

Bacterial Pharyngitis

→ Pharyngitis = sore throat (URI)

→ mainly viral pharyngitis

→ agents causing bacterial pharyngitis

- *Streptococcus pyogenes* (most common)
- *Corynebacterium diphtheriae*
- Other β -hemolytic streptococci (group C & G)
- *Mycoplasma pneumoniae*
- *Neisseria gonorrhoea*

Streptococcal Pharyngitis

→ *Streptococcus pyogenes* [Group A streptococcus - GAS]

→ transmission: through respiratory droplets

Clinical Manifestations: localised tonsillitis or diffuse pharyngitis

→ erythema & swelling of pharyngeal mucosa with purulent exudate formation

→ younger children (< 3 years): fever, malaise, lymphadenopathy without exudative pharyngitis

Suppurative Complications: may occur due to:

- direct extension of infection from pharynx to deeper tissues causing peritonsillar abscess (or quinsy) & retropharyngeal abscess
- very rarely, downward spread to the respiratory tract causing pneumonia or empyema
- hematogenous spread leading to bacteremia & meningitis

Non-suppurative Complications: develop 2-3 weeks following streptococcal sore throat:

- ARF (acute rheumatic fever)
- PSGN - usually develops following streptococcal pyoderma

Differential Diagnosis of streptococcal sore throat:

- Diphtheria - differentiated from streptococcal pharyngitis by the presence of membrane over the tonsil
- viral pharyngitis - differentiated by presence of concomitant rhinorrhoea, oral ulcers, cough, hoarseness.

Scarlet fever: mediated by streptococcal pyogenic exotoxins (SPE-A, B, C)

→ characterized by pharyngitis + rash (with sandpaper feel), strawberry tongue (enlarged papillae on a coated tongue)

→ rash in the skin folds are called Pastia's lines

Laboratory Diagnosis:

→ Specimen: throat (oropharyngeal) swab

• **Culture:** on blood agar → small pinpoint colonies with a wide zone of β -hemolysis

• **Culture Smear:** gram +ve, spherical cocci, arranged in short chains

• **Identification:** catalase negative, susceptible to bacitracin

• **Rapid Antigen Detection Test (RADT):** detects Group A carbohydrate antigens from throat swab by latex agglutination or enzyme immunoassay

• **ASO Titer:** Anti-streptolysin O (ASO) antibodies

• **Molecular Test:** commercial PCR

Treatment:

• For pharyngitis - benzathine penicillin G 1m single dose or oral penicillin V for 10 days

• For recurrent pharyngitis - clindamycin or amoxicillin-clavulanate

• For concomitant pneumonia & empyema - Penicillin G + drainage of empyema

Diphtheria: highly infectious childhood disease

→ caused by *Corynebacterium diphtheriae* which primarily infect the throat & produces diphtheria toxin that causes an exudative pharyngitis & membranous tonsillitis.

→ gram +ve, non-capsulated, non-sporing, non-motile bacillus

→ irregularly stained & frequently shows club-shaped swellings

→ *Corynebacterium diphtheriae* typically shows two features which differentiates it from other corynebacterium species.

- **Chinese Letter / Cuneiform arrangement:** V or L shaped

∴ bacterial cells divide & daughter cells tend to lie at acute angles to each other (this type of cell division is called snapping type of cell division)

- **Metachromatic / Volutin Granules / Polar Bodies / Babes-Ernst Bodies:** present at ends/poles of bacilli

→ storage granules composed of polymetaphosphates

→ stained strongly gram +ve

→ take up bluish purple metachromatic color when stained with Loeffler's methylene blue

→ better stained with Albert's, Neisser's, Ponder's stain.

[*Corynebacterium diphtheriae* is aka Klebs-Loeffler bacillus]

Virulence Factors: Diphtheria toxin (DT)

↳ DT is a polypeptide chain & comprises of 2 fragments - A (active) & B (binding)

→ Fragment B binds to host cell receptors & causes internalization of fragment A

→ Fragment A internalization → ADP-ribosylation of elongation factor (EF-2) → inhibition of EF-2 → inhibition of translation (protein synthesis)

Factors regulating Toxin Production:

• **Phage Coded:** DT is coded by a bacteriophage β -corynophage carrying tox gene

• **Iron Concentration:** optimum iron concentration = 0.1 mg per litre

Toxoid is Used For Vaccination: DT is antigenic & antitoxins are protective

→ DT is virulent ∴ cannot be given directly for vaccination

→ Toxoid is formed from DT (which is avirulent but remains antigenic) by formalin, acidic pH, prolonged storage

→ **Limit of Flocculation Unit (LF):** 1 LF unit is the amount of toxin which flocculates most rapidly with one unit of antitoxin.

Pathogenicity: Bacilli are non-invasive, present only at local site (pharynx), secrete the toxin which spreads via bloodstream to various organs.

Clinical Manifestations:

• **Respiratory Diphtheria:** most common form of diphtheria

→ tonsil, pharynx, nose, larynx can be involved

→ incubation period = 3-4 days

- **Faucial Diphtheria:** DT produces an inflammatory response that leads to necrosis of epithelium & exudate formation

→ formation of mucosal ulcers lined by a tough leathery greyish white pseudomembrane coat.

→ In severe cases, pseudomembrane may extend into larynx & bronchial airways which may result in fatal airway obstruction causing asphyxia (this mandates tracheostomy)

→ **Bull-neck appearance:** massive tonsillar swelling & neck edema - presentation: foul breath, thick speech, stridor

• **Cutaneous Diphtheria:** presents as punched-out ulcerative lesions with necrosis

→ most commonly occurs on the extremities

→ it is not toxin-mediated & is caused due to the organism itself

• **Systemic Complications:** polyneuropathy, myocarditis, pneumonia, renal failure, encephalitis, cerebral infarction, pulmonary embolism

- **Neurologic Manifestations:** toxin-mediated non-inflammatory demyelinating disorders → peripheral neuropathy, ciliary paralysis.

- **Myocarditis:** associated with arrhythmias & dilated cardiomyopathy.

Laboratory Diagnosis:

→ specimens: throat swab containing fibrinous exudates, portion of pseudomembrane, nose or skin specimens

• **Direct Smear Microscopy:**

- **Gram stain:** club-shaped gram +ve bacilli with Chinese letter / cuneiform arrangement

- **Albert's stain:** green bacilli with bluish-black metachromatic granules.

• **Culture Media:** *C. diphtheriae* are fastidious, aerobic & facultative anaerobe, does not grow on ordinary medium; grows best in blood agar, chocolate agar, Loeffler's serum slope

- **Enriched Media:**

① **Blood agar:** small, circular, white & sometimes hemolytic

② **Loeffler's serum slope:** small, circular, glistening, white with a yellow tinge

- **Selective Media:** for isolation of *C. diphtheriae* from both cases & carriers

③ **Potassium Tellurite Agar (PTA):** *C. diphtheriae* reduces tellurite to metallic tellurium giving them black colour

• **Identification:** - sugar fermentation test using Hiss' serum sugar media

- Pyrazinamide test: negative

- Urease test: negative

• **Toxin Demonstration:**

- **In vivo test (Animal Inoculation):** by inoculation of culture broth into guinea pig

- **In vitro test:**

① **Elek's gel precipitation test:** a type of immune-diffusion in gel

→ strain isolated is streaked onto a media containing a filter paper soaked with antitoxin

→ if the strain is toxigenic, it liberates the toxin which diffuses in the agar & meets with antitoxin to produce an arrow-shaped precipitation band.

→ Types of precipitation bands:

- Cross-over with each other - indicates unrelated strain
- Spur formation - partially related strain
- Fused with each other - identical strain.

② **Other in vitro tests:**

- detection of tox gene by PCR

- detection of diphtheria toxin by ELISA or immuno-chromatographic test (ICT)

- cytotoxicity produced on cell lines

• **Typing of *C. diphtheriae*:** 4 biotypes -

- gravis
- mitis
- intermedius
- belfanti.

→ humans are the only reservoir of *C. diphtheriae*

→ common in children aged 1-5 years

→ newborns are usually protected due to maternal antibodies

Treatment: should be started immediately on clinical suspicion of diphtheria

• **Antidiphtheric Serum or ADS (antitoxin):** passive immunization with antidiphtheric horse serum

→ a test dose should be given to check for hypersensitivity

→ 1m or 1v

• early stage (< 48 hours): 20,000 - 40,000 units

• if pharyngeal membranes present: 40,000 - 60,000 units

• late stage (> 3 days, with bull neck): 80,000 - 120,000 units

• Antibiotics: DOC = Penicillin or Erythromycin

↳ play a minor role once toxin is secreted ; useful :

- if given early (< 6h of infection), before toxin release
- prevent further release of toxin by killing the bacilli
- treatment of cutaneous diphtheria
- treatment of carriers

Prophylaxis: patient should be placed in isolation room

→ for close contacts, booster dose of diphtheria vaccine + penicillin G (single dose) or erythromycin (7-10 days)

→ Diphtheria toxoid is used for vaccination

• Vaccination: a protective titer of more than 0.01 Unit/mL of antitoxin can prevent all forms of diphtheria

→ Vaccine is not effective for:

- Prevention of cutaneous diphtheria
- Elimination of carrier state.

• Single Vaccine: prepared by incubating toxin with formalin & then the toxoid is adsorbed onto alum.

• Combined Vaccine:

① DPT: diphtheria toxin + pertussis (whole cell) + tetanus toxoid

② DaPT: contains DT, TT, acellular pertussis (aP)

③ Td: tetanus toxoid & adult dose (aP) of diphtheria toxoid

④ Pentavalent Vaccine: DPT + hepatitis B + Haemophilus influenzae

Dosing schedule:

• Children: total 7 doses

- 3 doses of pentavalent vaccine at 6, 10, 14 weeks
- 2 booster doses of DPT at 16-24 months & 5 years
- 2 booster doses of Td at 10 years & 16 years

• Pregnant woman: 2 doses of Td at 1 month interval

→ IM at anterolateral aspect of thigh (gluteal region is not preferred)

→ DPT should be kept at 2-8°C

→ dose: - children = 25 LF units of diphtheria toxoid

- adults = 2 LF units of diphtheria toxoid

→ Two commercial preparations of DPT are available [One dose (0.5ml) of vaccine]:

• Gilaxo: 25 LF (DT), 5 LF (TT)

• Kasauli: 30 LF (DT), 10 LF (TT)

Schick Test: toxin-antitoxin neutralization test

↳ to test susceptibility of individual to diphtheria before starting immunization (now obsolete)

Diphtheroids: (aka Coryneforms)

↳ non-diphtherial corynebacterium

→ normal commensals in throat, skin, conjunctiva, etc.

→ differentiated from C. diphtheria by features:

- stains more uniformly than C. diphtheriae
- show palisade arrangement
- absence of metachromatic granules.

Rare Causes of Bacterial Pharyngitis:

• Gonococcal Pharyngitis: *Neisseria gonorrhoeae*

↳ sexually-transmitted

→ causes urethritis ; rarely can cause pharyngitis following transmission by orogenital sexual contact

→ Diagnosis: nucleic acid amplification test (NAAT) of throat swab

→ Treatment: Ceftriaxone + azithromycin

Bacterial Lobar Pneumonia

→ Bacterial pneumonia $\begin{cases} \text{typical / lobar} \\ \text{atypical / interstitial} \end{cases}$

Lobar / Typical Pneumonia: involves infection of lung parenchyma & its alveoli.

→ characterized by consolidation, productive cough with purulent sputum

- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Haemophilus influenzae*
- Gram-negative bacilli

Interstitial / Atypical Pneumonia: occurs in the interstitial spaces of the lungs

→ cough is non-productive

- *Mycoplasma*
- *Chlamydia*
- *Legionella*

Clinical Features: fever with chills, sweats, tachycardia

→ tachypnoea, increased use of accessory muscles of respiration

→ dyspnoea

→ pleuritic chest pain (if pleura are involved)

→ GI symptoms seen in 20% patients: nausea, vomiting

→ myalgia, headache, fatigue, arthralgia

→ severely ill patients may develop septic shock & multiorgan failure

Pneumococcal Pneumonia (*Streptococcus pneumoniae*)

aka pneumococcus → leading cause of lobar pneumonia, otitis media in children & meningitis in all ages

→ *S. pneumoniae* ⇒ α -hemolytic

Virulence Factors & Pathogenesis:

• **Capsular polysaccharide:** principle virulence factor

→ protects cocci from phagocytosis.

• **C-carbohydrate Antigen [C-polysaccharide]:** this antigen precipitates with C-reactive protein (CRP).

• **Pneumolysin:** membrane-damaging toxin

→ inhibits neutrophil chemotaxis & phagocytosis (similar to streptolysin-O)

• **Autolysin:** an amidase enzyme which cleaves its own peptidoglycan leading to autolysis of cells.

→ responsible for characteristic bile solubility & draughtsman appearance of colonies.

• **Pneumococcal surface proteins (PspA, PspC)**

• IgA protease

• Neuraminidase

• **Pneumococcal surface adhesin A (PsaA)**

Clinical Manifestations: pneumococci first colonize the human pharynx

• **Lobar pneumonia:** productive purulent cough, fever, chest pain

→ dullness on percussion due to consolidation & crackles on auscultation

• **Empyema & parapneumonic effusion:** may occur as complications of pneumococcal pneumonia

• **Invasive pneumococcal Disease (IPD):** bloodstream infection, pyogenic meningitis, osteomyelitis, septic arthritis, endocarditis, pericarditis, primary peritonitis

• otitis media, sinusitis

Epidemiology: source of infection: human upper respiratory tract of carriers

→ transmission by inhalation of contaminated droplet nuclei

→ infection usually leads to colonization & carrier state; disease occurs only when host resistance is lowered due to presence of associated risk factors.

Risk Factors:

• **Children (<2 years):** because of their inability to produce adequate antibodies against the capsular antigen (immature immune system).

• **Splenectomy, Sickle Cell Disease & other Hemoglobinopathies:** since spleen is the site of destruction of capsulated bacteria

• **Underlying Comorbidities:** chronic lung, heart, kidney & liver disease, diabetes mellitus, immunosuppression

• **Viral URTI:** underlying viral upper respiratory tract infections (eg: influenza)

Laboratory Diagnosis:

→ **Specimens:** depending on the site of infection ⇒ sputum, CSF, pleural fluid, aspiration of sinus material, ear discharge

• **Direct Smear Microscopy:** shows numerous pus cells & lanceolate or flame-shaped gram +ve cocci in pairs surrounded by a clear halo (due to capsule); non-motile

• **Antigen Detection:**

→ **Capsular antigen:** in CSF by latex agglutination test using latex beads coated with anticapsular antibodies

→ **C-antigen:** detection of C-polysaccharide antigen in urine by immunochromatographic test (ICT)

• **Culture:** fastidious, does not grow in basal media

→ **Blood Agar:** initially ⇒ small dome-shaped surrounded by a green zone of α -hemolysis; on prolonged incubation, they undergo autolysis to produce a central depression with an elevated rim ⇒ draughtsman-shaped or coccum coin appearance

→ **Chocolate Agar:** greenish discoloration (bleaching effect)

• **Identification:** catalase negative

→ pneumococci can ferment insulin to form acid

→ pneumococci are sensitive to optochin disk & produce a wide zone of inhibition

→ pneumococci are soluble in bile due to autolysin activity

• **Typing of *S. pneumoniae*:** Quellung or Neufeld Reaction ⇒ used to be done in the past at bedside, directly from sputum samples

→ when specimen is treated with type-specific antiserum along with methylene blue dye ⇒ capsule becomes swollen, sharply delineated & refractile

→ currently, serotyping is done by latex agglutination test

• **Molecular methods:** PCR

→ common genes targeted are: *lytA* (autolysin gene), *ply* (pneumolysin) & *psaA*

• **Nonspecific Findings:** elevated acute phase reactant

→ leukocytosis

→ chest X-ray shows infiltrates & lobar consolidation

Treatment: for pneumococci infection -

• **pneumonia:** oral amoxicillin for 5 days

→ alternatives: IV penicillin or ceftriaxone, oral quinolone, clindamycin, azithromycin

• **meningitis:** ceftriaxone / cefotaxime ± vancomycin for 10-14 days

• **other invasive infections:** penicillin, cefotaxime / ceftriaxone

→ in critically ill-patients: same Tx as meningitis

• **Otitis Media:** oral amoxicillin for 7-10 days.

Drug Resistance in *Pneumococcus*:

• **Penicillin resistance:** due to alteration of PBP to PBP2b; altered PBP2b has low affinity for β -lactam drugs.

• **Multidrug Resistant (MDR):** non-susceptible to ≥ 3 antibiotic classes

→ penicillin, tetracycline, erythromycin, sulfonamides, clindamycin

→ serotype 19A is most commonly MDR.

→ some serotypes undergo capsule switching (change in serotype) which may contribute to development of antibiotic resistance.

Pneumococcal Vaccines:

- pneumococcal polysaccharide vaccine [PPSV23]

- pneumococcal conjugate vaccine [PCV13]

• **Schedule:**

→ **PCV13:** 3 primary doses at 6, 10, 14 weeks & booster at 15 months

→ **Age ≥ 65 years:** only PPSV23 or PCV13 followed by PPSV23 with ≥ 1 year gap

→ **19-64 years:** given only in presence of underlying risk factor.

	PPSV23	PCV13
Serotypes included	23 serotypes of <i>S. pneumoniae</i>	13 serotypes of <i>S. pneumoniae</i>
Immunogenicity	Less immunogenic ($\because$ capsular antigen is T cell independent)	More immunogenic (protein conjugate acts as adjuvant)
Duration	short term immunity (3-5 years)	longer immunity
Herd effect on carriers	promotes herd immunity doesn't provide mucosal immunity $\therefore$ not effective for carriers	does not promote provides mucosal immunity $\therefore$ can be used to eradicate carriage from nasopharynx
Cost	Less expensive	More expensive

Features	<i>S. pneumoniae</i>	<i>Viridans streptococci</i>
Arrangement	gram +ve cocci in pairs	gram +ve cocci in long chains
Morphology	Lanceolate-shaped	Round/oval
Capsule	Present	Absent
On blood agar	Draughtsman or coccum coin colony	minute colony
Bile solubility	soluble	insoluble
Insulin	fermented	not fermented
Optochin	sensitive	resistant

Haemophilus Influenzae Pneumonia

- Haemophilus species: oxidase +ve, capsulated, pleomorphic, gram -ve bacilli that require special growth factors present in blood, such as factor X & V.
- H. influenzae: most pathogenic species; causes pneumonia & meningitis in children
- H. ducreyi: causes a STD called chancroid
- H. haemolyticus & H. parahaemolyticus: produce hemolysis on blood agar

→ H. influenzae is aka Pfeiffer's bacillus

Virulence Factors:

- Capsular polysaccharide: principle virulence factor
 - acts by inhibiting phagocytosis
- Bacterial endotoxin
- Outer membrane proteins
- IgA1 proteases
- pili & other adhesion proteins

Serotyping: based on capsular polysaccharide ⇒ 6 serotypes (a to f).

- some strains lack capsule & are nontypeable strains
- H. influenzae b (Hib) is most virulent
- Hib capsule is made up of polyribosyl phosphate (PRP) antigen which is strongly immunogenic & is ∴ used for vaccination

Clinical Manifestations:

- Central Nervous System Infections: pyogenic meningitis, subdural effusion
- Epiglottitis: cellulitis of the epiglottis & supra-glottic tissues
 - can lead to acute airway obstruction
- Community Acquired Bacterial Pneumonia: pneumonia in infants
 - pleural involvement is more common in Hib infection
- Intracranial Focus: sinusitis & otitis from which the infection may spread to meninges
- Less common invasive conditions:
 - cellulitis of neck & head
 - pericarditis
 - osteomyelitis, septic arthritis
 - empyema, bronchiectasis
 - UTI
 - orbital cellulitis, endophthalmitis
 - bacteremia

Non-Typeable H. influenzae: cause disease by local invasion of mucosal surfaces

- Clinical manifestations:
- childhood otitis media
 - exacerbation of COPD
 - pleural sepsis
 - neonatal bacteremia
 - sinusitis

Epidemiology: H. influenzae is exclusively a human pathogen

- transmitted through respiratory tract; by droplet inhalation or contact with infected secretions or fomites
- common in children < 5 years & adults > 65 years.

Laboratory Diagnosis:

- Specimen: depending upon the site of infection - CSF, blood, sputum, pus, aspirates
- H. influenzae is highly sensitive to low temperature ∴ specimens for culture should never be refrigerated
- Gram staining: pleomorphic, gram -ve coccobacilli
- Capsule detection (Quellung reaction): capsular swelling occurs when a drop of CSF is mixed with type b antiserum & methylene blue
- Antigen Detection: of b capsular antigen by
 - latex agglutination test
 - direct IF test
 - ELISA

- Culture: H. influenzae is highly fastidious, requires 2 accessory growth factors (factor X & V) in blood for their growth
 - Factor X is a hemin
 - Factor V is nicotinamide adenine dinucleotide [NAD]
 - no growth on basal media due to lack of factors X & V
 - growth is scanty on blood agar ∴ only factor X is available (factor V is only present inside RBCs)
 - grows well on chocolate agar (∴ when blood is poured into molten agar at 75°C, RBCs get lysed releasing factor V)
 - grows well on blood agar with S. aureus streak line (this phenomenon is called satellitism)
 - special media: Fildes agar, Levinthal's agar
 - Haemophilus selective medium contains bacitracin, sucrose
 - non-motile bacilli
- Biochemical Tests: catalase +ve, oxidase +ve
 - Haemophilus species vary in their X & V requirement

- ① Growth surrounding X & XV disks only: require X
 - H. ducreyi
 - A. aphrophilus
- ② Growth surrounding V & XV disks only: require V
 - H. parainfluenzae
 - H. parahaemolyticus
 - A. paraphrophilus
- ③ Growth surrounding XV disk only: require both X & V
 - H. influenzae
 - H. aegyptius
 - H. haemolyticus

→ based on biochemical properties, H. influenzae has 8 biotypes (I - VIII)

• Molecular Method: multiplex PCR, BioFire FilmArray

Treatment:

- For invasive infections due to Hib: ceftriaxone, cefotaxime, etc for 1-2 weeks
 - alternatively: ampicillin + chloramphenicol
- Non-typeable strains: often resistant to β -lactams [due to β -lactamase production]
 - usually susceptible to quinolones (levofloxacin) & macrolides (azithromycin)

Hib Conjugate Vaccine: ∴ capsular antigens are poorly immunogenic to children, they are conjugated with adjuvants such as diphtheria toxoid, tetanus toxoid, N. meningitidis outer membrane proteins

- Hib vaccine is given in combination with DPT & hepatitis B (pentavalent vaccine) at 6, 10, 14 weeks of birth; 1m at anterolateral side of mid-thigh

Chemoprophylaxis: Oral rifampicin; indicated to:

- household contacts
- healthcare workers (if ≥ 2 cases occur within 60 days in the hospital)

Staphylococcal Pneumonia: S. aureus

- Infant Pneumonia: S. aureus is the leading cause of pneumonia in newborns & infants
 - presents with: fever, dyspnea, respiratory failure
 - chest X-ray reveals characteristic pneumatocele (shaggy, thin-walled cavities)
- VAP in adults: in hospitalised ventilated patients (ventilator acquired pneumonia)
- Post-viral CAP: community acquired pneumonia following viral infections (eg: influenza)
- CA-MRSA: necrotizing pneumonia

Gram -ve Bacilli Pneumonia:

- increasingly associated with causing lobar pneumonia, particularly VAP
- most are MDR

- Non-fermenters: - Pseudomonas aeruginosa - Acinetobacter
- Enterobacteriaceae: - E. coli - Klebsiella pneumoniae
- Enterobacter - Serratia marcescens

Rare Causes of Bacterial Pneumonia:

① Pulmonary Anthrax: Wool Sorter's disease ⇒ Bacillus anthracis

- transmission via inhalation of spores during exposure to contaminated animal products
- seen in workers of a wool factory
- Haemorrhagic pneumonia: presents initially with prodromal stage of fever, myalgia, lethargy followed by abrupt onset of haemorrhagic pneumonia leading to respiratory failure
- Chest X-rays show mediastinal widening &/or pleural effusions.
- Treatment with Anthrax Immunoglobulin: Raxibacumab & Obiltoximab neutralizes anthrax toxin

② Pneumonic Plague: Yersinia pestis

- Three clinical forms:
 - bubonic (most common)
 - pneumonic
 - septicemic
- Manifestations: fever, headache, cough, hemoptysis, dyspnea, chest pain
- Treatment: Gentamicin or streptomycin

Bacterial Interstitial Pneumonia

→ cough is non-productive

Agents of Atypical Pneumonia:

- **Respiratory Viruses:** - Coronavirus - Influenza virus
- EBV - adenovirus
- **Bacterial agents:** - Mycoplasma pneumoniae (aka walking pneumonia)
- Chlamydia - Legionella - Coxiella burnetii

Mycoplasma Pneumonia:

Mycoplasma pneumoniae → causes primary atypical pneumonia

- mycoplasmas are the smallest microbes capable of free-living in the environment
- they are filterable by bacterial filters
- lack a rigid cell wall ∴ completely resistant to antibiotics acting on cell wall such as β -lactams
- highly pleomorphic
- poorly gram -ve
- non-spore, non-flagellated, usually non-motile (some species show gliding motility)
- previously called pleuropneumonia-like organisms [PPLO] & Eaton's agent

Classification: Family Mycoplasmataceae has 2 genera: - Mycoplasma - Ureaplasma

- Mycoplasma pneumoniae causes atypical pneumonia
- Urogenital infections are caused by:
 - Mycoplasma hominis • Ureaplasma urealyticum
 - Mycoplasma genitalium • Ureaplasma parvum.

Pathogenesis: attachment of M. pneumoniae to respiratory mucosa mediated by its membrane bound adhesion proteins (eg: cytoadhesion P1 protein)

Epidemiology: transmitted by respiratory droplets
→ incubation period = 2-4 weeks

Clinical Manifestations:

• **Upper Respiratory Tract Infections (URTI):**

- pharyngitis, tracheobronchitis, rarely otitis media
- acute onset

• **Pneumonia:** onset is usually gradual

- wheeze or rales, dry cough, peribronchial pneumonia with thickened bronchial markings & streaks of interstitial infiltration on chest X-ray

• **Extrapulmonary Manifestations:**

- **Neurologic:** meningoencephalitis, encephalitis, aseptic meningitis, Guillain-Barre Syndrome
- **Dermatologic:** skin rashes including erythema multiforme major (Stevens-Johnson Syndrome)
- **Cardiac:** myocarditis, pericarditis
- **Rheumatologic:** reactive arthritis
- **Haematologic:** anemia, hypercoagulopathy.

Laboratory Diagnosis:

- **Specimens:** throat swabs, nasopharyngeal aspirates, bronchial brushings, bronchoalveolar lavages (BAL), lung biopsies
- **Transport media:**
 - Standard Mycoplasma fluid medium [containing fetal bovine serum, gelatine, penicillin]
 - Viral transport medium with ampicillin & cefotaxime

Culture:

- In liquid medium [containing PPLO broth]: turbidity & a color change
- In solid medium [containing PPLO Agar]: fried egg appearance of colonies
- Special stain: Diene's staining
- **Antigen Detection:** by direct immunofluorescence test & ELISA
- **Specific Antibody Detection Tests:** - immunofluorescence assays
- latex agglutination assays - ELISA using protein P1 antigens
- **Nonspecific Antibody Detection Tests:** heterophile agglutination tests such as:
 - Cold agglutination test - Streptococcus MG test
- **Molecular Methods:** PCR targeting M. pneumoniae specific 16S rRNA gene & P1 adhesion gene
- Treatment:** DOC = macrolides (azithromycin oral, 500 mg on day 1, 250 mg on days 2 to 5)
→ Alternatives: doxycycline, respiratory fluoroquinolones such as levofloxacin, moxifloxacin, gemifloxacin

Chlamydia Pneumonia: 3 chlamydia species:

- C. pneumoniae • C. psittaci • C. trachomatis
- Psittacosis:** C. psittaci → pathogen of parrots & other psittacine birds

- transmitted to humans by inhalation of aerosols from avian nasal discharges
- incubation period = 5-14 days
- mild influenza, interstitial pneumonia, septicemia, typhoid-like symptoms
- **Treatment:** tetracycline (DOC) → 250 mg, 4 times a day for at least 3 weeks to avoid relapse
- alternative: erythromycin → 500 mg, 4 times a day orally

Chlamydia Pneumoniae: C. pneumoniae is exclusively a human pathogen, transmitted by inhalational route

- **Clinical Manifestations:** atypical pneumonia, URTI, atherosclerosis, asthma & COPD.

Chlamydia Trachomatis [Infant Pneumonia]: C. trachomatis serotypes D to K can cause infant pneumonia

- develops within 3 weeks to 3 months of birth

Legionellosis: Legionellae → fastidious, pleomorphic, gram -ve short bacilli

- cause Legionnaire's Disease

Classification: L. pneumophila is the most important species which has 15 serogroups
→ Legionella inhabits aquatic bodies

Transmission: Multiple routes →

- Aspiration (predominant mode): via oropharyngeal colonization or directly via drinking of contaminated water
- aerosols from contaminated air conditioners, nebulizers, humidifiers
- direct instillation into lungs during respiratory tract manipulations
- no man-to-man transmission

Predisposing Risk Factors:

- smoking, alcoholism → advanced age
- chronic lung disease → immunosuppression

Pathogenesis: Legionellae reach the lungs → engulfed by alveolar macrophages by coiling phagocytosis → invasion & growth within alveolar macrophages by inhibiting phagosome-lysosome fusion → activation of cell-mediated immunity

Clinical Manifestations:

- **Pentec Fever:** acute flu-like illness [incubation period: 24-48 hours]
 - malaise, fever, headache
 - self-limiting, never develops into pneumonia
- **Legionnaire's Disease (Pneumonia):** incubation period = 2-10 days
 - non-productive cough, dyspnoea, chest pain, high fever, diarrhoea
 - chest X-ray shows pulmonary infiltrates
- **Extrapulmonary Legionellosis:** results from blood-borne dissemination from the lung
 - most common extrapulmonary site: heart (myocarditis, pericarditis, prosthetic valve endocarditis)
 - sinusitis, peritonitis, pyelonephritis, skin & soft tissue infection

Laboratory Diagnosis:

- **Specimen:** sputum, BAL fluid, bronchial wash, pleural fluid
- **Direct microscopy:** gram stain reveals numerous neutrophils but no organisms (∴ Legionella are poorly-stained → appear as faint pleomorphic gram -ve rods or coccobacilli)
 - Silver impregnation & Giemsa stain can be used.
- L. micdadei is weakly acid fast
- **Culture:** Buffered Charcoal Yeast Extract (BCYE) agar
- **Antibody Detection:** indirect immunofluorescent antibody test & enzyme immunoassays
 - antibodies usually appear late after 12 weeks
- **Urine Antigen Test:** enzyme immunoassays
 - highly sensitive, rapid, cheaper
 - antigen in urine is detectable shortly after onset of symptoms
- **Molecular methods:** BioFire FilmArray
- Treatment:** Macrolides (especially azithromycin) & respiratory quinolones (levofloxacin)

Pertussis:

Bordetella pertussis: highly fastidious, very small, gram -ve coccobacilli, non-fermenter

→ Family Alcaligenaceae

→ B. pertussis: causes whooping cough in children

[paroxysmal cough ending in a high-pitched inspiratory sound described as whoop]

→ B. parapertussis: milder form of whooping cough

→ B. bronchiseptica: pathogen of domestic animals

→ Whooping cough: violent paroxysmal productive cough in children

Virulence Factors:

- Tracheal Cytotoxin: part of cell wall peptidoglycan
→ cause damage to cilia of respiratory epithelial cells

- Pertussis toxin [PT]: most imp. virulence factor

- similar to cholera toxin in structure & mechanism

- it causes activation of adenylyl cyclase → ↑ conc. of cAMP → effects of whooping cough

- Adhesins: play a role in bacterial attachment

- Filamentous Hemagglutinin (FHA)

- Pertactin (outer membrane protein)

- Fimbriae / pili / agglutinogens

- Adenylyl cyclase toxin

- Deamonoecrotic toxin

- Bacterial endotoxin

Pathogenesis: bacteria attach to ciliated epithelial cells of nasopharynx, multiply by producing toxins to cause local mucosal damage & ∴ host defense mechanism.

Clinical Manifestations: 3 stages

[Incubation period = 7-10 days]

- Catarrhal Phase: lasts for 1-2 weeks

→ common cold-like nonspecific symptoms ⇒ coryza, lacrimation, mild cough, low-grade fever, malaise

→ highly infectious stage

- Paroxysmal Phase: lasts for 1-6 weeks, less infectious

→ specific symptoms ⇒ whooping cough, post-tussive vomiting

- Convalescent Stage: lasts for 1-3 months

- frequency & severity of coughing gradually decreases

→ antibodies may appear in serum

- each paroxysm consists of bursts of 5-10 repetitive violent spasmodic coughs, often with a single expiration which ends with an audible sound or whoop ⇒ whoop occurs due to rapid inspiration against a closed glottis at the end of the paroxysm

- episodes are often worse at night

- during a spasm, there may be visible neck vein distension, bulging of eyes, tongue protrusion & cyanosis

- weight loss may be seen (fever is uncommon)

Complications: more common among infants than older children or adults

- Pressure effects: subconjunctival haemorrhage, hernias, pneumothorax, rib fracture, petechiae

- Pneumonia: in old age (due to secondary infection)

- Neurological complications: convulsions, encephalopathy, coma

Differentials for Whooping Cough:

- Mycoplasma pneumoniae
- Influenza virus
- Chlamydia pneumoniae
- Rhinovirus
- Adenovirus

→ Whooping cough is a highly communicable disease.

→ Host: exclusively human disease (no animal reservoir)

→ Source: early cases [Catarrhal stage]

→ Age: predominantly a disease of pre-school children below 5 years (∴ maternal antibodies are not protective).

→ Shift of Median Age: pertussis has shifted from infants to older children & adolescence in countries with high vaccine coverage (indication that immunization or natural infection do not provide lifelong immunity)

→ transmission via inhalation of droplets or rarely through direct contact

Laboratory Diagnosis:

→ Specimens: nasopharyngeal secretions

→ Types of Swabs Used: for culture, alginate swabs are best followed by dacron swabs

- For PCR, only dacron or rayon swabs

- Cotton swabs are not satisfactory as fatty acids present in cotton may inhibit growth of bacilli

- Charcoal-impregnated cotton swabs [Stuart's] may be useful

- it is recommended to collect 6 swabs at 1-2 days intervals

→ Transport: if delay is expected, then suitable charcoal-based medium [Amies, Regan-Lowe, Jones-Kendrick transport media]

- Direct Detection: from nasopharyngeal secretions by direct immunofluorescence test using fluorescein labelled polyclonal or monoclonal antibodies

- Culture: nasopharyngeal culture ⇒ gold standard

→ B. pertussis is a strict anaerobe, fastidious

→ it requires special complex media for primary isolation, such as — Regan & Lowe medium

— Bordet-Gengou glycerine-potato-blood agar

→ colonies are greyish white, convex with a shiny surface ⇒ Mercury Drops or Bisected Pearl Appearance

- Culture Smear: ovoid coccobacilli, arranged in loose clumps with clear spaces in between ⇒ Thumb print Appearance

- Serum antibody Detection: Enzyme immunoassays [EIAs]

- Molecular Methods: PCR remains +ve in first 4 weeks of onset of symptoms

- Other Findings: lymphocytosis in young children

Treatment: antibiotics are less useful since pertussis is toxin-mediated

→ DOC = macrolides (Azithromycin for 5 days or erythromycin for 7-14 days)

→ Alternative: cotrimoxazole

→ Cough suppressants are not much effective

Chemoprophylaxis: Erythromycin

Vaccine:

① Whole-cell Pertussis Vaccines: prepared by heating followed by chemical inactivation & purification of whole B. pertussis bacilli.

DPT Vaccine: whole-cell (WC) pertussis vaccine along with diphtheria toxoid & tetanus toxoid.

→ 3 doses of pentavalent vaccine at 6, 10, 14 weeks

→ 2 boosters of DPT at 1½ years & 5 years

Contraindications of WC Vaccine:

- children more than 5-6 years age

- Any associated progressive neurological conditions

- Children with strong family history of epilepsy

- Hypersensitivity to previous dose.

② Acellular Pertussis Vaccine: composed of pertussis toxoid & 2 or more other bacterial components such as FHA, pertactin or fimbriae

→ efficacy is same as WC vaccine; associated with fewer side effects & can be safely given after 5-6 years

→ available as DaPT ⇒ recommended for older children who have missed their primary doses of DPT.

PARASITIC INFECTIONS

- *Paragonimus westermani*
- Hookworm
- *Ascaris*
- *Strongyloides*
- Filarial nematodes causing tropical pulmonary eosinophilia (TPE)

Paragonimiasis: *Paragonimus westermani* (trematode)
aka Oriental Lung Fluke $\Rightarrow$ causes endemic hemoptysis.

$\rightarrow$ endemic in North-East states of India.

Life Cycle: similar to other trematodes (except schistosomes)

• **Morphology:** - Adult worm [leaf-like], operculated eggs, larvae (5 stages)

• **Hosts:** 3 hosts -

- Definitive host $\Rightarrow$ humans

- Intermediate host $\Rightarrow$ snails, crabs

• **Transmission:** humans get infected by ingestion of second intermediate host carrying metacercaria larvae (infective form)

$\rightarrow$ larvae penetrate the intestinal wall to reach peritoneal cavity & then lungs where they develop into adult worms

$\rightarrow$ Adult worms undergo fertilization to produce eggs which are passed in sputum or rarely swallowed & excreted in feces

$\rightarrow$ eggs $\Rightarrow$ diagnostic form

Pathogenesis: due to multiplication of adult worm in lungs or occasionally at other extrapulmonary sites.

Clinical Manifestations:

• **Pulmonary Paragonimiasis (Endemic Hemoptysis):**

$\rightarrow$ adult worm initially causes eosinophilic granulomatous inflammation in lungs & subsequently form cysts surrounding the worms.

$\rightarrow$ productive cough with brownish blood tinged rusty sputum with an offensive fishy odour.

$\rightarrow$ hemoptysis with peripheral blood eosinophilia.

$\rightarrow$ bronchitis, bronchiectasis, pneumonia, lung abscess

• **Extrapulmonary Paragonimiasis:** worms migrate from ruptured cysts to various sites such as liver, spleen, etc & cause - Cerebral paragonimiasis

- Cutaneous paragonimiasis.

Laboratory Diagnosis:

• **Sputum Microscopy:** early morning, deeply coughed sputum sample

$\rightarrow$ saline mount of sputum sample (particularly blood-tinged flecks) is examined for characteristic operculated eggs.

$\rightarrow$ sputum can be concentrated by formalin-ether sedimentation technique (not by floatation method)

$\rightarrow$ stool microscopy may be done to detect eggs

$\rightarrow$ eggs are oval, operculated & golden-brown in colour; they are unembryonated when laid

• **Serological Tests:**

$\rightarrow$ DIGFA [Dot-immunogold filtration assay] for detection of serum antibodies

$\rightarrow$ ELISA to detect parasite specific IgG or IgE.

$\rightarrow$ Western blot test using adult worm homogenate.

$\rightarrow$ Antigen detection: Dot ELISA

• **Other Tests:** - peripheral blood eosinophilia

$\rightarrow$ MRI & CT scan are preferred to locate the cysts in the CNS & other sites.

Treatment: Praziquantel (25 mg/kg/dose, 3 doses per day for 2 days)

$\rightarrow$ surgical management may be needed for pulmonary or cerebral lesions.

Prevention: - sanitary disposal of sputum - health education
- control of snails - treatment of cases

Tropical Pulmonary Eosinophilia [TPE]:

- *Wuchereria bancrofti*
- *Brugia malayi*

$\rightarrow$ aka occult filariasis or Weingarten's syndrome

$\rightarrow$ it differs from classical filariasis.

Pathogenesis: represents a hypersensitivity reaction to microfilaria antigen

$\rightarrow$ microfilariae are rapidly cleared from the blood stream & filtered, lodged & destroyed in the lungs initiating an allergic response

$\therefore$ microfilariae are not detected in peripheral blood

Clinical Features: nocturnal paroxysmal cough & wheezing, weight-loss, low-grade fever

$\rightarrow$ occasional hepatosplenomegaly & lymphadenopathy. [called as Meyers Kouwenaar Syndrome]

Laboratory Diagnosis: occult filariasis is diagnosed by:

• blood eosinophilia (absolute eosinophil count $> 3000/\mu L$)

• Chest X-ray shows diffuse infiltration

• Elevated serum IgE levels

Treatment: diethylcarbamazine [DEC], 4-6 mg/kg for 14 days.

Characters	Classical Filariasis	Occult Filariasis
Probable etiology	Inflammatory changes to adult worm.	Hypersensitivity reaction to microfilaria antigen
Diagnostic form	Microfilaria in blood & in fluid.	Microfilaria absent in blood
Organs affected	Lymph nodes & lymphatic vessels.	Lungs, liver & spleen.
Pathology	Lymphangitis & lymphadenitis.	Eosinophilic granuloma.
Serology	Antibody detection is not diagnostic	IgE antibody detection is diagnostic

Migratory Stage of Intestinal Nematodes:

$\rightarrow$ Intestinal nematodes - *Ascaris*, hookworm & *Strongyloides* pass through the lungs during their life cycle

In Ascariasis: pulmonary symptoms are observed in the 2nd week after ingestion of eggs.

$\rightarrow$ immune-mediated hypersensitivity response

$\rightarrow$ non-productive cough, chest discomfort, fever

Loeffler's Syndrome: in severe cases, patients develop dyspnea & transient patchy infiltrates seen on chest X-ray along with peripheral eosinophilia.

Diagnosis: Presence of Charcot-Leyden crystals in sputum & stool.

FUNGAL INFECTIONS

• **Opportunistic Fungal Agents:**

- *Pneumocystis jirovecii* pneumonia

- Penicilliosis

- Zygomycosis

- Aspergillosis.

• **Fungi Causing Systemic Mycoses:** - *Blastomyces dermatitidis*

- *Histoplasma capsulatum*

- *Coccidioides immitis*

- *Paracoccidioides brasiliensis*

• **Yeast:** *Cryptococcus neoformans*

Pneumocystis Pneumonia [PCP]:

Pneumocystis: previously thought to be a protozoan, now classified under fungus (*Pneumocystis jirovecii* was previously called *P. carinii*.)

Pathogenesis: *Pneumocystis* exists in cyst & trophozoite forms.

$\rightarrow$ both cysts & trophozoites are found in human tissues

[trophozoites contain 4-8 sporozoites]

$\rightarrow$ once inhaled, cysts are carried to lungs where they transform into trophozoites stage $\rightarrow$ trophozoites induce inflammatory response $\rightarrow$ recruitment of plasma cells $\rightarrow$ formation of frothy exudate that fills the alveoli $\therefore$ this condition is aka plasma cell pneumonia.

Laboratory Diagnosis: histopathological examination of lung tissue/fluid

$\rightarrow$ Gomori's methenamine silver (GMS) staining is the method of choice to demonstrate cysts of *P. jirovecii*; cysts resemble black coloured crushed ping-pong balls, against the green background.

$\rightarrow$ PCR assay

$\rightarrow$ Chest X-ray depicts classical finding of bilateral diffuse infiltrates.

$\rightarrow$ CT of lung may reveal ground-glass opacities at early stage.

Treatment: cotrimoxazole (trimethoprim/sulfamethoxazole)

given for 14 days in non HIV patients & 21 days in patients with HIV

$\rightarrow$ It is also the recommended drug for primary & secondary prophylaxis.

Zygomycosis: caused by aseptate fungi of Phylum Zygomycota

$\rightarrow$ This phylum has 2 orders:

• **Order mucorales:** causes mucormycosis

- *Rhizopus*

- *Rhizomucor pusillus*

- *Mucor racemosus*

- *Lichtheimia corymbifera*

- *Apophysomyces elegans*

• **Order entomophthorales:** causes entomophthoromycosis

- *Basidiobolus ranarum*

- *Conidiobolus coronatus*.

Mucormycosis:

Pathogenesis: transmission occurs via inhalation, inoculation or rarely ingestion of spores

$\rightarrow$ spores develop into mycelial form which have wide aseptate hyphae which are angioinvasive in nature.

Predisposing Factors: • conditions with increased iron load

• Diabetic ketoacidosis [DKA]

• End-stage renal disease

• Patients taking iron therapy or deferoxamine

• Defects in phagocytic functions

Clinical Manifestations: 6 types of clinical presentations

• **Rhinocerebral mucormycosis:** occurs in patients with DKA

$\rightarrow$ starts as eye & facial pain

$\rightarrow$ may progress to cause orbital cellulitis, proptosis, vision loss

• **Pulmonary Mucormycosis:** occurs in patients with leukemia

$\rightarrow$ dyspnea, cough, chest pain, variable fever

$\rightarrow$ angioinvasion can lead to necrosis, cavitation, hemoptysis

$\rightarrow$ Chest X-ray: lobar consolidation, isolated masses, nodular disease, cavities, wedge-shaped infarcts

$\rightarrow$ **Differential Diagnosis:** mucormycosis needs to be differentiated from pulmonary aspergillosis; presence of ≥ 10 pulmonary nodules, pleural effusion or concomitant sinusitis favours diagnosis towards mucormycosis

• **Cutaneous mucormycosis**

• **Gastrointestinal mucormycosis:** necrotizing enterocolitis

• **Disseminated mucormycosis:** brain is the most common site

• **Miscellaneous forms:** affects bones, trachea, kidneys, etc.

Laboratory Diagnosis:

$\rightarrow$ histopathological staining such as H&E or methenamine silver

$\rightarrow$ culture on SDA at 25°C shows characteristic white

cotton woolly colonies with tube filling growth [called lid lifter]; Rhizopus colonies become brown-black later due to sporulation giving rise to salt & pepper appearance
→ LPCB mount of colonies reveals broad aseptate hyaline hyphae

→ Rhizoid: some species bear a unique root like growth arising from hyphae called rhizoid

- Rhizopus bears nodal rhizoids
- Lichtheimia bears internodal rhizoids
- Mucor: rhizoids absent

Treatment: Amphotericin B deoxycholate

→ alternatively: posaconazole or isavuconazole.

Entomophthoromycosis: includes subcutaneous lesions

Aspergillosis:

→ invasive & allergic diseases caused by a hyaline mold called Aspergillus

- A. fumigatus
- A. flavus
- A. niger.

Pathogenesis: transmission occurs by inhalation of airborne conidia

Risk Factors:

- Glucocorticoid use
- Profound neutropenia
- Neutrophil dysfunction
- Underlying pneumonia
- Anti-tumor necrosis factor therapy

Clinical Manifestations:

→ incubation period: 2-90 days

• Pulmonary aspergillosis:

- allergic bronchopulmonary aspergillosis (ABPA)
- severe bronchial asthma
- extrinsic allergic alveolitis
- Aspergilloma
- Acute angioinvasive pulmonary aspergillosis
- Chronic cavity pulmonary aspergillosis

• Other forms of Aspergillosis:

- invasive sinusitis
- cardiac aspergillosis (endocarditis, pericarditis)
- cerebral aspergillosis (brain abscess, haemorrhagic infarction, meningitis)
- ocular aspergillosis (keratitis, endophthalmitis)
- ear infection (otitis externa)
- cutaneous aspergillosis
- nail bed infection: onychomycosis
- Mycetoxycosis: liver carcinoma.

Laboratory Diagnosis: specimens - sputum & tissue biopsies

• Direct examination: KOH [10%] mount

• Culture: specimens are inoculated onto SDA
→ species identification is done based on macroscopic & microscopic (LPCB mount)

• Antigen Detection:

- B-D-glucan antigen assay: marker of invasive fungal infections; raised in most invasive fungal infections
- Galactomannan antigen: Aspergillus specific antigen
→ detected by ELISA in patient's sera/urine
- Antibody Detection: detection of serum antibodies is very useful for chronic invasive aspergillosis & aspergilloma
→ in allergic syndromes such as ABPA & severe asthma, specific serum IgE levels are elevated.

• Skin Test: positive skin test to various antigen extracts of Aspergillus indicates hypersensitivity

Treatment:

- For invasive aspergillosis: voriconazole

• For ABPA: itraconazole

• For single aspergilloma: surgery

• For chronic pulmonary aspergillosis: itraconazole or voriconazole

• For prophylaxis: posaconazole

Pulmonary Cryptococcosis: *Cryptococcus neoformans* ⇒ capsulated yeast that causes meningitis in HIV-infected individuals
→ infection is acquired by inhalational route.

Tuberculosis:

Mycobacteria: are acid fast, i.e., they resist decolorization by dilute mineral acids due to:

- presence of high content of mycolic acids in cell wall
- integrity of cell wall

→ non-motile, non-spore, non-capsulated, weakly gram +ve, straight or slightly curved rod-shaped, obligate aerobes
→ they sometimes show branching filamentous forms resembling fungal mycelium.

Classification:

- **M. tuberculosis complex**
- **M. leprae** [Hansen's bacilli]
- **Non-tuberculous mycobacteria (NTM)** → cause opportunistic infections

→ Tuberculosis affects the lungs primarily; other organs are also involved.

M. tuberculosis complex:

- **M. tuberculosis** • **M. bovis**
- **M. africanum** • **M. microti** • **M. magerit**

Antigenic structure:

- **Cell wall (insoluble) Antigens:** cell wall has several layers:
 - Peptidoglycan layer
 - Arabinogalactan layer
 - Mycolic acid layer: confers very low permeability & is responsible for acid fastness & reduces the entry of most antibiotics

- Outermost layer: consists of lipids, glycosides, mycosides
- Proteins
- Plasma membrane: contains **lipid-arabinomannan (LAM)**; also used as a target antigen for TB diagnosis

- **Cytoplasmic [soluble] Antigens:** antigen 5, antigen 6, antigen 6D

Pathogenesis:

- Source of infection: - human (cases of pulmonary TB)
- bovine source (consumption of unpasteurized infected milk)

Mode of Transmission:

- **Air-borne:** transmitted by inhalation of aerosols generated while coughing, sneezing of infected patients.
- inoculation through direct skin contact
- Ingestion: swallowing of sputum (in infants) or consumption of unpasteurized (infected) milk.

Risk Factors:

- sputum +ve patients (sputum showing acid-fast tubercle bacilli on microscopy) transmit more efficiently than sputum -ve patients.
- **Bacillary load:** At least 10^4 bacilli/mL in sputum is required for effective transmission [adult patients with cavitary lesions in lung have more bacillary load in sputum]
- overcrowding in poorly ventilated rooms
- Following infection, only a minor proportion of people develop progressive disease due to presence of endogenous risk factors:
 - low cell-mediated immunity (immunocompromised)
 - Comorbidities (post-silicosis, post-transplantation, DM, IV drug abuse, smoking, CRF)
 - Age: late adolescence, early adulthood, elderly are at greater risk
 - Risk is higher in women at 25-34 years of age while at older ages, men have a greater risk.

Sequence of Pathogenic Events:

[i] Droplet nuclei containing tubercle bacilli are inhaled:

- they are trapped in upper airways & expelled out by ciliary action of mucosal cells

→ only a small fraction of droplets reach the alveoli

[ii] **Adhesion to Macrophages:** Mycobacterial LAM binds to complement receptors & mannose receptors present on surface of macrophages

→ this leads to internalization of bacilli.

[iii] **Phagocytosis by Macrophages:** enhanced by complement (C3b) mediated opsonization of bacilli

[iv] **Survival Inside the Macrophages:** due to bacterial cell wall LAM which impairs phagosome-lysosome fusion by inhibiting increase in intracellular Ca^{2+} & phosphatidylinositol 3-phosphate.

→ If bacilli are successful in inhibiting phagosome-lysosome fusion, then it replicates inside the macrophage → macrophage eventually ruptures → releases its bacillary content which infect other uninfected phagocytes

Host Immune Response:

• Cell-Mediated Immune Response:

→ Macrophages present mycobacterial antigens to T_H cells → activation into T_H1 & T_H2 → T_H1 releases cytokines (IL-2, IFN- γ) → activation of monocytes & macrophages
∴ activation of T_H1 cells leads to 2 kinds of responses — macrophage-activating response & tissue-damaging response; the balance between these 2 determines the outcome of infection

[i] Macrophage-Activating Response:

→ IFN- γ activates resting alveolar macrophages into activated macrophages which are capable of killing & digesting tubercle bacilli.

→ these activated macrophages aggregate around the centre of lesion & form characteristic granuloma called **Tubercle***

* **Tubercle:** essential pathological finding in TB.

- **Hard tubercle:** initially hard, composed of a central zone containing activated macrophages & a peripheral zone of fibroblasts & lymphocytes
- **Soft tubercle:** later, central part of lesion undergoes caseous necrosis
- Growth of M. tuberculosis is inhibited within the necrotic environment because of low oxygen tension & low pH.
- eventually the lesion heals & calcifies
- the viable bacilli may remain dormant within the macrophages/necrotic material for many years without causing tissue destruction.

[ii] **Tissue-Damaging Response:** when macrophage-activity response is weak & the bacilli are more virulent.

→ mycobacterial growth can be inhibited only by an intensified delayed hypersensitivity reaction [DTH] leading to lung tissue destruction

→ caseous necrosis becomes liquefied & further spreads by 3 ways:

- direct draining into airways
- lymphatic spread & ∴ reseeding into same/opposite lung
- hematogenous spread to other organs.

• **Humoral Immune Response:** ∴ M. tuberculosis is intracellular ∴ humoral immunity plays a minor role
→ the anti-LAM antibodies play a role in preventing dissemination in children

Clinical Manifestations: TB is classified as pulmonary & extra-pulmonary TB.

• **Pulmonary TB (PTB):** 60-90% of all TB cases

→ further classified into primary & post primary/secondary.

• **Extra Pulmonary TB [EPTB]:** results from hematogenous dissemination of tubercle bacilli to various organs in 10-40% of all TB cases.

- **Tuberculous lymphadenitis:** most common form (mainly in children)

→ posterior cervical & supraclavicular lymph nodes

→ painless swelling in the neck region without warmth or colour change

• **Pleural tuberculosis:** presents as pleural effusion

→ tuberculous empyema is less common which develops due to rupture of a cavity into pleural space with spillover of tubercle bacilli which may form a bronchopleural fistula.

• **Tuberculosis of Upper Airways:** larynx, pharynx, epiglottis
→ hoarseness, dysphonia, chronic productive cough.

• **Genitourinary tuberculosis:** - renal tuberculosis

- genital tuberculosis: in females, involvement of fallopian tubes & endometrium causing infertility; in males, epididymis is affected.

• **Skeletal tuberculosis:** weight-bearing joints such as spine [Pott's disease / tuberculous spondylitis], hips, knees are affected

→ Kyphosis & paravertebral cold abscess may form

• **Tuberculosis of CNS:** common in children

→ tuberculous meningitis & tuberculoma.

• **GI tuberculosis:** affects terminal ileum & caecum

→ TB peritonitis may occur

• **Tuberculous pericarditis:** occurs as a direct extension from adjacent lymph nodes or following hematogenous spread

• **Tuberculous skin lesions:**

→ **Sarcoidosis:** tuberculous involvement of skin by direct extension, usually from underlying tuberculous lymphadenitis

→ **Lupus vulgaris:** apple jelly nodules are formed over the face in females

• **Miliary or Disseminated Tuberculosis:** hematogenous spread of tubercle bacilli leading to formation of yellowish 1-2 mm size granulomatous lesions resembling millet seeds in various organs (common in HIV-infected people)

• **Post TB Aspergillosis:** chronic pulmonary aspergillosis may develop due to colonization of Aspergillus fumigatus in the residual TB cavities; presents as respiratory impairment, hemoptysis, weight loss.

• **HIV-associated Tuberculosis:** one of the most common opportunistic diseases among HIV-infected persons due to immunosuppression

→ common presentations: lymphatic, disseminated, pleural TB & meningitis.

Epidemiology: highest burden of TB in southeast Asia, India having the largest share

→ TB is one of the top 10 causes of death worldwide & the leading cause among infectious diseases.

Laboratory Diagnosis:

Specimen Collection: in pulmonary TB, 2 sputum samples — spot & early morning collected 1 day apart (alternatively, collect 2 spot samples 1 hour apart)

• collect gastric aspirate in children

• In EPTB, specimens vary depending on the site involved

• patient should be advised to inhale deeply (2-3 times) & cough out deep from the chest during exhalation & then to spit the sputum into a wide-mouthed screw capped container

• sputum should be at least 3-5 mL in quantity, thick & purulent (yellowish mucus).

Digestion, Decantation & Concentration of Sputum Sample: 2 methods:

- Modified Petroff's method [4% NaOH]
- NALC [N-acetyl-L-cysteine] + 2% NaOH.

Direct Microscopy:

(i) Zn Staining (Hot method): smears are prepared from thick mucopurulent part of sputum or with sediment obtained after concentration.

→ **Negative result**: at least 100 oil-immersion fields (OIF) must be examined for 10-15 minutes before giving negative report

→ **Positive result**: M. tuberculosis appears as long, slender, beaded, less uniformly stained rod-coloured acid-fast bacilli (AFB)

No. of AFB Seen	OIF to be screened	Grading	Result
No AFB	100	0	Negative
1-9/100 OIF	100	Scanty	Positive
10-99/100 OIF	100	1+	Positive
1-10/OIF	50	2+	Positive
>10/OIF	20	3+	Positive

(ii) Kinyoun's Cold Acid-Fast Staining:

→ it differs from ZN stain by that:

- heating is not required
- phenol concentration in carbol fuchsin is increased
- duration of carbol fuchsin staining is more

(iii) Fluorescence (Auramine) Staining: uses

auramine-phenol solution (for 7-10 mins) as primary stain, 0.5% acid alcohol (for 2 min., twice) as decolorizer & 0.1% potassium permanganate (for 30 sec) as counter stain.

→ slide is examined under fluorescent LED microscope

→ bacilli appear brilliant yellow against the dark background.

• **Culture Methods**: Gold standard (more sensitive than microscopy, indicates viability)

→ RNTCP recommended culture media include both conventional & solid media

(Lowenstein-Jensen (LJ) Medium) & automated liquid culture such as Mycobacterium Growth Indicator Tube (MGIT).

Conventional Solid Media (LJ medium):

→ composed of coagulated hen's eggs, mineral salt solution, asparagine & malachite green.

→ incubated for 6-8 weeks because of the slow growing nature of tubercle bacilli (long generation time of 10-15 hours)

→ **Colonies**: M. tuberculosis ⇒ typical rough, tough & buff-coloured colonies

• M. bovis ⇒ smooth, moist & white coloured colonies that break up easily when touched.

Automated Liquid Culture: positive growth gets detected within 3-4 weeks; negative result is reported 6 weeks of incubation

→ use of liquid broth such as Middlebrook 7H9 medium.

• **Serology**: antigen & antibody detection are not recommended because of low sensitivity

• Molecular Methods:

(i) PCR: Nested PCR targeting IS6110 gene, MPT64 gene, 65 kDa & 38 kDa genes

(ii) Automated Real Time PCR:

- Cartridge based Nucleic acid amplification Test [CBNAAT]

(eg: GeneXpert)

- Chip Based real time PCR (eg: Truenat)

→ CBNAAT simultaneously detects MTB complex DNA & rifampicin resistance

(iii) Line Probe Assay: involves probe-based detection of amplified DNA

Diagnosis of Latent TB: by demonstration of delayed or type IV hypersensitivity reaction against the tubercle bacilli antigens

→ 2 methods: ⇒ tubercle skin test (TST)

⇒ IFN- γ release assay

(i) Tuberculin Skin Test [TST]:

→ **Antigens used**: preparation of purified protein derivative [PPD-23]

→ **Dosage**: [Tuberculin unit = TU] 1TU = 0.00002mg of PPD

→ **Procedure**: by method of Mantoux test

- 0.1ml of PPD containing 1TU is injected intradermally into flexor surface of forearm

→ **Reading**: taken after 48-72 hours

→ at the site of inoculation, induration surrounded by erythema is produced; width of induration:

≥ 10 mm | Positive

6-9 mm | Equivocal / doubtful reaction

< 5 mm | Negative reaction

→ Positive TST in adults only indicates present or past exposure with tubercle bacilli but does not confirm the presence of active stage of disease

→ In children, positive test indicates active infection & used as a diagnostic marker.

→ False positive tests after BCG vaccination & NTBM infection

→ False negative tests in case of early or advanced TB, miliary TB

(ii) IFN- γ Release Assay (IGRA): uses highly specific M. tuberculosis antigens such as CFP10 (culture filtrate protein) & ESAT6 (early secreted antigenic target-6) [both coded by RD1 genes]

Drug Susceptibility Testing (DST):

• **Phenotypic methods**: - MGIT

- Proportion method

• **Genotypic methods**: - GeneXpert

- Line probe assay

→ Universal - DST [U-DST] refers to testing all TB patients for resistance to at least rifampicin (by CBNAAT).

Treatment:

First-line drugs: for the treatment of drug susceptible TB (DS-TB)

Second-line drugs: treatment of drug resistant TB (DR-TB)

• **Multidrug Therapy**: combination of more than one drug for rapid & effective killing of tubercle bacilli

• **Short Course Chemotherapy**: lasting for 6 months for DR-TB

• **Two Phase Chemotherapy**: Intensive phase & continuation phase

• **DOTs strategy** [Directly Observed Treatment, Short Course]

• **Treatment should be planned only after the result of DST are available.**

Standard Regimen for DS-TB: 6 month course

→ Intensive phase: 4 drugs HRZE for 2 months

→ Continuation phase: 3 drugs HRE for 4 months.

Regimen for DR-TB: use of higher numbers of 2nd line agents, given for longer duration

→ Clinical follow-up ⇒ at least monthly

→ long term follow-up is carried out upto 2 years of completion of treatment

Newer Anti-Tubercular Drugs: Bedaquiline, delamanid

Bedaquiline: class Diarylquinoline

→ considered as miracle drug for treatment of XDR-TB.

→ acts by inhibiting mycobacterial ATP synthase

→ it is strongly mycobactericidal

→ it is recommended in MDR-TB or XDR-TB cases which are resistant to fluoroquinolones &/or 2nd line injectable drugs

→ contraindicated in pregnancy & age < 18 years.

Delamanid: class Nitroimidazole

→ acts by inhibiting mycolic acid synthesis of bacterial cell wall

→ used in treatment of all DR-TB

→ should not be used in - children < 6 years

- pregnant & breastfeeding women

- prolonged QT interval

- hypersensitivity

Mechanisms of Drug Resistance: resistance in tubercle bacilli is due to point mutation in the genome

→ there is development of drug resistance especially when monotherapy is used; this is effectively checked by multiple drug therapy.

• **Mono resistance**: resistance to 1 first-line ATD

• **Poly resistance**: resistance to > 1 first-line ATD other than isoniazid & rifampicin.

• **Rifampicin Resistance**: resistance to rifampicin with or without resistance to other ATDs.

• **Multidrug Resistance (MDR-TB)**: resistance to both isoniazid & rifampicin with/without resistance to other first-line ATDs.

• **Extensive Drug Resistance (XDR-TB)**: MDR-TB cases which are also resistant to:

- fluoroquinolones &

- at least one second-line injectable agent (amikacin / kanamycin / capreomycin)

• **Pre-XDR-TB**: resistance to either fluoroquinolones or second line injectable (SLI) agents.

Revised National Tuberculosis Control Programme [RNTCP]:

→ launched by the Government of India in collaboration with WHO & World Bank in 1992

→ In 2020, RNTCP was renamed to NTEP (National Tuberculosis Elimination Programme)

Vaccines against TB:

• Bacillus Calmette-Guérin Vaccine (BCG):

→ Danish 1331 strain of BCG is used

Reconstitution of BCG: BCG is available in lyophilized form & has to be reconstituted before administration; done by using normal saline as diluent; distilled water is never used as it is an irritant; once reconstituted, it has to be used within 1 hour

Administration of BCG: 0.1 mL (0.1 mg TV) of BCG vaccine is administered above the insertion of left deltoid intradermally using 26 gauge tuberculin syringe

→ If BCG is properly injected intradermally, then at the inoculation site:

- After 2-3 weeks | papule develops
- 5-6 weeks | shallow ulcer covered with crust
- 6-12 weeks | permanent tiny round scar
- 8-14 weeks | Mantoux test positive

→ Duration of immunity: 15-20 years

Indications of BCG:

- directly given to newborn soon after birth
- if not given at birth, it can be given later, maximum upto 2 years
- after performing tuberculin skin test

Contraindications of BCG:

- HIV positive child
- Child born to AFB positive mother
- Pregnancy
- Generalized eczema

• VPM1002: recombinant BCG vaccine under phase II trial

• Chemoprophylaxis: Isoniazid or ethambutol for 6 months

→ Shortcomings of chemoprophylaxis:

- expensive
- risk of developing tuberculosis is minimal in tuberculin reactors
- side effects of the drugs.

Nontuberculous Mycobacteria (NTMB) Infections:

→ formerly called atypical mycobacteria or mycobacteria other than tubercle bacilli (MOTT)

→ opportunistic pathogens

→ man-to-man transmission not known

Classification of NTM:

→ NTM are classified into 4 groups (based on pigment production & rate of growth)

• Photochromogens: produce pigments only when the colonies are exposed to light

- *M. marinum* - *M. abscessum* - *M. goodii*

• Scotochromogens: produce pigments both in light & dark - *M. chelonae* - *M. fortuitum*

• Non-photochromogens: do not produce pigments

- *M. avium-intracellulare* complex (MAC)

- *M. ulcerans*

• Rapid growers: grow within 1 week

- *M. chelonae* - *M. abscessus*