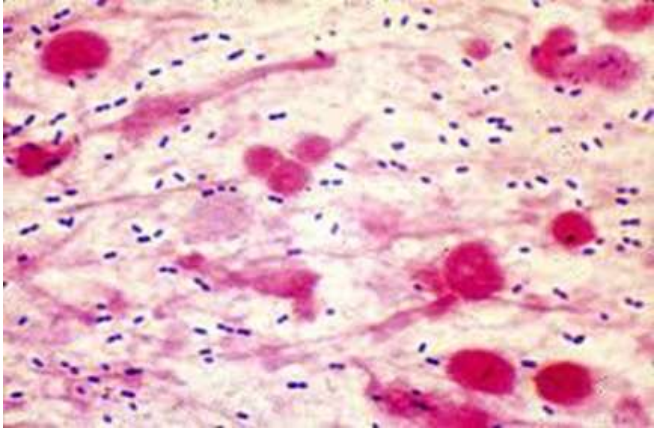


Clinical case

- A 25 year old boy presented with fever for 4 days, cough with expectoration, difficulty in breathing
- On examination his temp. was 101°F, crepitations on the lower parts of chest, tachycardia, tachypnoea
- His sputum was rusty and mucoid in appearance
- Sputum direct microscopy and culture was done
- Provisional diagnosis?



Pneumonia

Dr Nikunja K Das

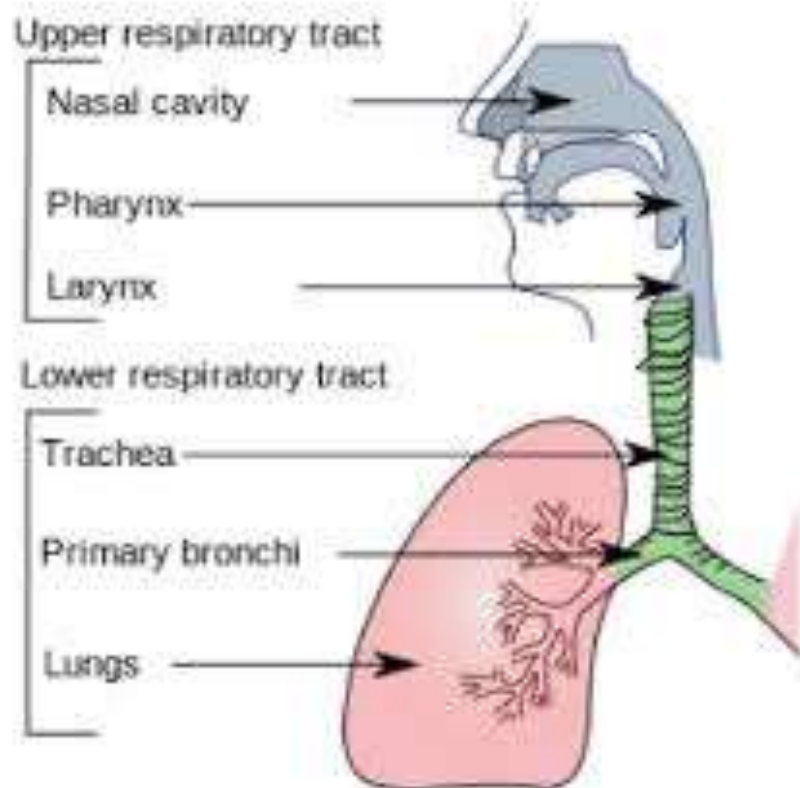
Department of Microbiology

Must know areas

- Lower respiratory tract infections
- Community acquired pneumonia
- Hospital/ Health care/ Ventilator associated pneumonia
- Pathogenesis of pneumonia
- Causative agents of pneumonia
- Laboratory diagnosis of pneumonia

What is a Pneumonia?

- Infection of pulmonary parenchyma
- Major cause of illness and death
- Misdiagnosed, mistreated, underestimated
- Once a microbe has invaded, disease follows
- CAP, HAP, VAP
- New category of Health care associated pneumonia (HCAP)



Immediate

Physical barrier

- Anatomic barrier (nares, glottis, etc.)
- Epithelial monolayer and tight junctions
- Cough
- Mucociliary transport

Early

Innate immunity

- Airways surface liquid and antimicrobial compounds
- Pulmonary and alveolar macrophages
- Airway mast cells
- Activation of epithelial and endothelial cells
- Activation of complement

Late

Adaptive immunity

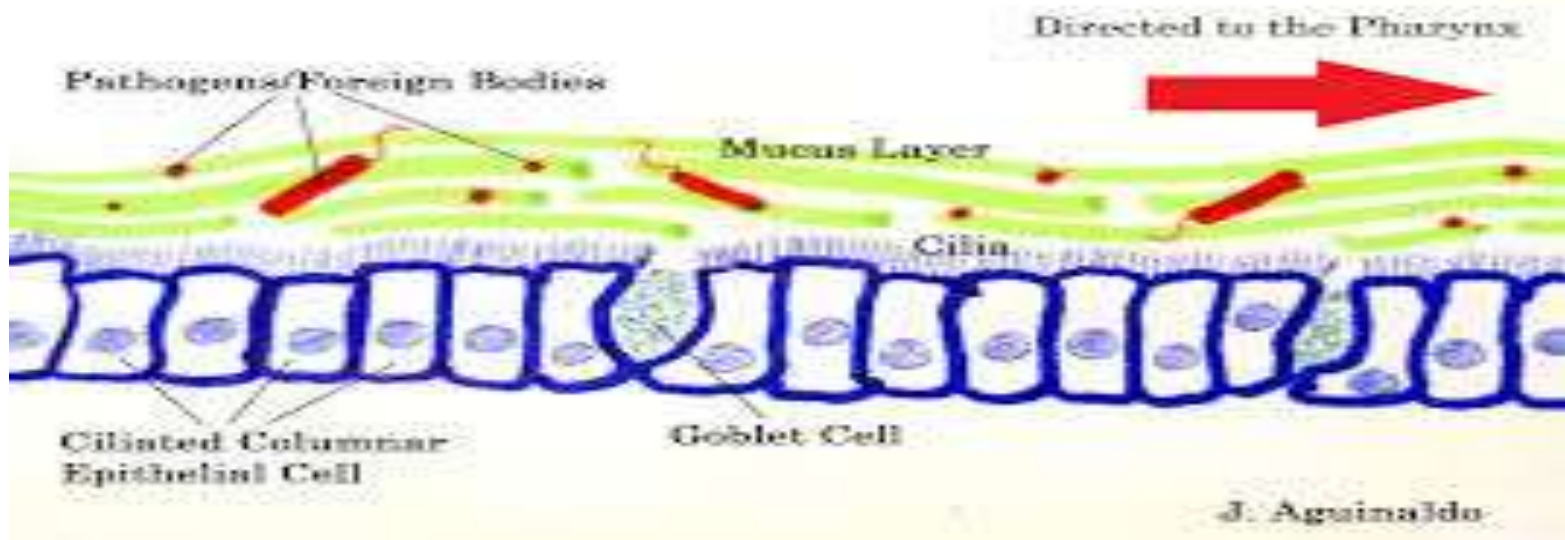
- Neutrophil recruitment and activation
- Lymphocyte recruitment and activation



Defense mechanisms

Hair and turbinates	Large inhaled particles
Branching architecture of tracheobronchial tree	
Muco-ciliary clearance, local antibacterial factors	
Gag reflex, cough mechanism	Aspiration
Normal flora	
Local Macrophages	
Local proteins (Surfactant, protein A)	
TNF, IL-8, GM-CSF	

Mucociliary Escalator



Respiratory defense mechanism

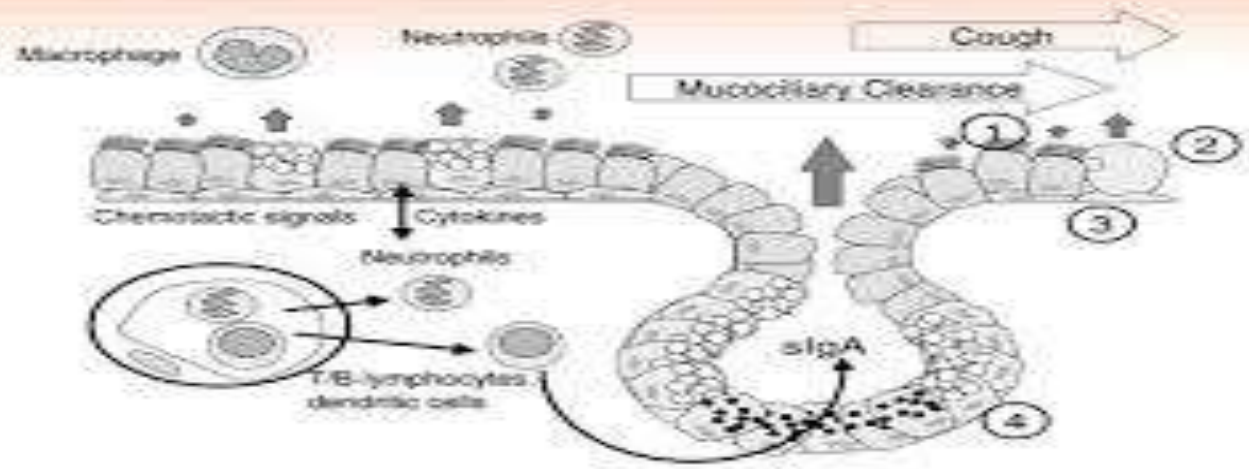


Table 1: Pathophysiological modes of spread

Mechanism	Examples
Aerosols Inhalation	<i>Mycoplasma pneumoniae</i> , <i>Chlamydophila psittaci</i> , <i>Chlamydophila pneumoniae</i> , <i>Legionella pneumophila</i>
Oropharyngeal secretions Aspiration	<i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , anaerobes, gram-negative bacilli
Haematogenous spread	<i>Staphylococcus aureus</i>
Reactivation of latent microorganisms	<i>Mycobacterium tuberculosis</i> , <i>Pneumocystis jiroveci</i>

Pathogenesis

- Upper airway colonization/ infection
- By aspiration (small volume)
- By inhalation of airborne droplets
- Seeding of the lungs by blood
- Infection from contiguous area
- Viruses destroy normal epithelium causing superadded bacterial infection

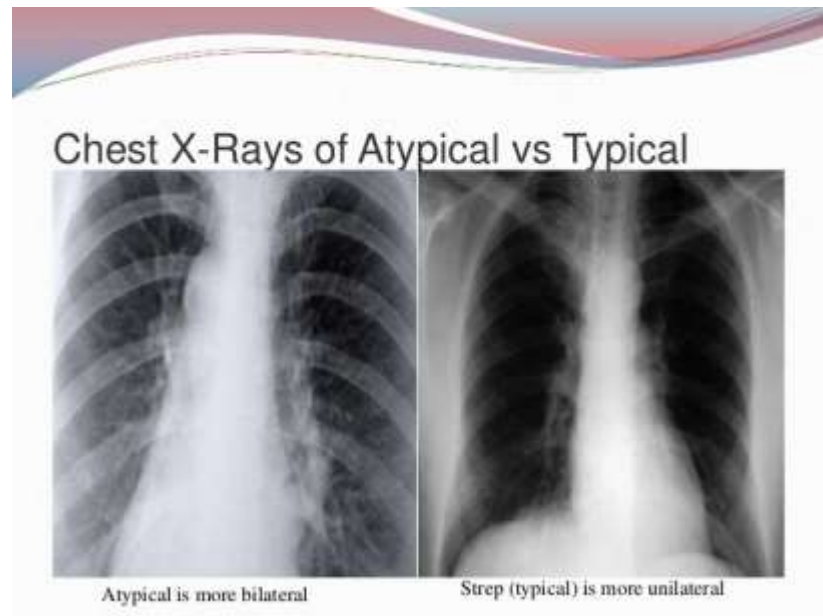
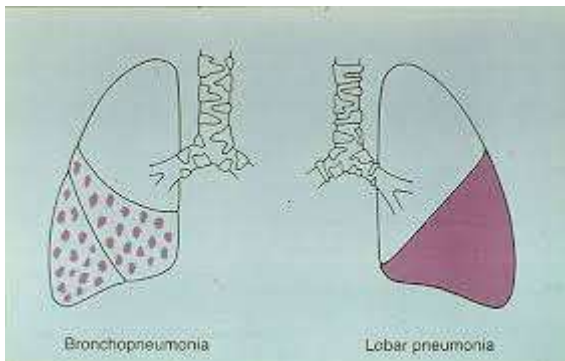
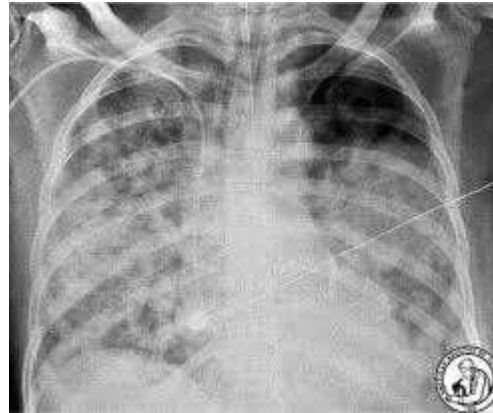
Pathogenesis

- In health care associated pneumonia the environment, and the transfer between the patients and staff or other patients serve as source of pathogens
- Through aspiration, leakage from endotracheal tube
- Biofilms in endotracheal tubes are a source

Pathology

- **Edema**
- **Red hepatization**
- **Gray hepatization**
- **Resolution**
 - Lobar pneumonia
 - Respiratory bronchiolitis in VAP
 - Bronchopneumonia in nosocomial pneumonia

Pics of X ray



Clinical manifestations

- Fever
- Chills
- Chest pain
- Tachycardia, Tachypnoea
- Cough
- Typical or atypical based on cough productive or not

Causative agent (bacteria)

<i>Common agents</i>	<i>Less common agents</i>
<i>Streptococcus pneumoniae</i>	<i>Serratia</i>
<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
<i>Haemophilus influenzae</i>	<i>Legionella</i>
<i>Mixed anaerobic infection</i>	<i>Acinetobacter</i>
<i>Bacteroides</i>	<i>Actinomyces</i>
<i>Fusobacterium</i>	<i>Bacillus</i>
<i>Peptostreptococcus</i>	<i>Moraxella</i>
<i>Peptococcus</i>	<i>Campylobacter</i>
<i>Prevotella</i>	<i>Enterococcus</i>
<i>Enterobacteriaceae</i>	<i>Proteus</i>
<i>E.Coli</i>	<i>Streptococcus pyogenes</i>
<i>Klebsiella pneumoniae</i>	<i>Nocardia</i>
<i>Enterobacter</i>	

Causative agents (virus)

Children	Adults
Respiratory syncytial virus	Influenzae A
Parainfluenzae virus 1, 2, 3	Influenzae B
Influenzae A	RSV
Adenovirus types 1, 2, 3, 5	Human metapneumovirus
Influenzae B	Adenovirus 4, 7
Rhinovirus	Rhinovirus
Coxsackie virus	Enterovirus
Echovirus	Echovirus
Measles	Coxsackie
Hantavirus	Epstein-Barr virus
	CMV
	Varicella-zoster, measles, HSV, Corona

Causative agent (Fungus)

Histoplasma capsulatum

Coccidioides immitis

Rhizopus

Absidia

Mucor

Cunninghamella

Aspergillus

Candida

Other agents

<i>Coxiella burnetti</i>	<i>M. abscessus</i>
<i>Rickettsia rickettsiae</i>	<i>M. Avium complex</i>
<i>Mycoplasma pneumoniae</i>	<i>M. Kansasii</i>
<i>Chlamydia psittaci</i>	<i>M. Chelonae</i>
<i>Chlamydia trachomatis</i>	<i>M. Fortuitum</i>
<i>Chlamydia pneumonia</i>	<i>M. Xenopi</i>
<i>Mycobacterium tuberculosis</i>	<i>Ascaris lumbricoides</i>
	<i>Pneumocystis jiroveci</i>
	<i>Strongyloides stercoralis</i>
	<i>Toxoplasma gondii</i>
	<i>Paragonimus westermani</i>

Community acquired pneumonia

- Patients are believed to have acquired the infection outside the hospital setting
- >80% in children are caused by viruses
- <20% in adults

Community acquired pneumonia

- Neonates- (*C.trachomatis, P.jiroveci*)
- 2 months-5 years (RSV, metapneumo, influenzae)
- Young adults- *M pneumoniae, Chlamydiae, S pneumoniae, H influenzae*

Community acquired pneumonia

Outpatients	Non-ICU	ICU
<i>Streptococcus pneumoniae</i>	<i>S.pneumoniae</i>	<i>S.pneumoniae</i>
<i>M.pneumoniae</i>	<i>M.pneumoniae</i>	<i>S.aureus</i>
<i>H.Influnzae</i>	<i>C.pneumoniae</i>	<i>Legionella spp</i>
<i>C.pneumoniae</i>	<i>H.Influnzae</i>	<i>H.Influnzae</i>
<i>Respiratory viruses</i>	<i>Respiratory viruses</i>	
<i>SARS, Hantavirus, metapneumovirus, coronavirus</i>		
<i>CA-MRSA</i>		
<i>Polymicrobial</i>		<i>Anaerobic infections</i>

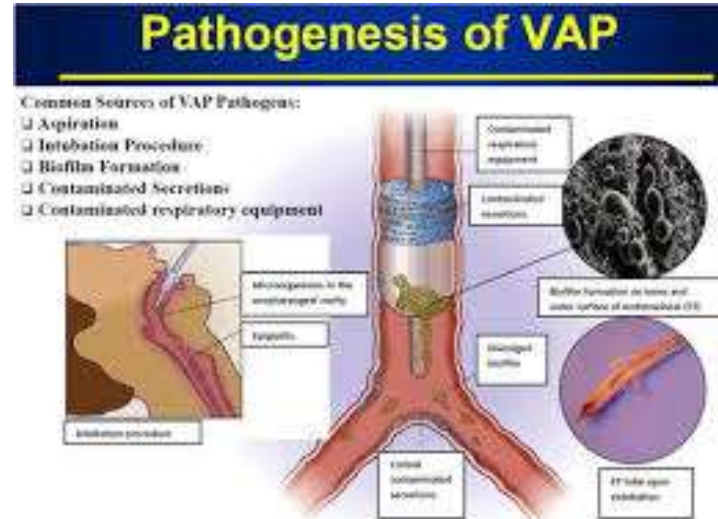
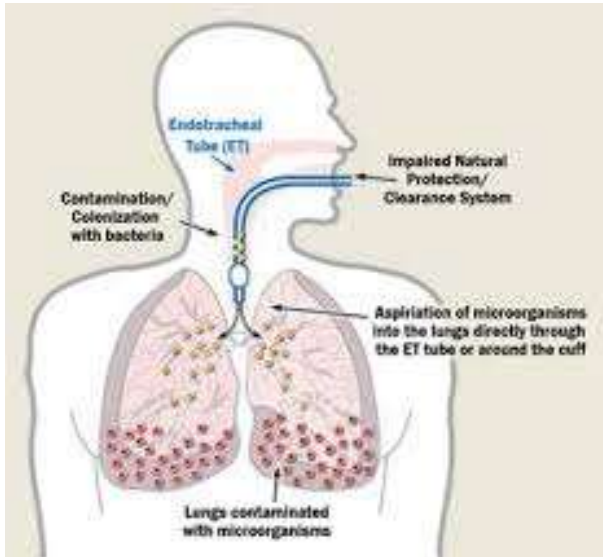
Health Care-Associated pneumonia

- Hospitalised within 2 to 90 days
- Persons presenting as outpatients but infected with MDR agents earlier associated with HAP
- Oral antibiotics, IV line, earlier shift of acute illness patients to home, immunomodulatory therapies

Hospital/Ventilator associated pneumonia

- Patients are believed to have acquired the infection within the hospital setting
- Usually at least 2 days following admission

Pic of ventilator



Ventilator associated pneumonia

<i>Early VAP</i>	<i>Late VAP</i>
<i>S pneumoniae</i>	<i>P aeruginosa</i>
<i>H influenzae</i>	MRSA
MSSA	<i>Acinetobacter spp</i>
<i>Enterobacteriaceae</i>	<i>Antibiotic resistant Enterobacteriaceae</i>
<i>E coli</i>	<i>ESBI positive strains</i>
<i>K pneumoniae</i>	<i>K spp</i>
<i>Proteus spp.</i>	<i>Legionella</i>
<i>Enterobacter</i>	<i>Burkholderia cepacia</i>
<i>Serratia</i>	<i>Aspergillus</i>

Etiology as suggested by history

Anthrax	Cattle, goat, swine, hair, wool exposure
Brucellosis	Cattle, goat, pigs; abattoir worker, unpasteurized milk
Melioidosis	Travel to endemic area
Plague	Bioterrorism, ground squirrel, rabbit
SARS	Outbreak, epidemic region
Hantavirus	Rodent dropping, urine, saliva
Q fever	Exposure to infected goats, cattle, sheep
Legionnaires' disease	Contaminated aerosols
Leptospirosis	Wild rodents, dogs, cats, rats
Psittacosis	Exposure to birds
Tularemia	Rabbits, hares, foxes (skinning)

Atypical pneumonia

- Not caused by traditional organisms, clinical feature is different from classical pneumonia
- Moderate amounts of sputum, no consolidation, mild or no leucocytosis, no alveolar exudate
- Do not respond to lactams, sulphonamides
- Very mild symptoms but consolidation is there and restricted to a smaller area than a lobar pneumonia

Atypical pneumonia

- *Mycoplasma pneumoniae*
- *Legionella pneumophila*
- *Chlamydiae pneumoniae*
- *Viruses and protozoa*
- Treatment will depend upon the causative agent as the infective agent is atypical

Diagnosis

- Suggested by clinical features derived from clinical history, physical examination, radiographic chest imaging
- Role of laboratory test is to identify the specific etiology although causative agent found in about 50% cases
- Helps the clinician to narrow antibiotic spectrum
- Guide about antibiotic resistance
- May have a pathogen not covered with empirical therapy

Laboratory diagnosis (specimen collection)

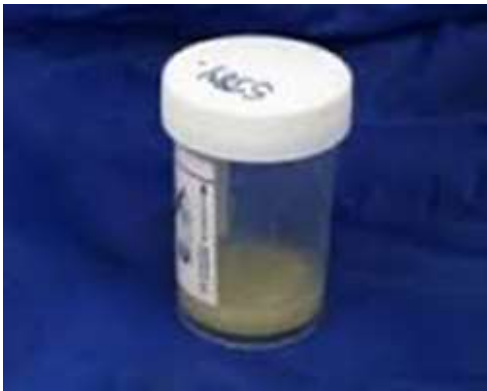
- Bronchoalveolar lavage (BAL)
- Bronchial brush
- Bronchial wash
- **Sputum** (least clinically relevant) as chances of contamination is there but most often taken

Lab diagnosis (specimen collection)

- **Sputum:** Primary mean of determining the cause of bacterial pneumonia
- May be contaminated with saliva
- **Expectorated:** Deep coughed out specimen, no food 1-2H prior, mouth rinsed with water
- Material collected in clean wide mouth universal container, immediate transport to lab

Specimen collection

- **Induced:** In patients unable to produce sputum:
Postural drainage, thoracic percussion
- Aerosol induced (15% NaCl, 10% glycerin), nebuliser is used, specially useful in *P.jiroveci*
- **Gastric aspirate** for AFB in children, collected in morning
- **Endo-Tracheal aspirate:** in patients on ventilators



Sample collection

- **Bronchoscopy:** bronchial mucosa directly visualised and can be taken for biopsy, Broncho Alveolar Lavage, Bronchial Brush, Transbronchial biopsy
- **Transtracheal aspirate:** percutaneous needle is inserted in trachea through cricothyroid membrane
- **Thoracocentesis:** for patients of empyema
- **Thin needle aspiration, Biopsy**

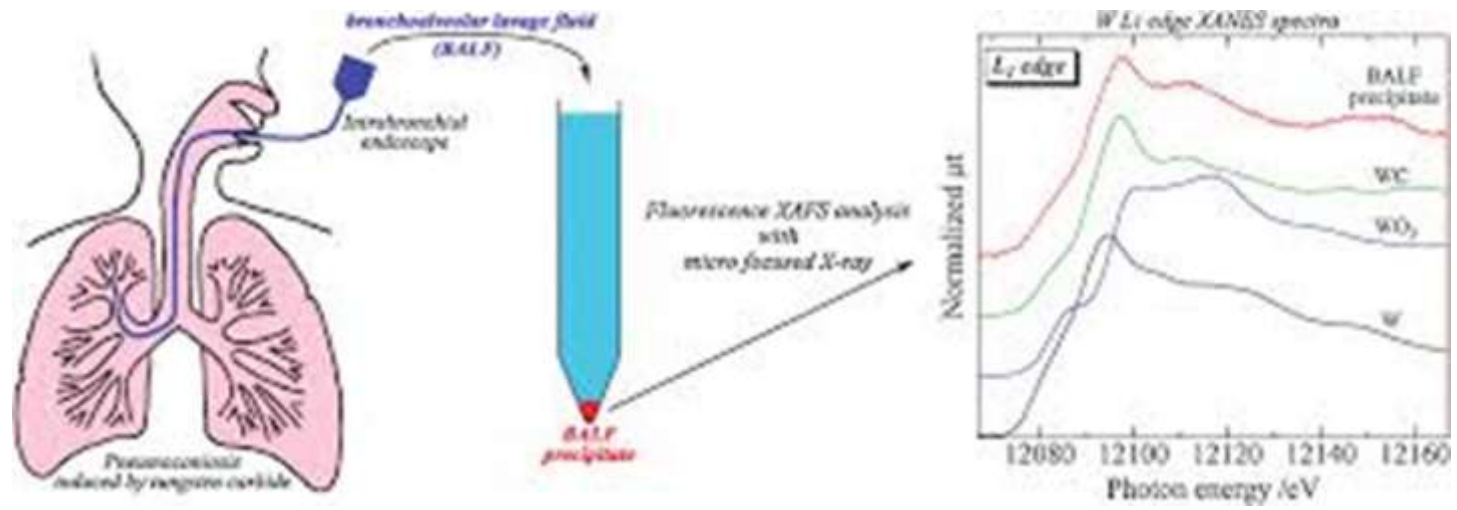
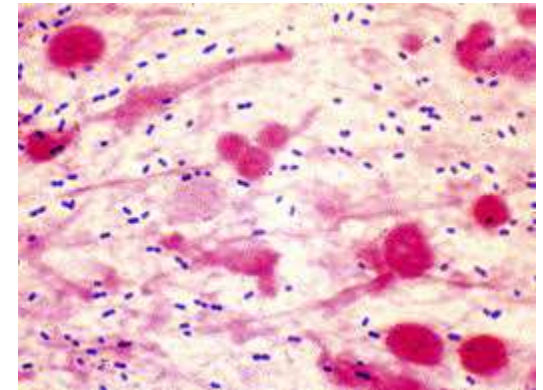
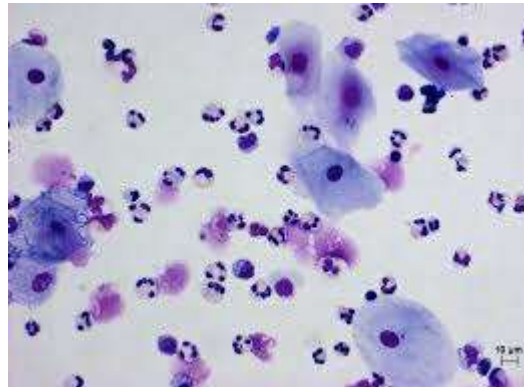
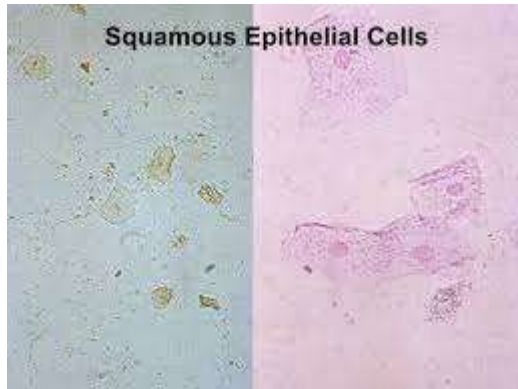


Figure 1. Diagnostic thoracocentesis producing purulent pleural fluid

Sample collection

- Collect the sample before any antibiotic therapy
- Early morning specimen
- Transport within 2 hours
- Supplemented with nasopharyngeal aspirate, nose and throat swabs for viruses, mycoplasma

Culture



Direct visual examination

- Direct wet mount for parasite
- 10% KOH for fungal elements, PAS stain
- Calcoflour white with ultraviolet light
- Gram stain for quality of the specimen, bacteria and yeast
- Acceptable specimen has <10 sq epi cells and >25 pus cells/ low field

Direct visual examination

- Acid fast staining
- Auramine-rhodamine stain
- Toluidine O *Pneumocystis*, *Nocardia*
- Direct fluorescent antibody staining
- Modified ZN for *Cryptosporidium spp*
- Modified Gomori methenamine stain for *Nocardia*,
Actinomyces, *fungi*, *parasites*

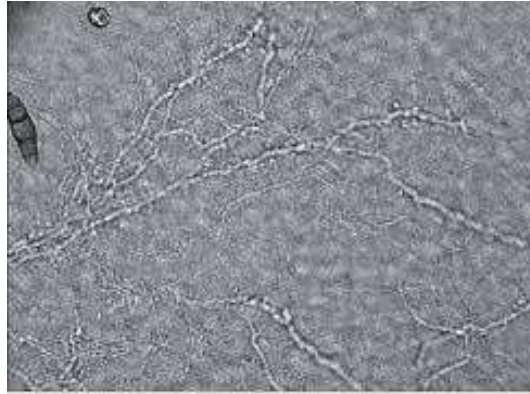
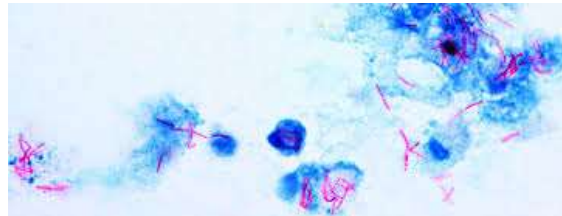
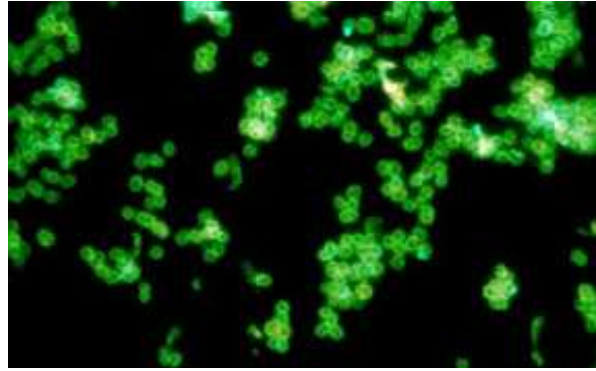
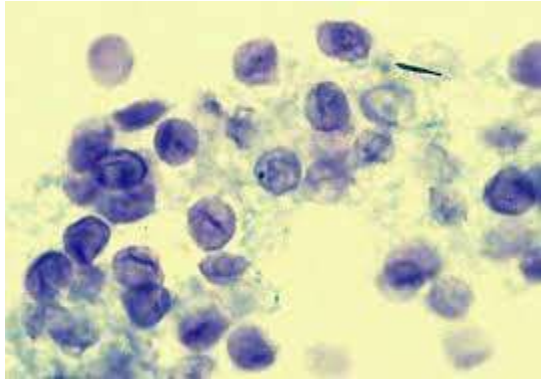


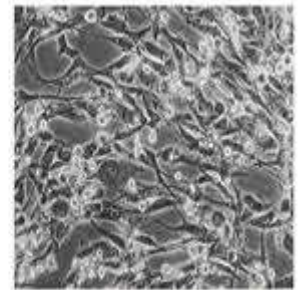
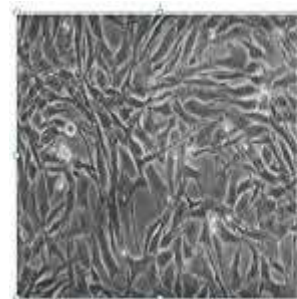
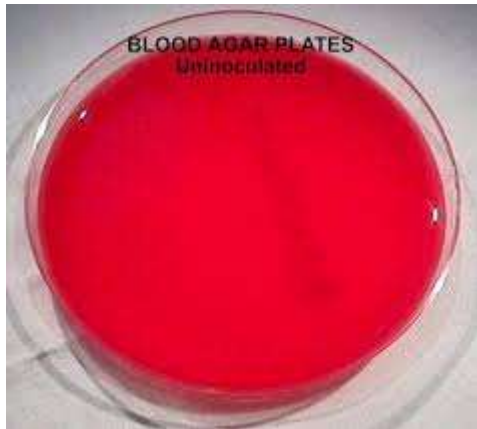
Figure 1: Skin scraping and KOH mount showing branching fungal hyphae in dermatophyte infection





Culture

- According to the suspecting organism
- Blood agar, chocolate agar, MacConkey agar
- Thioglycollate, RCM for anaerobic samples
- Sabourauds dextrose agar
- Lowenstein-Jensen media, BCYE
- Tissue culture/ egg inoculation



Laboratory diagnosis

- **Blood culture:** can be done
- Increases the credibility of the organism isolated from sputum
- Collected at the time of sputum collection
- Before antimicrobial treatment given
- Helps in finding of drug susceptibility tests
- Neutropenia, asplenia, complement def, CLD

Laboratory diagnosis

- Antigen tests: *L.pneumophila I* ags in urine
- Direct fluorescent antigen test for Herpes, CMV, Adenovirus, influenzae virus, RSV, *C trachomatis*
- Pneumococcal antigen test in urine
- Influenzae virus antigen

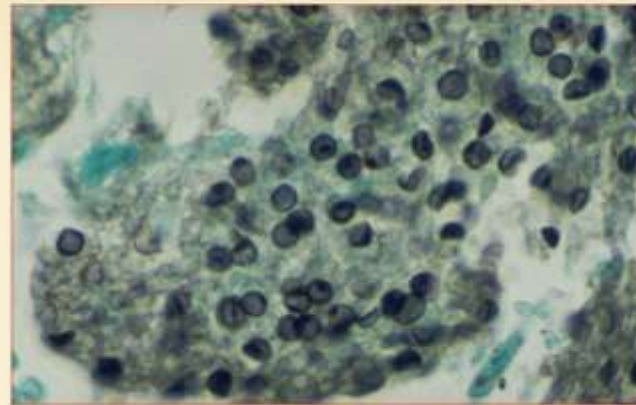
- **Polymerase chain reaction:**
- *L.pneumophila, M.tuberculosis, M.pneumoniae, C.pneumoniae*

- Serology:
- Four-fold rise in specific antibody in paired sera
- Specially useful in atypical pathogens like *Coxiella burnetti*

Other methods

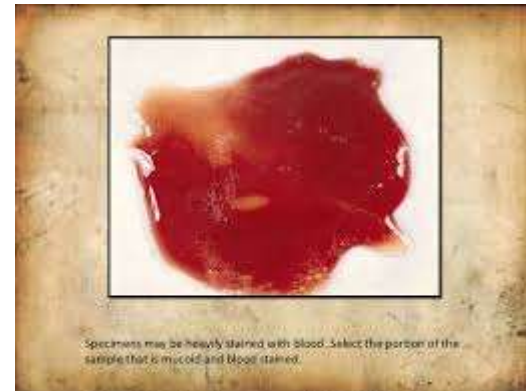
- If diffuse infiltration in immunocompromised patients: *Pneumocystis carinii*: Gomori's methanamine silver stain used

Gomori methenamine silver stain at high magnification demonstrates cysts of *Pneumocystis jiroveci* in lung



Potential clues to etiology

- Pneumatocele on chest X-ray : *S.aureus*
- Red currant jelly sputum: *Klebsiella pneumoniae*



- What is a pneumonia?
- What is a community acquired pneumonia
- What are the samples that are needed to be collected in a suspected case of pneumonia
- What are the criteria to reject a sputum sample in a laboratory