, acute respiratory diseases (ARD), UTIs and eye infections etc.

- Most of the adenovirus serotypes were recovered from AIDS patients.

Laboratory diagnosis

- **Specimens:** Throat swab, Eye swab and Stool etc.

- **Cell culture:** Cell cultures of human origin, such as human embryonic kidney cell culture, HeLa (or) HEP-2 cell culture, are useful for adenovirus isolation.

- **Cytopathic changes** may take several days to develop and consist of **cell rounding** and **aggregation** into **grape like clusters**.

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- Electron microscopy is used for adenovirus detection.
- Adenovirus particles are observed with icosahedral symmetry under transmission electron microscopy.
- Other tests, including immunofluorescence and PCR, are widely used for adenovirus identification.

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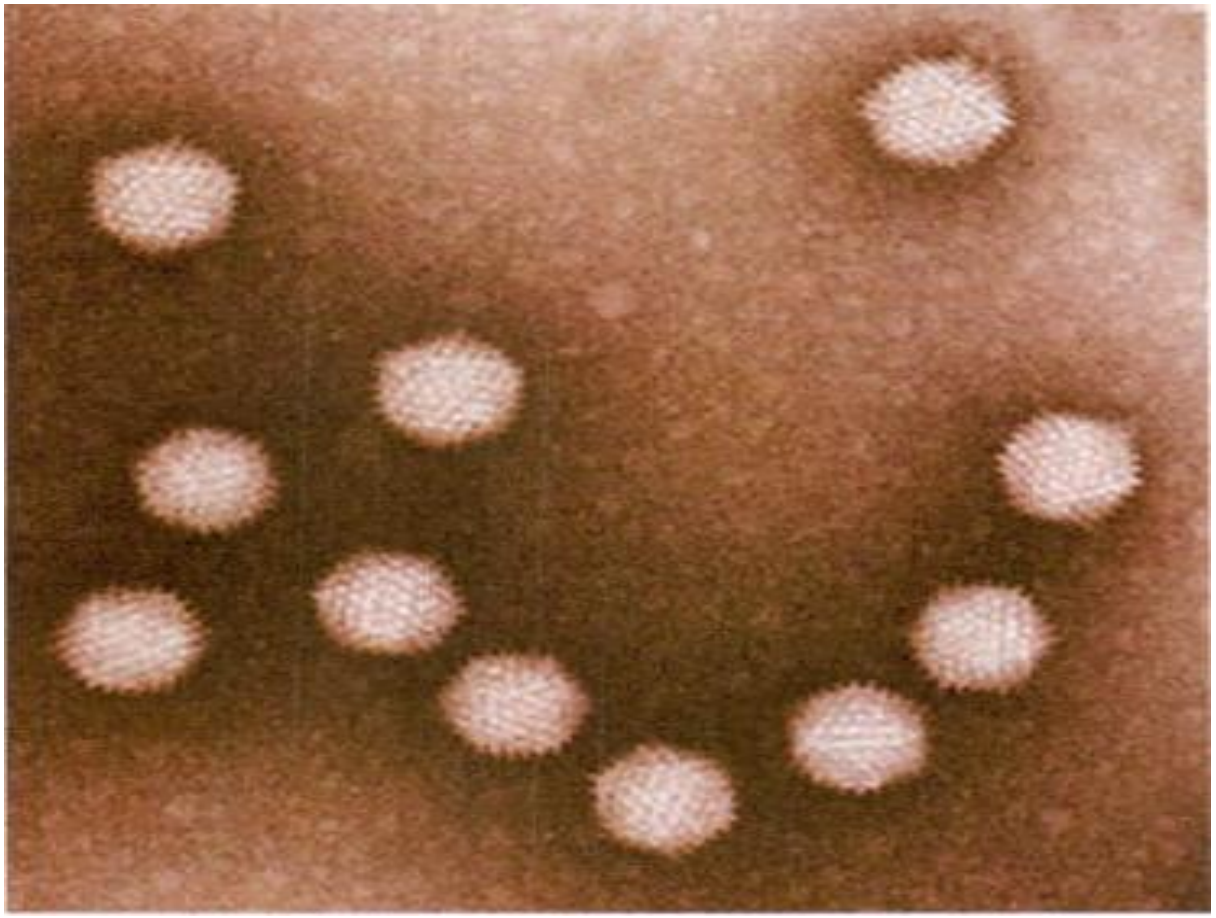
Transmission electron micrograph of Adenovirus particles



Parasitic pulmonary infections

- **The following parasites are causing pulmonary infections in humans:**

- *Ascaris lumbricoides*
- *Ancylostoma duodenale*
- *Entamoeba histolytica*
- *Toxoplasma gondii*
- *Plasmodium* species

Ascaris lumbricoides

- **Ascaris lumbricoides** (roundworm) causes **ascariasis** and is an **intestinal parasite**.

- It has worldwide distribution and it is more prevalent in the countryside than in the city.

- It **infects** the **gastrointestinal tract** and also **causes pulmonary infections**.

- **Morphology:** Egg, larvae, male and female worms.

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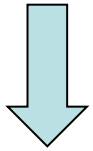
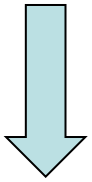
- **Infection** is initiated by swallowing **infective stages of eggs** and **larvae**



multiplication in the intestine

blood stream



lung migration  Pulmonary infections

Laboratory diagnosis

- **Specimens:** Sputum, stool and blood.

- **Eggs, larvae, male** and **female worms** can be **observed** by using **saline wet mount** and **iodine wet mount techniques**.

- The **hemoccult test** shows the **presence** of **blood** in the **stool**.

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- **Blood tests** often reveal **elevated eosinophils**.
- Low RBC count.
- CT scan and biopsy.
- **Molecular diagnosis:** PCR.

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Ascaris lumbricoides

Egg

Adult Male & Female worm

LEARNING OUTCOMES

At the end of the lecture, students should be able to:

- Define pulmonary infections.
- Enlist pulmonary infection causing microbes.
- Describe various types of laboratory diagnosis for the identification of pulmonary microbial infections.

REFERENCE BOOKS

- David Greenwood *et al* (2007), Medical Microbiology (7th edition). Churchill Livingstone Elsevier.
- J. C. Pommerville (2004), Alcamo's Fundamentals of Microbiology (7th Edition). Jones and Bartlett Publishers.
- Mims *et al*, (2008) Medical Microbiology (4th Edition). Mosby Elsevier.
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Thank you