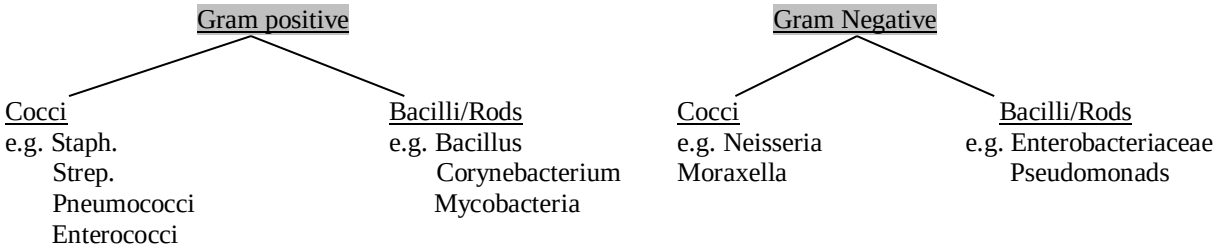
SYSTEMIC BACTERIOLOGY

Bacteria can be broadly divided based on oxygen requirements of different organisms

Classification	Important Genera
Obligate aerobes	Mycobacterium, Pseudomonas Brucella, Bacillus , Nocardia
Microaerophilic	Campylobacter Helicobacter
Facultative anaerobes	Most bacteria
Obligate anaerobes	Non sporing anaerobes Clostridia

AEROBIC BACTERIA



GRAM POSITIVE COCCI .

Can be divided into two types:

Micrococcaceae family-	Streptococcaceae family-
• Catalase positive • GPC cluster/tetrad • Staphylococcus, Micrococcus	Catalase negative • GPC chain/pair • Sterptococcus , Pneumococci, Enterococcus

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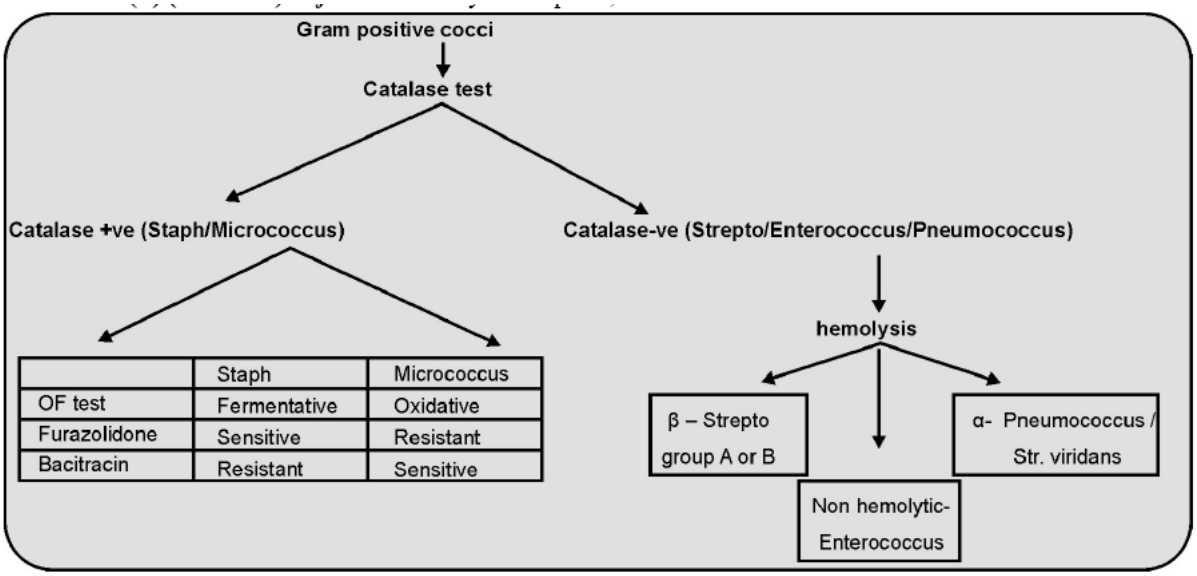


	Staph	Micrococcus
OF test	Fermentative	Oxidative
Furazolidone	Sensitive	Resistant
Bacitracin	Resistant	Sensitive

STAPHYLOCOCCUS

Staphylococcus are differentiated from Micrococcus by

- *Hugh Leifson's oxidative fermentative* test – (Staph. - fermentative, Micrococcus- oxidative)
- *Furazolidone sensitivity* (Staph – sensitive, Micrococcus- Resistant)
- *Bacitracin* sensitivity (Staph – Resistant, Micrococcus- sensitive)

Coagulase positive (Tube & slide)	Coagulase Negative
Staphylococcus aureus	*S. epidermidis*
Staph hyicus & intermedius	*S. saprophyticus*

Antigenic structure &Virulence factors

Cell wall associated Structures	**Activity**
Capsular polysaccharide	Inhibits opsonisation
Peptidoglycan	Confers cell rigidity & induces inflammatory response
Teichoic acid	Helps in adhesion to mucosal surfaces & prevent opsonisation
Clumping factor/ Bound coagulase	Responsible for slide coagulase reaction
Protein A	Binds to Fc of IgG leaving Fab free to bind to Ag - Co agglutination
Extracellular factors	**Activity**
α hemolysin	• Inactivated at 70^0C reactivated **paradoxically** at 100°C • Lyses rabbit > sheep and human red blood cells • Leucocidal, Cytotoxic, dermonecrotic, lethal
β Hemolysin	• Sphingomyelinase • Lyses sheep RBC , but not human or rabbit RBC • Exhibits **hot-cold** phenomenon
γHemolysin	• Bicomponent proteins, Lyses rabbit sheep and human red blood cells
δHemolysin	• Detergent like, Lyses rabbit, sheep and human red blood cells
Leucocidins / Panton valentine toxin	• Two components F & S • Damage PMN & macrophages
Synergohymenotropic toxin	• Bicomponent toxin - γ & Leucocidins- Community acquired MRSA
Epidermolytic toxin (Exfoliative toxin)	• Scalded skin syndrome • Severe- **Ritter** disease (newborn), **TEN** (adult)

	• Milder- Pemphigus neonatorum, bullous impetigo
Enterotoxins	• Produced by **50%** of clinical isolates • Cause food poisoning • IP: **1-6hr** due to preformed toxin • Vomiting due to **Vagus N** & vomiting centre stimulation • **Heat stable** (not destroyed after heating food) • Multiple antigenic type (A-E, G-I, K-M) **(MC- type A)**
Toxic shock syndrome toxin	• Enterotoxins F/pyrogenic exotoxinC **(MC**) > Enterotoxins B,C • a/w use of vaginal tampon • Anti TSST1 Ab is protective • Rash, desquamation & Multi organ failure • T/T- Clindamycin (reduces toxin synthesis)
Extracellular enzymes	**Activity**
Coagulase Enzyme	- Enzyme is secreted into the surrounding - Requires coagulase reacting factor (CRF) present in plasma for action - Responsible for tube coagulase test
Others- Staphylokinase (fibrinolysin) , Hyaluronidase , Deoxyribonuclease , Lipase, Protease	

Superantigens –

- Act on **Vβ** of TCR
- Dont require antigen presentation by macrophage, directly stimulate non specific T cells
- Leads to massive release of cytokines & polyclonal B cell activation
- Example :
    - Staph- TSST, Exfoliative toxin, Enterotoxins.
    - Strept- Str pyrogenic exotoxinA,B,C
    - Mycoplasma arthritidis &Yersinia enterocolitica
    - Virus – EBV,CMV, HIV, Rabies nucleocapsid
    - Fungus- Malassezia furfur

MRSA → Methicillin resistant S*taphylococcus aureus* –

- Resistance is chromosomally mediated (**mec A** gene) that codes for- altered PBP 2a

Penicillin binding Protein (PBP)-

- Normal protein present in all bacterial cell wall.
- Transpeptidase in nature
- Required for peptidoglycan layer cross linking
- Also site for- beta lactam drugs to bind & inhibit
- In MRSA- PBP gets altered to PBP2a (less affinity for beta lactam)

Treatment for MRSA-

- Vancomycin is DOC
- Others- Teichoplanin, linezolid, streptogramin,
- cipro/levofloxacin, cotrimoxazole, clindamycin, mupirocine, minocycline
- Newer – daptomycin, **ceftobiprole**, tigecycline, oritavancin

Type of MRSA-

- Community acquired-(mec A IV)& PV Toxin,
- Hosp acquired -(mecA I,II,III)

Detection of MRSA –

- Using 5µg methicillin or 1µg oxacillin disc or cefoxitin disc
- On media containing 4% nacl
- Incubation at lower temperature of 30^0 , full **24hr** incubation

VRSA- Vancomycin Resistant S*taphylococcus aureus* –

VISA - Vancomycin Intermediate S*taphylococcus aureus* -

- Mechanism – due to increase cellwall thickness
- Treatment – same drugs given for MRSA except Vancomycin & Teichoplanin.

Staphylococcus aureus:

• Coagulase +ve • Heat stable thermonuclease • DNAse	• Phosphatase (also S.epidermidis) • Golden yellow pigmentation • Hemolysis	• Ferment mannitol • Black colony on K tellurite agar • Gelatin liquefy • Protein A

Media

- 5% sheep blood agar – shows pin head shaped colony with narrow zone of β haemolysis
- MacConkey agar – minute pink colonies – means Lactose fermenting
- Selective medium
  - Salt milk agar (8-10% NaCl)
  - Mannitol salt agar-with 7.5% NaCl
  - Ludlam's lithium chloride & tellurite media
- Golden yellow pigmentation (due to carotene)

S.aureus is the MC agent for :

- Skin & soft tissue infection
- Botryomycosis
- Tropical pyomyositis – S.aureus , (acute bacterial myositis – GrA Streptococcus) (Overall - S.aureus)
- Osteomyelitis, septic arthritis
- postoperative parotitis
- Paronychia
- Epidural abscess
- Surgical wound infection

Endocarditis -

- MC cause of Native valve endocarditis – MC : S.aureus
- MC cause of Early prosthetic valve endocarditis(<12m) - S.epidermidis
  late prosthetic valve endocarditis (>12m)- Str Viridans
- Endocarditis in IV drug users – Rt sided – MC S.aureus, Lt sided – MC: Enterococcus > S.aureus
- Subacute endocarditis – MC agent Str Viridans

Points to remember:

- MC method for typing of S.aureus – Phage typing (pattern method)
- Plasmid mediated beta lactam resistance in S.aureus transferred by – Transduction(MC) > conjugation
- MC site of colonization – Skin
- MC way to of hospital spread- Hosp staff to Pt
- MC way to prevent the hospital spread- Hand washing

Coagulase Negative Staph. (CONS) → normal flora of skin

1. S. epidermidis (MC CONS)- produce polysaccharide glycocalyx (slime) (Biofilm production)

- Endocarditis with insertion of valvular **prosthesis**
- Ventricular shunt infections
- Stitch absess

2 .S. saprophyticus - causes UTI in young sexually active female. **novobiocin resistant**

STREPTOCOCCUS

Catalase –ve, gram positive cocci arranged in chains

Classification based on the basis of Hemolysis divided into 3 groups:

- ∝ haemolytic – Viridans group, common in throat.
- β haemolytic – lysis of RBCs due to Streptolysin
  Most of the pathogenic Streptococci fall into the group of β haemolytic
- γ haemolytic – no hemolysis seen, include Enterococci

Lancefield grouping:

On the basis of group specific **carbohydrate antigen** in cell wall β haemolytic streptococci are further divided in to 20 serological types A to V except **I & J**;

Griffith Typing- Strep. Group A is subdivided based on **protein M**

Species	Lancefield	Important Lab Characteristics
S. pyogenes	A	Bacitracin – sensitive PYR test positive
S. agalactiae	B	Bacitracin – resistant Hippurate hydrolysis, CAMP test positive

Media :

- Selective media : Crystal violet- Blood agar & PNF medium (Polymyxin Neomycin Fusidic acid)
- Transport media- Pike's media

Strep. pyogenes (group A)

Structural components of *Strept pyogenes* & Human tissue with which it cross react

Structural components of *Strept pyogenes*	Human tissue
Capsular hyaluronic acid	Synovial fluid
Cell wall protein **M**	**M**yocardium
Cell wall **c**arbohydrate	**C**ardiac valves
Cytoplasmic membrane	Vascular intima

Peptidoglycan	Skin antigens

Virulence factor	Activity
Lipoteichoic acid	• Mediates adherence to fibronectin receptors on epithelial cells
M protein	• Mediates adherence to epithelial cells, inhibits phagocytosis
Hyaluronic acid capsule	• Inhibits phagocytosis
Erythrogenic toxin (SPE)	• 3types (SPE A, B and C). • A&C - bacteriophage coded, B -chromosomal • Superantigens • Scarlet fever • Toxic Shock Syndrome
Streptolysin O	• Produced by group A, C and G streptococci. • **Oxygen labile**, Serum stable • Inactive in oxidized form and reactivated by treatment with reducing agents. • Also Pneumolysin, tetanolysin, perfringolysin • Acts by binding to cholesterol in the cell membrane and producing hole in it. • It is strongly antigenic and rise of **ASO >200u**, indicates recent streptococcal infection
Streptolysin S	• Oxygen stable, **Serum soluble** • Hemolysis on the surface of an aerobic blood agar plate. • Not antigenic.
Streptokinase	• **Fibrinolysin** (activate plasminogen) • Rapid spread of infection by preventing the formation of fibrin barrier. • This property is also used therapeutically in treatment of **coronary thrombosis**.
Deoxyribonuclease	• **Streptodornase** (4 types- A,B,C,D) • **Anti-DNAase B** > 300-350u is useful for the retrospective diagnosis of skin infections(pyoderma) & AGN where ASO is usually low • Liquify thick pus. Applied therapeutically in empyema (Streptodornase& streptokinase)
Hyaluronidase	• M type 4 & 22 (noncapsulated)
Serum opacity factor	• Lipoproteinase enzyme

Pathogenicity →

- Pharyngitis/sore throat – MC infection.
  - A/w lower M types.
  - Complication- otits media, quinsy, Ludwig's angina, meningitis, pneumonia(post viral)
- Scarlet fever & TSS
- Impetigo –Seen in young individual. A/w Higher M types. (frequently nephritogenic strains)
- Erysipelas – Seen in older. Affects superficial lymphatics.
- Puerperal sepsis
- Cellulitis
- **Non suppurative sequelae**
  - ARF (Acute rheumatic fever)
  - Acute glomerulonephritis – can occur either after skin or throat infection. Produced by gp A also group C may be involved; Most cases of AGN occur about one week after group A type 12 infections.
  - Erythema nodosum
  - Reactive arthritis
  - Guttate psoriasis

	Acute rheumatic fever	AGN

Site	Throat	Skin> Throat
Prior sensitization	Essential	Not
Serotype	Any	Pyodermal- 49,53-55, 59-61 Pharyngitis – 1,12
Immune response	Marked	Moderate
Complement level	Unaltered	Low
Genetic susceptibility	Present	No
Repeated attack	Common	Not so
Penicillin prophylaxis	Indicated	Not so
Course	Progressive	Spontaneous resolution
Prognosis	Variable	good

Group B Streptococci

***S. agalactiae* causes**

Early onset & late onset ***neonatal meningitis***

- Early onset – first 5 days, usually due to ascending infection in utero > colonization during birth
- Late onset → after 1 wk, acquires infection from hospital personnel & colonization during birth

Presumptive identification

- CAMP +ve
- Hippurate hydrolysis test +ve
- Bacitracin resistant
- Pigment production

Enterococci → important characteristic

- Growth in presence of **6.5% NaCI , 40% bile, at pH 9.6 and at 45^0C**
- Most strains are **resistant to penicillin**, aminoglycoside & sulfonamides.
- Combination therapy with penicillin & aminoglycoside is effective.
- Disease- UTI, endocarditis, intra abdominal infection, meningitis
- Most commonly associated with human disease *Enterococcus fecalis* & *Enterococcus faecium* (↑ resistant)
- Resistance in Enterococci : Vancomycin resistant– due to Van gene

Viridans strep-

- α hemolytic , mouth commensals, usually sensitive to penicillin
- Disease – *S.mutans* - dental caries, *S sanguis* - endocarditis

S.pneumoniae

α Hemolytic but under anaerobic condition – beβ hemolytic

	S pneumoniae	***S viridians***
Morphology	Lanceolate	Round / oval
Arrangement	Pairs	Chains
Capsule	+	-
B/A	Draughtsman	Convex
Liquid Medium	Uniform turbidity	Granular turbidity
Bile solubility	+	-
Inulin fermentation	+	-
Optochin	S	R
Animal Pathogenicity	Pathogenic	Non-pathogenic

Antigenic structure

- Capsular Ag – 90 types, SSS, typing by Quellung reaction
- Carbohydrate Ag – CRP precipitates with this Ag.
- Pneumolysin

- Autolysin – amidase – cleaves PG layer- leads to draughtsman shape colony & bile solubility

Pathogenicity

- Commensal in nasopharynx– 5%(adult), 20%(children)- in throat
- Pneumonia – In adults – type 1 – 8 (most virulent type-**3**), In Children – 6,14,19,23
- Meningitis
- Others – otitis media, sinusitis, peritonitis etc.

- *Penicillin resistance - due to altered PBP 2b(chromosomal)*

Vaccine

1. ***Polysaccharide vaccine*** **(23 valent) –**

- Not useful for <2yr as T independent
- C/I- lymph reticular malignancy, pregnancy
- Indication- spleenectomy, sickle cell patient, DM, chronic heart/lung/renal/liver disease

*2.****Conjugated vaccine*** (7 valent)- can be given <2yr

GRAM NEGATIVE COCCI

N meningitidis, N.gonorrhoeae, Moraxella, Veillonella

NEISSERIA

- Catalase & oxidase +ve Diplococci

N. meningitidis	***N. gonorrhoeae***
Capsulated	Non- Capsulated
Lens shaped (Diplococci with adjacent sides flattened)	Kidney shaped
Ferment Glucose and Maltose	Glucose only fermented
Rarely have plasmids	Usually possess plasmid & more drug resistant
Extra or intracellular	Mostly intracellular

N. meningitidis (Meningococcus)

Antigenic Determinants & Virulence factors

- *Capsular polysaccharide* → inhibits phagocytosis, are of 13 serogroups
  - Most meningococcal disease → A B, C , Y,29E, W 135
  - Group A- epidemics, C- sporadic, B-both
  - Serogroup further classified based on OMP
- *Endotoxin& LOS(Lipo oligo saccharide)* → produce Water House – Friderichsen Syndrome
- *IgA 1 protease* → IgA1 protease cleaves subclass IgA 1 & inactivates it.
- *Pili* → allow to adhere & antiphagocytic
- OMP - Outer Membrane Protein

Pathogenicity

- MC source – human carriers
- Nasopharyngeal carrier- children mainly - 5-10%(inter epidemic), 20-90%(during epidemic)
- MC Route- hematogenous > olfactory N > conjunctiva
- Deficiency of terminal complement components (C5- C9) → increase risk of meningococcal infection
- Outbreak in Hajji pilgrims in 1990 – due to group A, W135
- High prevalence – sub-Saharan belt of Africa
- Seasonal variation- MC in winter spring

Clinical feature:

- Fulminant meningococcemia – septic shock, DIC, rashes & Water House – Friderichsen Syndrome (DIC induced micro thrombi, hemorrhage, B/L adrenal hemorrhage)
- Meningitis- common in 3-5 yr age

Diagnosis

- Culture media – Chocolate agar, Muller Hilton agar, Thayer martin media - 35c at 5-10% CO2
- Sample – CSF, Blood and nasopharyngeal swab (for carriers)
- LDH & neuraminidase in CSF
- Antibody – retrospective evidence only

Treatment

- Cefotaxime & ceftriaxone → DOC
- Rifampicin or ciprofloxacin – for carriers & prophylaxis

Vaccine

- Polyvalent vaccine containing → A,C,Y ,W-135 immunity lasts for 3 years
- No vaccine for Gp B: as its capsule is poorly immunogenic & sialic acid residue in capsule cross react with human brain tissue (fatal autoimmune consequences)
- Not useful – below 3 yr (capsule is poorly immunogenic <3 yr as it is T independent Ag)
- C/I- pregnancy

N. gonorrhea

- Based on **Pili** divided to - 4 types (T_1-T_4)
- Auxotyping is done
- IP- 2-8days

Antigenic structure

- Pilli -imp role in attachment
- Outer membrane protein – protein I, II, & III
- IgA 1 protease – also present in Meningococcus, Pneumo, H.influenzae)
- LOS → Toxicity is due to endotoxic effect.
- Transferrin & lactoferrin binding protein

Clinical feature:

- Babies born to infected mother – causes ophthalmia neonatorum (in first 2 days of life, 30%), Ophthalmia neonatorum is also caused by Chlamydia trachomatis(after 1 week)
- MC of PID worldwide (India – TB)
- Male- MC is urethritis (doesn't involve testes)
- Female- cervicitis(adult vagina is resistant to Gonococcus, so Less severe in female)
- DGI(deep gonococcal Infection)- mainly arthritis- MC a/w Por A1 & AHU auxotype
- Complication – water can perineum, Fitz Hugh Curtis syndrome (perihepatitis), metastatic complications(Endocarditis, arthritis, nephritis)
- MC seen in people with Blood *group **B***

Lab diagnosis-

Sample –

- Urethral discharge,
- Endocervical swab (high vaginal swab not recommended),
- Rectal swab(if h/o anal sex)

Gram staining-

- Urethral discharge demonstrating intracellular diplococci – *for males* 90% sensitive
- But for females it is only 50-60% Sn due to presence of normal flora confounding interpretation.
- So, Endocervical culture is recommended– 90% Sn

Selective media: Difficult to grow than meningococci

- Thayer Martin (VCN)
- Modified Thayer Martin (VCNT)
- Modified New York city medium

Transport media-

- Charcoal impregnated swabs/ medium (stuart / Amies media)

Serology- CFT – limited success

Treatment - DOC-

- Cefotaxime & ceftriaxone
- Mostly resistant to Penicillin due to Penicillinase production

GRAM POSITIVE BACILLI

AEROBIC GPB are 2 types

1) Spore forming – *Bacillus, Clostridium*
2) Non spore forming – *Corynebacterium, Listeria, Erysipelothrix, Mycobacterium, Non sporing anaerobe-Actinomycetes, Propionobacterium, Bifidobacterium, Lactobacillus*

CORYNEBACTERIUM

- *C. diphtheriae* – Kleb Loeffler bacillus
- Club shaped, Chinese letter & cuneiform arrangement
- Possess metachromatic granules (Inorganic polyphosphates) -Babe Earnst or polar body
- Special stain- Albert , Ponder, Neisser stain

Metachromatic Granule Produced By
C.diphtheriae, C.xerosis
Gardenella vaginalis
Spirillium
Mycobacterium spp
Enterobacter aerogene
yeast

On the basis of morphology on tellurite medium *C. diphtheriae* has three biotypes

McLeod classification	Gravis	Intermedius	Mitis
Colonies on PTA	daisy head	Frogs eggs colony	poached egg
Fermentation of starch	+ve	-ve	-ve
Toxigenic strains	100%	95-99%	80-85%
	Epidemic	Epidemic	Endemic
complication	Paralytic& Hemorrhagic	Hemorrhagic	Obstructive
Hemolysis	Variable	non Hemolytic	Hemolytic
Morphology	Short, no granules	Long barred, poor granules	Long curved, prominent granules

Culture →

1. ***Loffler's serum slope (Enriched medium)→***
   a) Earliest growth (6-8hrs)
   b) Granules best developed
   c) Water of condensation can be used for animal pathogenicity
2. ***Selective medium***
   a) Potassium tellurite (0.04%) –black colonies in 2days due to tellurite reduction.
   b) Tinsdales, Hoyles medium, McLeod's medium

Clinical diphtheria *– caused by 3 species*

- All produce Diphtheria toxin & black colony on PTA
- *C. diphtheriae* (with four biotypes),
- *C. ulcerans &*
- *C. pseudotuberculosis* –

C. diphtheriae – (urease negative) but *C. ulcerans* & *C. pseudotuberculosis* – (urease positive)

Diphtheria toxin :

- Bacilli is non invasive but **DT** released locally & absorbed in circulation and produce systemic manifestation So diphtheria is toxemia but not bacteremia
- Skin /local lesions - by non Toxigenic strain strains, so vaccine has no role
- **Toxin has 2 fragments-**
  - A fragment → A ADP ribosylation of elongation factor2 → inhibit EF2 → inhibits protein synthesis
  - B Fragment – for transportation of fragment A inside cell
- Toxin expression- Depends on iron concentration (0.1mg per litre optimum)
- DT is coded by bacteriophage- β **phage (tox phage)**
- Toxoid produced – by formalin, acidic pH, prolonged storage
- 1 unit if antitoxin – neutralizes 100 MLD of toxin
- **Park William 8** strain of *C. diphtheriae* is used as a source of toxin for diphtheria toxoid (DPT vaccine)

	Vaccine	**Antibiotic**
Carrier	Not affective	Affective
Treatment of Diphtheria	Immunoglobulins are Affective	Not affective(Except early stage)
Cutaneous Diphtheria	Not affective	Affective

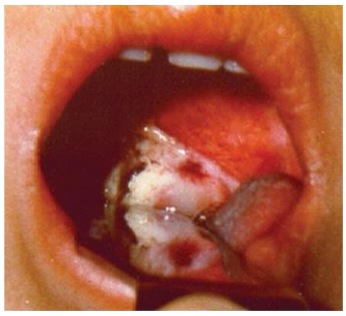
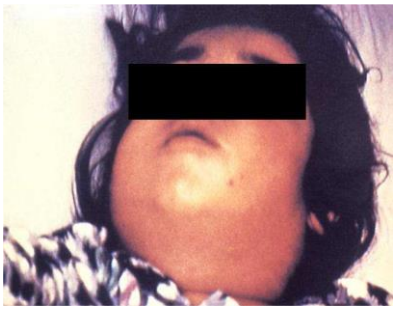
Shick test –

- Susceptibility test before starting immunization (now obsolete)
- Toxin given I/d on test forearm while the other forearm acts as control injected with heat inactivated toxin

Shick test	**Result**	**Interpretation**
Negative	No reaction in both arm	Immune - No Immunization Reqd
Positive	Erythema & indurations on test arm,↑ size by 7thday No reaction in control arm	Non Immune/susceptible- needs immunization
Pseudo	Erythema & indurations on test and control arm but disappears 5th day	Hypersensitive - No Immunization Reqd
Combined	Erythema & indurations on test and control arm but disappears only from control arm by 5th day	Non Immune & Hypersensitive - needs immunization

Pathogenicity :

- Natural infection only in man.
- Pseudo membrane formation on tonsil, posterior pharyngeal wall.
- Source- carriers (Nasal carries are particularly dangerous - shed large no. of bacilli)
- Faucial diphtheria– MC, laryngeal – dangerous, mandates tracheostomy
- 1st muscle involved – palatopharynges
- Ciliary but not papillary paralysis occurs – So blurred vision with intact light reflex
- Neurological complication- occurs in 1st / 2nd week
- Systemic- myocarditis, peripheral neuropathy
- Cardiac damage is permanent but Nerve recovery usually occurs
- Death- due to circulatory failure

Diagnosis –

- Usually clinical, not to wait for lab confirmation
- Gram staining of throat swab & culture followed by toxigenicity testing

Toxigenicity or Virulence test of C. diphtheria

- In vivo tests- Subcutaneous test & Intra dermal, **Guinea pig** or rabbit used, mice is resistant to diphtheria
- In vitro tests: Elek gel precipitation test, PCR detecting Tox gene, ELISA/ICT detecting toxin

Treatment

- *C. diphtheriae* sensitive to penicillin, Erythromycin, Rifampicin; but antibiotics do not neutralize circulating toxin. So, antibiotics + antitoxin given
- Erythromycin active for treatment of carriers

Prevention:

- Vaccine- active immunization by toxoid – to prevent diphtheria, but can't prevent carrier stage

DPT- DT, TT& pertussis (whole cell or acellular component)

Diphtheria toxoid – 2 types. Fluid toxoid(formalinized) and
Adsorbed toxoid- more immunogenic (aluminum phosphate > hydroxide)

- Both whole cell killed B.pertussis and acelluar pertussis component increases potency of DT(adjuvant)
- Whole cell killed B.pertussis is encephalogenic & short lasting immunity while acellual pertussis component (DTaP) is devoid of neurological complication.
- Acellual pertussis component consists of pertussis toxin, agglutinogen, fimbrial Ag.
- Quadruple vaccine – (DPT+ Hib) – DT & TT increases immunogenicity of Hib.

Non Diphtheria Corynebacterium

- *C. ulcerans* – mimic respiratory diphtheria, transmitted by cow milk
- *C. pseudotuberculosis (Preisz Nocard bacilli)* – affect sheep & horse
- *C . minutissimum (causes erythrasma)*
- *C. jeikeium*- multidrug resistant
- *C.parvum* – immunomodulator

BACILLUS

- Aerobic gram positive spore forming bacilli
- In human and animals → 2 species

B.ANTHRACIS

- *B.anthracis* – highly pathogenic, non motile, zoonotic disease
- All are motile except- B.anthracis
- *All Noncapsulated except*- B.anthracis

- Spores found in soil or in culture and never in animal body
- Agent of Bioterrorism → recently used by Afghanistan (spores enclosed in paper were mailed)
- Ascoli thermo precipitin test – to demonstrate anthrax Ag

Anthrax Vs Anthracoid bacilli-

- MC fadyean reaction – polypeptide capsule seen when stained with polychrome methylene blue
- All are motile except- B.anthracis
- Grams: bamboo sticks appearance, chain of bacilli
- On Agar plate- medusa head appearance colony (under low power microscope)
- Gelatin stab: inverted fir tree appearance
- Solid media with penicillin – String of pearl appearance
- Non –Hemolytic colony on blood agar
- Selective medium – PLET media

Virulence factor-

- **Anthrax toxin –** 3 fraction
  - *Edema factor* -↑cAMP,
  - *Protective factor*-binding(Ab is protective),
  - *Lethal factor*
  - Alum precipitated toxoid vaccine(containing protective antigen)
- **Capsule**-
  - Made up polyglutamate-
  - Plasmid mediated,
  - Live attenuated spore vaccine (Sterne, Mazucchi)

Anthrax: It is a zoonotic disease (cattle and sheep)
Man acquires infection through:–

- Cutaneous type- by small cuts or skin abrasion , use of shaving razors used for animal, insect bite
- Pulmonary type- by spore inhalation
- Intestinal type- by ingestion of meat (rarely)

Types of anthrax

Cutaneous anthrax	**Pulmonary anthrax**
Hide porter's disease	Wool sorter's disease
Cutaneous exposure to animal	Inhalation of spores
Malignant pustule (black eschar surrounded by non pitting edema)	hemorrhagic mediastinitis
Dock worker, butcher, abattoir, farmer	Wool factory
Most Common (95%)	Rare

Bacillus cereus

- Motile, lacks capsule, causes food poisoning
- Can also cause opportunistic infections like endocarditis, bacteremia, LRTI, meningitis
- Presence of B.cereus atleast $>10^5$ / gm of stool- considered diagnostic.
- Selective media- **MYPA**(mannitol, egg yolk, phenol red polymyxin agar)

B.cereus	**Diarrheal type**	**Emetic type**
IP	8-16 hr	1-5 hr (preformed toxin)
Food	Cooked meat/vegetable	Rice(Chinese fried rice)
Toxin	Resemble heat labile toxin of E.coli	Resemble heat stable toxin of E.coli
Clinical feature	Diarrhea, abdominal cramps , rarely nausea	Vomiting, abdominal cramps
Serotype	2,6,8,9,10,12	1,3,5

CLOSTRIDIUM

Gram Positive, spore forming, Anaerobes.

- All motile except- *Cl.tetaniVI & Cl perfringens*
- *All Noncapsulated except- Cl perfringens*
- Spore – Subterminal except
    - Spherical & terminal Drum Stick Appearance - *Cl tetani*
    - Oval & terminal – tennis racket shaped – *Cl. tertium*
    - *Central- C.bifermantans*
- Noncapsulated(except *Cl.perfringes*)
- Motility – stately
- Found normally in feces- Cl.perfringes, Cl. difficle

Cl. perfringens

- Non Motile, Spores oval, subterminal
- Toxigenic & invasive
- 4 Major (Lethal) Toxins - α,β, ε, i → 5 types (A-E)
- BA → Target Haemolysis
- Litmus Milk reaction (+)
- Naegler reaction (+) d/t θ toxin → opalescence on egg yolk media (inhibited) by antisera

Infections:

1. **Gas gangrene** –
    - Established causes - Cl. Perfringens type-A, Cl.novyi, Cl.septicum
    - No PMN in spite of extensive tissue damage
    - IP short for Cl. Perfringens
    - Pain & crepitus- characteristic
    - Spores- not produced in tissue/media
    - Gram staining-
        - GPB without spore- Cl. Perfringens
        - Citron body& boat shaped GPB)- Cl.septicum
        - GPB with oval sub terminal spore- Cl.novyi
    - Treatment-
        - Surgery,
        - DOC-Pn+clindamycin,
        - Hyperbaric O2 ,
        - Anti gas gangrene serum

2. Food Poison – Type A → Enterotoxin (Similar to cholera & EPEC)
3. Necrotising enteritis (Pigbel): d/t β toxin of type C

Cl. tetani –

Motile, swarming

Two toxins : -

- Tetanospasmin – Blocks release of inhibitory transmitter glycine and GABA→ spastic paralysis
- Telanolysin

Tetanus-

- 1st symptom-↑masseter tone(trismus/lock jaw) then→ descending tetanus
- Hands feet are spared
- Mentation unimpaired, deep tendon reflex↑
- Shorter IP→ graver the prognosis
- Noninfectious- no person to person spread
- Diagnosis is always clinical, microscopy is unreliable

Treatment: all type of wounds need surgical toilet followed by:

Immunity Category	Wound <6hr, clean , non-penetrating, no/negligible tissue damage	Other wounds
A	Nothing required	Nothing required
B	Toxoid 1 dose	Toxoid 1 dose
C	Toxoid 1 dose	Toxoid 1 dose + HTIG
D	Toxoid complete dose	Toxoid complete dose+ HTIG

A- Taken complete course of TT/booster with in past 5 yr
B- Taken complete course of TT/booster with in past > 5 yr- <10yr
C- Taken complete course of TT/booster with in past >10 yr
D- Not Taken complete course of TT/booster or immunity status not known

Antibiotic-

- To eradicate source of toxin- penicillin/metronidazole/Clindamycin/erythromycin
- No role after 6hr

Prevention:

- TT-3 doses at 1-2m interval followed by 2 boosters after 16-24months & 5yr then 2 doses of TT at 10 & 16 yr
- Others- DPT, HTIG, Combined immunization

Clostridium botulinum

- Botulinum toxin → Most toxic known to man (MLD → 1 μg)
- BT- Blocks release of acetylcholine→ flaccid paralysis
- 8 types (A-G), A,B & E → Human disease
- All except C2 are-neurotoxin, C2-enterotoxin
- Type C,D- bacteriophage coded
- Toxin used for t/t of strabismus, blepharospasm
- Symptoms- **5D-**
    - Diplopia,
    - Dysphasia,
    - Dysarthria,
    - Constipation,
    - Descending paralysis (symmetric)
    - ↓DTR

Types:

- Food borne botulism → Preformed toxin of Canned food
- Wound botulism
- Infant botulism (spore ingestion) - Floppy Child syndrome
  d/t ingestion of spores, toxin released inside, ***source-honey***

Cl. difficile

- Causes Pseudo membranous Colitis after antibiotics like Clindamycin, Ampicillin
- Rx –Metronidazole> Vancomycin
- Toxins: A→Enterotoxin B→ Cytotoxin
- Frank blood in stool is uncommon
- Culture is more sensitive but less specific
- More specific- Toxin démonstration, colonoscopy

LISTERIA MONOCYTOGENES

- Gram +ve coccobacilli
- Tumbling motility at 25c but nonmotile at 37c
- Growth improves if cultured in thioglycollate broth at **4c (cold enrichment)**

- It can grow in refrigerated food & can tolerate preserving agents

Pathogenicity

Enteric route→ bacterial internalin attaches to epithelial cell cadherin receptor → phagocytosed by epithelial cell → phagolysozome lysed by **listeriolysin**→ Listeria escapes to cytoplasm induces host cell **actin** polymerization→ Listeria propels to cell membrane → produces **listeriopods** in cell membrane through which it migrates to adjacent epithelial cell / macrophages

Bacteria uses host cell actin to spread - Listeria, Shigella, Rickettsia

Infections

Pregnancy & Neonatal infections – Before 20 wks is rare. May lead to abortion, still birth,

neonatal disease – Early onset & Late onset meningitis

Lab diagnosis →

- Media- Blood agar, chocolate agar, **PALCAM** agar
- Anton test – instillation to rabbit eye causes conjuntivitis

Treatment-

- DOC- Ampicillin (also penicillin)
- Alternate- cotrimoxazole(if allergic to penicillin)
- Cephalosporin- not affective

Erysipelothrix rhusiopathiae

- Gram+ve bacilli, causes erysipeloid skin lesion

ACTINOMYCETES

- Gram +ve filamentous bacteria
- Human pathogenic actinomycetes belong to four genes
- *Actinomyces, Streptomyces, Nocardia , Actinomadura*

Actinomyces	**Nocardia**
Non Acid fast	Partially acid fast
Anaerobe	Obligate aerobe
Found as Endogenous flora- mouth	Usual habitat- Soil, infections are exogenous
Disease occur in immunocompetent host also	Usually affects people with low immunity
Disease- Cevicofacial osteomyelitis, Lumpy jaw in cattle	**Disease**- Pulmonary, CNS, Mycetoma
DOC- Penicillin	DOC- sulfonamide and cotrimoxazole
Sulfur granules- Organism	Paraffin bait technique- for isolation

MYCOBACTERIUM

Acid fast organism

Acid fastness – due to mycolic acid & integrity of cellwall

Examples-

- *Mycobacteria* - TB/leprae/NTM
- *Nocardia*
- *Rhodococcus*
- *Spore*
- *Sperm head*

- *Legionella mecdedei*
- *Parasite – Cryptosporidium, cyclospora. Isospora, Tinea scolex*

Mycobacterium – Name was derived from their branching filamentous form & mould like pellicle in liquid media

M tuberculosis complex – include species

- *M. tuberculosis*
- *M. bovis* (bovine tubercle bacillus)
- *M. africanum* (intermediate between M tuberculosis & M bovis)
- *M. microti* (vole tubercle bacillus)
- Generation time – 20 hours
- Incubation – 6-8 week

M tuberculosis	M bovis
Shape- Curved,long,beaded,less uniformly stained	Straight, short, stout, uniformly stained
Rough, tough, buff colony	White , smooth, moist colony
Eugonic growth	Dysgonic growth
Niacin & nitrate +ve, Neutral red (+)	Niacin, Nitrate, NR (-)
Pathogenic to G. pig , but **Not to rabbit**	Pathogenic to both
Obligate aerobe	Microaerophilic
Both are equally pathogenic to human	

Pathogenicity

- Escape killing by macrophage by inhibiting phagolysozome fusion & HSN IV
- Virulence factor- cord factor, lipoarabinomannan, HSP

Clinical feature

Pulmonary TB-

- **Primary TB –**
  - Affect children,
  - Sub pleural focus in lower lobe of lungs (Ghon's focus)+ hilar LN↑= **primary complex**
  - Ghon focus + Fibrosis & calcification- **Ranke** complex
- **Post primary TB-**
  - Affect Adult,
  - upper lobe focus (**Simons** focus),
  - LN rare, necrosis, cavitations & hematogenous spread
  - Infra clavicular lesion- **Assman** focus

Extrapulmonary TB-

- MC site LN (cervical)>pleural TB
- Skeletal TB- MC Site is spine
- TB meningitis
- GI TB- MC site caecum, ileum
- TB pericarditis- MC cause of chr constrictive pericarditis

- Scrofuloderma- skin lesion d/t breakdown of underlying TB foci (usually lymph node)
- Lupus vulgaris- MC TB skin lesion, Female, face & neck, apple jelly nodule, ↑malignant(Sq cell Ca)

Lab methods

Digestion & Decontamination procedures for sputum -
- Petroff's method (4%Na OH)
- Others- N acetyl cysteine, Oxalic acid

AFB smear –
Ziehl Neelsen staining- detection limit 10^4 bacilli / ml of sputum
- RNTCP recommended
- Used for monitoring T/T, assessing the severity and infectiousness of the patients
- Two sputum samples are collected- early morning & spot samples

Fluorescent staining- Auramine /rhodamine- Used for rapid screening

Culture- detection limit 10 to 100 viable organism
SOLID MEDIA
(A) Egg Based- **Lowenstein Jensen** , Petragnani, Dorset
(B) Blood Based Media – e.g. Tarshis
(C) Agar Based Media –transparent; colonies observed earlier- Middle brook 7H11& 7H10
→ 7H11 is preferred over 7H10 – improves the recovery of INH resistant strains of M. tuberculosis
LIQUID MEDIA – Middle brook 7H9, Dubos ,Proskauer, Sula, Sauton – Used for drug susceptibility, Ag & vaccine

Automated culture method- detects growth faster
- BATEC
- BacT/ALERT

PCR → detecting IS6110 gene

Serology → Not much useful

Diagnosis of latent TB

1)Tuberculin test –
- Mantoux test - 0.1ml PPD(5TU) given ID-
- Others- Heaf test(multiple puncture), Tine test – disposable prong with dries PPD
- Induration : >10mm – always +ve, 6-9mm- equivocal (BCG/NTM), <5mm- always-ve
- Indicator of – 1.Active infection in infants, 2. Prevalence of infection, 3. Past exposure in adult,
- False –ve – early/adv. TB, miliary TB, measles , ↓ immunity
- False + → BCG vaccination, atypical Mycobacterial infection
- Repeated test at diff site – false-ve/equivocal reaction may turn +ve

2)**Interferon gamma assay**

Vaccine –
BCG → Bacilli Calmette Guerin
- 0-80% efficacy, immunity last for 10-15yr
- BCG strain- M bovis attenuated in glycerol bile potato medium
- Indication- newborn soon after birth, no need after 2yr
- C/I- infect with active HIV, AFB+ve mother, ↓Immunity, Generalized eczema

Sensitivity Testing
- Resistance Ratio
- Absolute concentration
- Proportion method – RNTCP recommended
- Radio metric method → based on proportion method
- Molecular methods

Drug Resistance- due to mutation

MDRTB- R/ INH& Rifampicin +/- R/other 1st line drug
XDRTB- MDRTB + R/quinolone+ R/aminoglycoside (amikacin/capreomycin/kanamycin)

Neonatal TB

- Before delivery – If mother chest X-ray & sputum AFB +ve – mother given ATT (HRE)
- After delivery –
  - **If mother chest X-ray & sputum AFB +ve then, mother to be given ATT(HRE) + baby (INH for 9-12m) + screening of household contacts**
  - Separation from mother or with hold of breast feeding is not recommended.

NTM/MOTT/ Atypical Mycobacteria

Runyon group		SPECIES
I Photo chromogen	Pigmentation only in light	*M kansasii, M marinum, M simiae, M.asciaticum* ***(MASK)***
II Scoto chromogen	Pigmentations only in light & dark	***SSG****-M. scrofulaceum, M szulgai , S.gordonae*
III Non-chromogen	No Pigmentation	*MAC, M xenopi M. ulcerans*
IV Rapid growers	Grows within a week	*M chelonei, M fortuitum*

Diseases caused by Atypical Mycobacterium

- **Post trauma injection abscess - *M chelonei , M fortuitum***
- **Swimming pool granuloma - *M marinum***
- **Buruli ulcer - *M. ulcerans***
- Mycobacteria Causing Johnes Disease -*M. paratuberculosis*
- Lymphadenopathy -, *M. scrofulaceum*
- *M avium intracellulare*- Pulmonary disease Disseminated disease
- *M kansasii- causes pulmonary disease like TB*

MYCOBACTERIUM LEPRAE

- Non cultivable on artificial medium,
- Generation time- 20days
- Grows well in cooler part of body(skin, testes, peripheral nerve, anterior eye)
- 5% H_2 SO_4 Acid Fast
- Intracellular, arranged as **cigar** like bundles of bacilli bound with lipid like glia (**globi**) present inside foamy macrophage(**Virchow's leprae cell**)
- Bacteriological index – total no of leprae bacilli
- Morphological index - The % of solid uniformly stained live bacilli in tissues
  MI is more meaningful for assessing the progress of patients on chemotherapy

Epidemiology:

- Transmission- nasal secretion
- IP- 2-5year
- Not highly communicable, intimate & prolong contact necessary
- Most affected area- Southeast Asia& Brazil- In India(MC-Bihar>Orissa>UP)

Animal model-

- foot pad of mouse(Shepard model)
- Nine banded armadillo is highly susceptible to leprosy, due to low body temperature.

Antigenic structure

(1) LAM-B (Lipoarabinomannan B) is highly immunogenic & used in serodiagnosis of leprosy.
(2) PGL – 1 (phenolic glycolipid 1)

Classification

- Ridley Jopling classification→ TT, BT, BB, BL, LL
- Madrid classification – LL, TT, borderline/dimorphous, indeterminate (early unstable type)
- Indian classification – Madrid + pure neuritic type

Lepromatous	Tuberculoid
AFB + ++ multibacillary	+/-
Skin lesion many, poorly marginated, multiple infiltrative nodules, Leonine facies	Few, sharply demarcated, symmetric
Nerve lesion- late	Early anesthetic skin lesion
Lepromin test -	+ve
Humoral- Auto Abs +	-
CMI low	normal
Biological false +ve VDRL test in syphilis	-
Involve any organ – except CNS & lungs, also warm area of skin(axilla, groin, scalp)	MC nerve involved- Ulnar >post auricular Medial popliteal N- never involved

Lepra Reaction Type I-

- HSN IV,
- a/w borderline
- If occurs before t/t – down grading reaction occurs towards LL
- If occurs after t/t – upgrading reaction occurs towards TT
- MC feature- edema
- ↑ Tcell (γδ TCR)
- MC nerve- Ulnar N
- t/t- DOC- glucocorticoid

TypeII reaction- (ENL- Erythema Nodosum Leprosum)

- HSNIII,
- a/w BL,LL
- Usually follows sulfonamide therapy, but may precede t/t
- MC- crop of painful erythematous papule
- Central cytokine involves- TNFα
- T/T- DOC- thalidomide & glucocorticoid, clofazemine & antipyretics

Anti leprosy Vaccines

- BCG
- Armadillo-derived killed *M leprae*
- BCG & Killed *M leprae*
- ICRC bacillus

Lepromin reaction →

- 0.1ml Lepromin given i/d
- Early/Fernandez **reaction** - 2-3days- Induration like tuberculin (DTH)- indicates Inf. in past(not useful)
- Late/Mitsuda **reaction** - 3-4 weeks → nodule, necrosis, ulcer
    - Measure of CMI induced by injected Lepromin (doesn't say about the past exposure not used in diagnosis))
    - Uses – 1.classify lesions of leprosy, 2. Assess prognosis, 3.Assess resistance to leprosy in individuals

Lab diagnosis:

- Sample- minimum 4 skin (slit skin)+ ear lobule+ nasal mucosa – grading of smear is done based on MI.
- Acid fast staining with 5% sulfuric acid

- Mouse food pad inoculation
- Ab to PGL1 (phenolic glycolipids)

Treatment-

- Paucibacillary- (I ,TT, BT)- Rifampicin(monthly)+ dapsone daily – for 6months
- Multibacillary- (BB,BL, LL)- Rifampicin(monthly)+ dapsone daily +clofazemine daily –till 2years or smear -ve
- Single lesion – (ROM)- rifampicin+ ofloxacin+ minocycline – single dose

GRAM NEGATIVE BACILLI

ENTEROBACTERIACEAE

Family character:

- Gram –ve bacilli
- Non fastidious
- Glucose fermenting,
- Catalase +ve, (exception is Shigella dysenteriae Ty I)
- Oxidase negative
- Reduce Nitrate
- Motile (exception *Shigella, Klebsiella*)
- Aerobic or facultative anaerobe

Classification: based on Lactose fermentation (pink colony on MacConkey)

- Lactose fermenter – Escherichia, Klebsiella
- Non Lactose fermenter- Shigella, Salmonella, Proteus, Yesinia
- Late Lactose fermenter – Shigella sonnei

ESCHERICHIA COLI

- Motile by Peritrichous flagella,
- 3 antigen- O Ag, H Ag, K Ag
- 3 Toxin- LT,ST, VT

LT: (heat labile)	ST(heat stable)	VT
Resembles cholera toxin in structure- A-↑ cAMP, B-binds GM1 ganglioside	↑ cGMP	Inhibit ribosome & protein synthesis
Plasmid coded	Plasmid coded	Phage coded

CLINICAL INFECTIONS :

UTI:-

- MC cause of UTI- E.coli
- "Early" O groups 1, 2, 4, 6, 7, 18,75
- Cystitis & lower UTI- mostly due to ascending infection, lack K Ag
- Pyelonephritis & upper UTI- mostly due to descending infection, possess K antigen
- Virulence factor- Hemolysin, P fimbriae, mannose resistant fimbriae
- Kass' concept of "significant Bacteriuria - $>10^5$ /ml of urine"

Diarrhoea:-

1)EPEC

- Diarrhea in infants and children, sporadic diarrhea in adults
- Nontoxigenic, non invasive
- Adhere to intestinal mucosa & disrupt brush border–attaching effacing lesions **(A/E lesions)**

2) ETEC

- Acute watery diarrhea in infants and adults: ***traveler's diarrhea***
- Pathogenesis: LT, ST (plasmid mediated) , Fimbrial proteins (Colonization Factor Ag), noninvasive
- Diagnosis –Typing, Demonstration of toxins

3) EIEC

- Dysentery like disease in all ages
- Pathogenesis: Epithelial cell invasion by ***virulence marker Ag(VMA)***
- Called atypical E coli(lactose Nonfermenter), resembles Shigella flexneri
- Diagnosis - HeLa & Hep2 cell invasion assay , Sereny's test

4) EHEC (VTEC)

- ***O157:H7*** & Bloody diarrhea in all ages
- Pathogenesis- VT inhibits protein synthesis,
- Complications- Capillary microangiopathy , Causes **HUS & HC**
- Diagnosis: Sorbitol MacConkey agar

5) EAEC

- Persistent diarrhea especially in developing countries
- Most are "O" un-type able but "H" type able
- Aggregated in a "Stacked Brick" formation on Hep2 cell lines
- EAST 1 (enter aggregative heat stable Enterotoxins)

Treatment:

- UTI- fluoroquinolones
- Diarrhea- fluid & electrolyte balance, No Antibiotics

KLEBSIELLA

- Capsulated, Nonmotile,
- LF, produce mucoid colonies
- *K. pneumoniae* – **Urease +ve** - causes pneumonia, UTI, abdominal, wound & surgical site infection, Friedlander Pneumonia → Multiple abscess formation with severe
- *K.ozaenae,* - causes ozaenae (foul smelling nasal discharge)
- *K.rhinoscleromatis* – causes rhinoscleroma

PROTEUS

- Motile, NLF,
- PPA+ve (phenyl alanine deaminase +ve) ,
- urease+ve, H_2S+ve, fishy/seminal smell
- Swarming on blood agar

- **Organism which swarm**- Proteus, Clostridium tetani, V parahemolyticus, V alginolyticus, Serratia
- Forms struvite stone in bladder in alkaline urine
- Non motile strains of Proteus (Basis of Weil felix)-OX 19, OX2 (Pr vulgaris), OX K (Pr mirabalis)

SHIGELLA

- NM, NLF, causes dysentery
- 4 Species- *Sh. Dysenteriae, Sh. Flexneri, Sh. Boydii, Sh. sonnei*
- *All are Catalase* +ve except - *Sh. Dysenteriae type 1*
- *All are* Mannitol fermenting except - *Sh. Dysenteriae*
- *All are anerogenic except- Sh. flexneri* biotypes-New castle, Manchester
- All are lactose non fermenter *except-- Sh. sonnei*
- *Ornithine decarboxylase+ve, ONPG+ve - Sh. Sonnei*
- *Most hardier- Sh. sonnei*
- *MC in world- Sh. sonnei*
- *MC in India- Sh. flexneri*

- Serotyping- Sh. *dysenteriae* -10, *Sh. flexneri* – 6+2 , *Sh. Boydii*-18 Serotypes, *Sh. Sonnei-**1***
- Colicin typing done for- *Sh. Sonnei*

Sh. Dysenteriae; Ty 1 –

- Produce shiga toxin
- Shiga bacillus (Catalase negative) ,
- Causes HUS, forms Shiga toxin

Ty 2 – Schmitzi – indole +ve
Ty 3-7 – Large & Sachs group

Pathogenicity

- Endotoxin – LPS – Diarrhea, ulcers
- Exotoxin – by Sh. Dys type – I
- All spp are Invasive - VMA (virulence marker antigen)

- Infective dose- 10 organisms(Salmonella & Vibrio- 10^6 10^8)
- Complications: Reactive Arthritis, Toxic Neuritis, Intussusception, HUS,TPP

Diagnosis:

- Specimen- mucus flakes of stool
- Transport media- Sach's buffered glycerol saline
- Selective media → DCA, XLD, SS, HE Agar
- Enrichment Broth – G.N broth, Selenite F broth

Treatment-

- Mild- no t/t,
- Severe- Ampicillin, Cotrimoxazole, ceftriaxone

BetaLactamase-

Ambler classification- (Molecular/ Structural)

- ***Class A-ESBL- (extended spectrum Beta lactamase)***
  - Resistant to all Penicillin + 1st/2nd/3rd generation cephalosporin + Monobactam
  - Overcome by b lactam + b lactamase inhibitor

- ***Class B-MBL- (MetaloBetalactamase)***
  - Resistant to AMPc spectrum + Carbapenams
  - Not overcome by b lactam + b lactamase inhibitor

- ***Class C- AMP C Beta lactamase-***
  - Resistant to ESBL spectrum + cephamycins like cefoxitin and cefotetan
  - Not overcome by b lactam + b lactamase inhibitor
- ***Class D- oxacillinase***

Bush Jacoby Medeiros classification- Functional (phenotypic) classification

SALMONELLA

- Motile (by means of Peritrichous flagella)- except *S. gallinarum pullorum*
- *Enteric fever* – S .Typhi, S Paratyphi A, B, C

Antigenic classification:

DNA hybd study- 7 group(all pathogenic-gr1)
Kaufmann White Scheme- 2399 serotypes-Based on- ***O , H,Vi Ag***

H antigen →

- Heat labile, alcohol labile. Formaldehyde stable,
- H Ag is stronger immunogenic,
- H Ab appears late, goes late
- H Ag when Reacts to H Ab- forms large loosed fluffy clumps

O Ag →

- Heat stable formaldehyde labile polysaccharide
- O Ag is Less immunogenic,
- O Ab appears early, goes early
- O Ag when Reacts to O Ab- forms granular chalky clumps

Vi Ag –

- Surface polysaccharide covering O Ag
- Heat labile when present it renders the bacterium inagglutinable by O antiserum
- Possessed by S Typhi & S. Paratyphi C, Citrobacter
- Poorly immunogenic, but protective
- Absence of Vi Ab- poor prognosis
- Persistance in coalescent stage – carier state
- Epidemiological typing of S.Typhi- by Vi specific bacteriophage

Lab diagnosis

Enrichment media –

- Tetrathionate broth, Selenite F broth, GN broth

Selective media →

- DCA, XLD, SS, HE Agar
- Need tryptophan as growth factor

Biochemical reaction

1. H_2S +ve except strains of *S. paratyphi A*
2. Citrate + except *S. Typhi, S. paratyphi A*
3. NLF
4. Gas +ve except S.Typhi
5. Indole, Urease -ve

Enteric fever: step ladder-fever, rose spots, coated tongue

1. Culture – blood, faeces, urine, bone marrow, rose spots etc
2. Demonstration of **circulating** Ag

3. Demonstration of Antibodies in serum

Week wise diagnosis of choice:

- 1st week –Gold standard- Duodenal content culture > BM culture>blood culture (best- combination of all 3)
- Blood culture sensitivity- 90% in 1st week,75% in2nd wk ,60% in 3rd wk
- Clot culture – higher sensitive than blood culture
- End of 1st week- widal
- 2nd & 3rd week- widal
- 4th week -stool & urine culture
- Stool culture- +ve in both case/carrier, +ve even if after antibiotic start

Detection of Antibodies (Widal)-

- Antibodies - appear by end of 1st wk, peaks 3rd wk then falls
- 4 fold rise - highly significant between 1st & 3rd week
- Anamnestic reaction- false+ve in unrelated infection
- Titre of 1: 100 of O agglutinins → significant, 1: 200 of H agglutinins → significant
- H agglutinins appears late, goes late
- Rise in O indicate recent infection
- Pts treated with chloramphenicol – false –ve

Carriers →

- Chronic carrier- >1year, 3% in India,
- Mainly in biliary tract, gall bladder & rarely urinary tract
- Fecal carriers MC, but Urine carriers- more dangerous than fecal carriers
- Detected by- Vi agglutinins (1:10) or fecal/urine culture

Treatment –

- Ciprofloxacin, ceftriaxone, alternate (Azithromycin)
- MDR S.Typhi- Resistant to/ Ampicillin, cotrimoxazole, chloramphenicol
- DOC for MDRS- quinolone
- Nalidixic acid resistant S Typhi- DOC-higher or longer doses of cipro/ofloxacin

NonTyphoidal Salmonella-

- Salmonella septicemia – typically caused by *S. cholerasuis*
- Salmonella gastroenteritis
  - Zoonotic food poisoning
  - Food like meat, egg , milk
  - S. Typhimurium, commonest species (30-40%)
  - Others are S. Enteritidis, S Dublin, S. Newport, S. Heidelberg

Vaccine

- Parental –
  - Killed **Whole Cell**
  - **TAB** vaccine
  - Purified **Vi**
- Oral –**Ty 21a** (Gal E mutant) 3 doses on alt day

YERSINIA

Yesinia pestis

- Safety pin appearance **(Bipolar** staining) with ***Wayson's / methylene blue stain***
- Optimum temp-**27^{0}C** but capsule grows best at **37c**
- In broth – stalactite growth
- Production of pigmented colonies on medium containing haemin

Plague-

- Zoonotic in rodents
- Reservior- wild rat
- Type of plague- Bubonic (MC), Septicemic, Pneumonic Plague
- Transmitted by →
    - Rat flea bite (Bubonic & Septicemic)
    - Inhalational (Man to Man) in Pneumonic plague
- India – outbreak in 1994 in Beed-Latur district (Maharashtra), then in 2002(Simla)
- Incubation Period –
    - Bubonic & septicemic (2-7d),
    - Pneumonic(1-3d)
- Highly infectious & fatal- Pneumonic

Lab diagnosis-

- Smear from bubo- bipolar staining (methylene blue staining)
- Fluorescent Ab staining
- Culture- blood & bubo aspirate
- Ab to F1 Ag ->128

Treatment- Streptomycin(DOC)
Vaccine-

- Killed (Haffkine institute, Mumbai) – immunity lasts for 6months &
- Live attenuated

Yersiniosis:

- Yesinia inf other than Y.pestis – i.e *Y. pseudotuberculosis & Y.enterocolitica*
- Both motile at 25c , not at 37c
- Cold enrichment done for culture

Y. pseudotuberculosis

- Zoonosis
- grows at 22c, urease+ve
- **C/F** - Mesenteric lymphadenitis, resembling appendicitis, Erythema nodosum

Y.enterocolitica

- Causes acute enterocolitis and terminal ileitis, lymphadenitis.
- Reactive arthritis in HLA B27 pts.

Pasteurella multocida

- Zoonotic, wound infection, flu like

Francisella tularensis

- Tularemia: a disease of rodents, originally described in California
- Mode of transmission-
  Direct contact with rodents> Tick bite
- Fastidious, filamentous, filterable
- Divide by budding& binary fission
- Francis blood dextrose cystine agar
- *Human disease:*
    - Local ulceration with lymphadenitis (most common form)**,**
    - Typhoid like fever ,
    - Flu like

VIBRIO

- Gram negative curved rods(comma shaped)

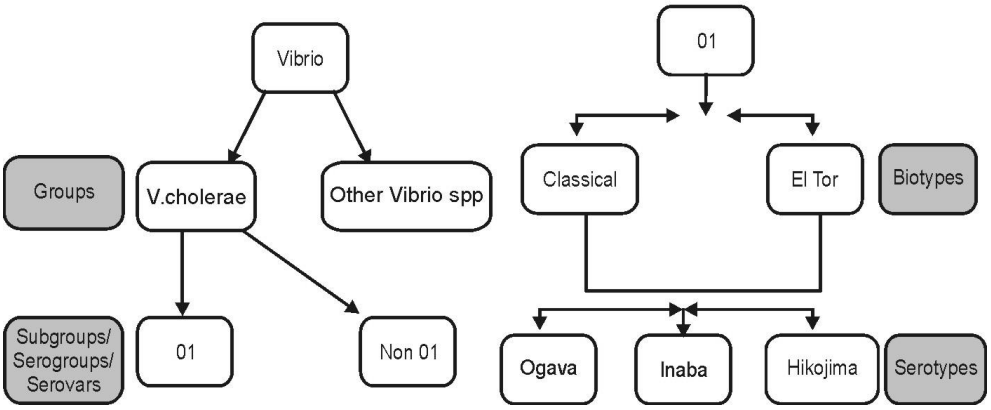
- All are halophilic except V.cholerae, mimicus
- Single polar flagella (darting motility) – Also by Campylobacter
- Oxidase positive , Non/late lactose fermenter but sucrose+ve (V.cholerae)
- Indole +ve & Nitrate+ve (cholera red reaction)
- String test+ve with Na deoxycholate

Culture media:

Transport media- Venkatraman Ramakrishnan (VR) medium Cary blair medium Autoclaved sea water
Enrichment – APW (alkaline peptone water) Monsurs taurocholate tellurite peptone water
Selective – Bile salt agar (BSA) Monsurs gelatin taurocholate trypticase tellurite agar (GTTA) Thiosulphate citrate bile sucrose (TCBS) – yellow(V cholera), green(V parahemolyticus)

Classfication

Gardner and Venkataraman Classification



Classfication

Points to remember:

- 1st 6 pandemics- started in Indian subcontinent & due to classical Vibrio
- 7th pandemic- started in Indonesia, due to Eltor, less severe illness & more carriers
- Eltor is much hardier/resistant than classical, but produce less severe cholera,
- Eltor has more carrier than cases
- MC Serotype - Ogawa >Inaba> hikojima (agglutinated by both antisera)

O139 Vibrio- (Bengal strains) - 1st in 1992 from Chennai

- Capsulated, might be invasive
- Arise from Eltor by horizontal transfer
- Clinically & epidemiologically indistinguishable from O1
- Not neutralized by O1 antisera
- Currently – both O1 Eltor & O139 coexist. Classical Vibrio still reported in Bangladesh.

Biotypes of V.cholerae O1	Classical strain	El Tor
Hemolysis	-	+

Chick erythrocyte agglutination	-	+
Polymyxin B (50 u)	+	-
Group IV phage susceptibility	+	-
Eltor Phage V susceptibility	-	+
VP	-	+
CAMP	-	+

Cholera:

- **Cholera**: occurs only in man (rice water stools)
- Maintained-
  - Interepidemics- in sea water
  - During epidemic- In carriers
- Acid labile (alkali stable), so require high infective dose of 10^6
- Age-
  - Interepidemics – all age equally
  - During epidemic- More children
- Heat labile, but can resist refrigeration

Virulence factor-

- TCP- toxin co-regulatory pilus- adhesion
- Cholera Toxin (CT)- resembles heat labile toxin of *E.coli* (LT)
  - A unit – ADP ribosylation of G protein→↑Adenyl cyclase →↑cAMP,
  - B unit - binds to ganglioside receptors
- LPS- has no role in pathogenesis

Treatment-

- DOC in adult- Doxycycline (if resistant, then cipro)
- DOC in children- cotrimoxazole
- DOC in pregnancy- Furazolidone
- DOC for chemoprophylaxis- Tetracycline

Halophilic Vibrio – ↑NaCl tolerance- *V. parahemolyticus (7%), V. alginolyticus (10%), V. vulnificus (8%)*
Causes food poisoning (sea fish), green colony on TCBS(sucrose-ve)

V. parahemolyticus – Shows kanagawa phenomenon on wagatsuma agar
Capsulated, bipolar staining, Peritrichous flagella

V. vulnificus- lactose fermentative

Vaccines - Killed whole organism & Oral Vaccine – Killed & Live

NON FERMENTERS

Pseudomonas aeruginosa

MC nosocomial pathogen because:
Ubiquitous water and soil organism
Wide temp range 5c to 42c
Produce biofilm
Resistant to disinfectant, antiseptics & multidrug resistance(d/t resistant plasmids)
Virulence factor-
Exotoxin A- NADase like, inhibiting protein synthesis (like diphtheria toxin)
Exotoxin S- Ribosylation of GTP binding protein
Others- Capsule/slime layer, Exotoxin S,U,Y, protease, elastase, Hemolysin, Endotoxin

Distinguishing Characteristics

- Oxidase – positive, non – fermenting
- Motile with polar flagella
- Pigments: Pyocyanin (blue – green) by P.aeruginosa & Fluorescein (greenish yellow- by all spp)
- Pigmentation is Produced in **King'**s media
- Selective media- **cetrimide** agar
- Grape – like fruity odor
- Slime layer (alginate)
- Non – lactose fermenting colonies on MacConkey

Infections:

- Burn patients infection
- Malignant otitis media
- Shanghai fever
- Cellulitis (blue – green pus)
- Neutropenic Patients –causes Pneumonia and septicemia
- Chronic Granulomatous Disease (CGD)
- Ecthyma gangrenosum
- UTI in Catheterized patients
- Cystic fibrosis - by high slime/ alginate producing strains (mucoid)

AntiPseudomonal drugs:

Penicillin- Piperacillin, Mezlocillin,Ticarcillin Cephalosporin – Ceftazidime,cefoperazone,cefipime Carbapenems- imipenem, meropenem Monobactam- Aztreonam	Aminoglycoside- Tobra,Genta,Amikacin Quinolone- Cipro, Levofloxacin Other- PolymyxinB, Colistin

Burkholderia

Both- GNB, NF,OX+ve, Resistant to polymyxinB,

- Bipolar stained
- Zoonotic, agent of bioterrorism,
- Occupational spread, t/t- ceftazidime / Carbapenems

B. pseudomallei

- Causative agent of *melidiosis* (Glanders like disease)
- **Motile**
- Zoonotic - rodents
- Humans - Pulmonary infections like TB(MC), Multiple abscesses , LN↑
- k/a- Vietnam Time-Bomb

Burkholderia mallei

- Non-motile,
- Zoonotic- horse
- Mallein test- like tuberculin test
- **Glanders**: nodules in Respiratory system
- **Farcy**: skin with prominent lymphatics underneath

HAEMOPHILUS

H. influenzae

- Blood loving , Oxidase + Catalase +
- Fastidious, Refrigeration kills
- Requirement for –
  - X (haemin in blood) &
  - V factor(NADP inside RBC)
- Pfeiffer's bacillus
- Pleomorphism
- Capsular polysaccharide -
  - 6 serotypes – (a-f) –
  - 95% by type b (**PRP** –poly ribosyl ribitol phosphate)
- **Satellitism** on blood agar - around Staph streak providing V factor
- Media- Levinthal's agar Field's agar ,chocolate agar
- Antigen-Capsule, OMP. LOS
- H.infl & H.pylori- complete genome is mapped

Pathogenicity:

Capsulated strains	Non typeable strains
Invasive- meningitis, epiglottitis, pneumonia, bacteremia, endocarditis	Non-invasive - otitis media(MC in adult) LRTI(adult)
Children	Adult
Hematogenous spread	Contagious spread
Vaccine available for type B only	Not available

Vaccine- Hib PRP (for type 2) vaccine: not effective in <2 years of age, ↑immunogenicity by DT&TT
No vaccine for other H.infl types
Prophylaxis- Rifampicin

***H. aegyptius* -**

- Koch's –Week's bacillus
- Pink eye**,**
- Brazilian purpuric fever

H. ducreyi

- ***Chancroid/soft sore:*** painful lymph node, tender nonindurated genital ulcer
- School of fish" or "rail road track" appearance , bipolar staining
- Medium used: chocolate agar with 1% isovitalex, Vancomycin , also grow on CAM

HACEK group of organisms

- Fastidious slow growing bacteria, normal or oral cavity , causes endocarditis
  - *Haemophillus species- parainfluenzae,aprophilus,paraaprophilus*
  - *Actinobacillus actinomycetemcomitans.*
  - *Cardiobacterium hominis,*
  - *Eikenella corrodens*- corrodes on blood agar
  - *Kingella kingae*

BORDETELLA

- Gram negative coccobacilli. Thumbprint appearance
- Culture → Bordet Gengou (glycerol, potato extract) - bisected pearls or mercury drop colonies

Virulence factors →

Pertussis toxin-↑cAMP(only by B.pertussis)	heat labile toxin,	LPS
Agglutinogen	Tracheal Cytotoxin	Pertactin (OMP)
Filamentous hemagglutinin	Adenylate cyclase	

Pathogenesis

- Source of infection -patient in early stage
- No healthy carrier
- Secondary Attack rate of more than 90%
- 3 stages-
  - Catarrhal- nonspecific symptom, highly infectious, +ve culture& smear
  - Paroxysmal – whooping cough, vomiting, usually culture& smear -ve
  - Convalescent stage
- Specimen – per nasal swab for nasopharynx
- Whooping cough- also by Mycoplasma, Adenovirus
- **Vaccine**- DPT/ DTaP (See C.diphtheriae section)

BRUCELLA

- Zoonosis
- gram -ve coccobacilli , Strict Aerobe
- *B. melitensis* – Sheep, Goat *B. abortus* – Cattle
- *B. suis* – Pig *B. canis* – Dog

Brucellosis –

- Malta/Undulant fever
- Intracellular affecting RES
- Transmitted by – Raw milk intake (MC) > contact with animal feces/urine
- B melitensis- most pathogenic (M Ag > A Ag)
- B abortus- A Ag> M Ag
- Tbilisi phage typing is done

Diagnosis-

- *Culture* → Blood (Castaneda method). & BM culture (↑Sn)
- Erythritol- improves growth
- Catalase +ve, Ox +ve, Urease+ve

SEROLOGY –

Standard Aggl Test (SAT)

- IgM is detected using B abortus Ag- **NOT** useful for chr brucellosis
- False –ve : due to blocking Ab (removed by prior heating / 4%Nacl / coomb test)
  Also d/t prozone phenomena
- False +ve : cross reacting Ab in Yesinia. cholera, tularemia- removed by aggl absorption test

CFT & ELISA - for chronic infection

Animal brucellosis →

- Rose Bengal Card test,
- Milk Ring Test,
- Whey aggl test

SPIROCHETE

- Pathogenic spirochetes- Treponema, Borrelia, Leptospira
- Possess- endoflagella

Treponema

1.	*T. pertenue*	:	Yaws
2.	*T. endemicum*	:	Endemic Syphilis (Bejel)
3.	*T. carateum*	:	Pinta
4.	*T. pallidum*	:	Syphilis

Syphilis

- IP-9-90days
- ***Primary Syphilis-***
  - MC(genitalia)> mouth, nipple,
  - Characterized by Hard/hunterian chancre(painless LN↑) + painless ulcer
- ***Secondary Syphilis -***
  - Skin rashes
  - Condyloma lata at mc junction
  - Mucosal patches-
  - Highly infectious
  - Serology 100% sensitive
- ***Latent Syphilis -***diagnosis by serology only
- ***Tertiary Syphilis –***
  - CVS- aneurysm + AR
  - Gummata
  - CNS- GPI & tabes dorsalis

Diagnosis-

- Dark ground- detection limit 10^4/ml
- DFA - ↑Sn (uses mAb to T pallidum)-
- Staining- Silver Impregnation -Levaditi (tissue), Fontana (smear)
- Culture-
  - Pathogenic species cannot be cultivated on artificial media
  - Maintained in ***rabbit testes***
  - Non pathogenic spp (Reiter Treponema) culture tried- Smith Noguchi medium

Serology :

Non Treponemal Test:

- Cardiolipin (Diphosphatidyl Glycerol) used.
- Older test- Wasserman (CFT), Kahn Test – Tube Flocculation

- Newer test- VDRL & RPR

VDRL –

- Slide Flocculation test
- Ag used- Cardiolipin + Lecithin+ Cholesterol
- Biological false +ve (1%)- acute infection/infl, chr-leprosy, malaria, hepatitis
- Prozone phenomena
- Other Non Treponemal Test – RPR (rapid plasma regain), TRUST(toludine red unheated serum test)

VDRL	**RPR**
Result read microscopically	Macroscopically-microscope not required as carbon particle added to Cardiolipin Ag
Once reconstituted, should be used in 24 hr	Can be stored – ADTA used as stabilizer
Preheating of serum is required- to remove inhibitors	Preheating of serum not required- as choline chloride used to remove inhibitors
Sample- blood, plasma, serum, CSF	Cant be used for CSF
Rotation for 4 min	Rotation for 8 min
78% Sn in primary syphilis	86% Sn in primary syphilis

Specific/ Treponemal test →

- TPI (Immob.) -using live Nicholl's strain
- TPA (agg)-(Killed)
- TPHA – using extract, most specific
- FTA, FTA-ABS (most sensitive)

Points to remember:

- For monitoring the response to treatment - VDRL>RPR
- Primary- Most Sn : TPPA> RPR/TRUST > FTA-ABS
- Secondary – all equally sensitive (100%)
- Latent & late - Most Sn : TPPA & FTA-ABS
- Overall most specific- TPPA & FTA-ABS
- First test to be +ve - FTA-ABS
- Rapid & mass screening- VDRL

Congenital syphilis-

- 19s IgM FTA
- Serial IgG in baby- If maternal transfer→ titre falls in 3m, If Congenital → titre rises in 3m
- Baby serum> cord blood> Maternal serum

BORRELIA

Relapsing fever →

- Epidemic relapsing fever –
  - Caused by *B recurrentis* –
  - Louse borne
  - Exclusively human
- Endemic relapsing fever –
  - Tick borne
  - Caused by *B. duttoni, B. hermesii*-
  - Natural host is rodents
- Shows antigenic variation- reason for relapse

Lymes disease –

- *B. burgdorferi* – Tick borne
- Clinical triad-
- **Erythema migrans, dissemination, persistent infection**

Lab Diagnosis

- Diagnosis mainly on clinical ground.
- Isolation from skin lesions or blood- modified Kelley's medium
- Microscopic detection – Dark ground, phase contrasts, Immunfluroscence, **silver staining**
- Antigen in urine

Borrelia vincentii

- Commensal of mouth
- **Vincent's angina-** ulcerative gingivostomatitis (predisposing conditions such as malnutrition, viral infection)
- Often associated with **fusiform bacillus** (*Fusobacterium fusiformis)*

LEPTOSPIRA

- Leptospira – obligate aerobes
  - *L. interrogans* – parasitic strains - >23 serogroup & >230 serovars
  - *L. biflexa* – free living saprophytic strains
- 3R- Zoonotic (rat) + Rice field + Rain
- Transmission- direct contact with animal(occupational)
- India- MC in TN, Andaman, Kerala
- Clinical Stage-
  - Anicteric (90%)- fever, conjunctival stuffiness
  - Icteric-(10%)- (**Weil's/hepatorenal hemorrhagic syndrome**)

Diagnosis-

- Sample- blood, CSF & urine(later stage)
- **Microscopy-** dark field- detection limit 10^4 /ml, phase contrast, silver impregnation
- **Culture**- EMJH, Fletcher, Korthoff
- Incubation at 28-30c for 13 wk, tedious, Sn less than serology
- **Serology-**
  - Genus specific – CFT, ELISA
  - **Microscopic aggl test**(MAT)- gold standard(Sero group & serovar specific)

RICKETTSIACEAE

- Obligate intracellular pathogen
- Transmitted by arthropod
- Not cultivable in cell free media- except Bartonella

Group	Species	Disease	Vector
Typhus group	*R. prowazekii*	Epidemic typhus & Brill Zinsser	Louse **(LET)**
	R. typhi	Endemic typhus	Flea **(FEN)**
Spotted fever group	*R. rickettsii*	Rocky mountain spotted fever	Tick **(TRI)**
	R.conori	Indian tick typhus	tick
	R. akari	Rickettsial pox	Mite(gamasid) **(PSM)**
Scrub typhus	*O.tsutsugumashi*	Scrub typhus	Mite (trombiculid)

- Most severe- Rocky mountain spotted fever
- Mild- R pox
- Eschar Seen-Scrub , R.pox, fever boutonneuse.
- Vesicular /vericelliform rash- Rickettsial pox

- Transovarial transmission occurs only in tick- i.e. Rocky Mountain spotted fever, Indian tick typhus

Lab diagnosis

- Isolation – in lab animals, hens egg & cell cultures
- Direct detection of the organisms & their antigens - giminez, Machiavello & Giemsa stain

Neil mooser Reaction –

- Male guinea pig intraperitoneal inoculation
- Rocky mountain spotted fever- scrotal necrosis
- Epidemic typhus- only fever , but negative tunica reaction
- R.typhi/conori/akari - fever and positive tunica reaction(testicular inflammation)

Serology - Weil Felix reaction

- Cross reacting alkali stable polysaccharide Ag of P.vulgaris (OX2,X19) & P.mirabilis (OXK)
- False +ve if proteus infection present

Rise in titre more meaningful

Weil Felix	**OX2**	**OX19**	**OXK**
Epidemic typhus	+	++++	-
Endemic Typhus	+	++++	-
RMS Fever	+ to ++++	++++	-
Scrub typhus	-	-	+++

Scrub typhus-

- Mite & chigger borne
- Affect rats in deserts, rain forest
- Eschar at site, rash, LN↑
- ↑OXK titre in Weil Felix

Coxiella burnetii –

- It survives holders method of pasteurization of milk
- Transmitted without arthropod (respiratory mode)
- No skin rash in Q fever.
- Zoonotic (wild animal)
- Produces interstitial pneumonia, shows latency

Ehrlichia → multiply with in membrane bound vacuoles in phagocytes (morula)

Bartonella

- Involve RBC
- *B.bacilliformis* –
  - Oroya fever ,
  - Verruga peruana,
  - Carrion's dis,
- *B. quintana* –
  - Trench fever ,
  - Louse borne
  - 5 days fever
- *B. hensalae* –
  - Cat scratch diseases ,
  - Lymphadenopathy,
  - Bacillary angiomatosis (in HIV)

CHLAMYDIA

- Obligate intracellular gram negative bacteria.

- Doesn't grow on artificial media
- Modified peptidoglycans
- Can't produce their own ATP(Energy parasite)

Species	Character	Serotype	Disease
C.trachomatis	Form compact inclusions with glycogen matrix Sn to sulfonamide Natural parasite of human Leave the host cell with a scar	Biovar TRIC -A, B, Ba, C	Trachoma
		Biovar TRIC - D-K	Inclusion conjunctivitis infant pneumonia Genital chlamydiasis
		Biovar LGV - L1,L2,L3	LGV
C. *psittaci*	Form diffuse vacuolated inclusions without glycogen matrix Natural parasite of birds Leave host cell by lysis	Many	Psittacosis (Pneumonia) Inhalational route Poultry ingestion- no role Occupational spread to human
C.pneumoniae TWAR agent	Exclusively human pathogen	Only 1	Atypical pneumonia a/w atherosclerosis,asthma,sarcoidosis Hep2 cell most affective cell line

Life cycle – divide by binary fission without eclipse phase
Takes 24-48 hr

Elementary body	Extracellular, infectious form, rigid cellwall, small size electron dense nucleoid, DNA=RNA
Reticulate body	Intracellular , replicating form, fragile cell wall,larger size, no electron dense nucleoid, RNA> DNA

Antigen-

- LPS- genus specific, used for CFT
- Envelop surface Ag- species specific,
- Major OMP- Serovar serotype specific- used for micro-IF test

Diagnosis:

Microscopy-

- Stain by Casteneda , Macchiavello, Giminez
- Lugol's I2-(only for C.trachomatis)
- IF using monoclonal Ab- ↑Sn, detect both inclusion & elementary body

Culture →

- Mice (infective by only C.psittaci & LGV)
- Yolk sac, cell culture (McCoy Cells & HeLa)
- Hep2 for *C.pneumoniae*

Serology-

- MicroIF (serovar specific),
- CFT(genus specific)
- High tite seen in – LGV, infant pneumonia, salpingitis

PCR- more Sn & Sp

Treatment of chlamydial infections →

- C.trachomatis- Azithromycin
- C.psittaci- tetracycline
- C.pneumoniae- erythromycin/tetra

C.trachomatis

- MC cause of STD worldwide
- MC cause of ophthalmic neonatorum, NGU, epididymitis
- NGU complications- Reiter syndrome (conjunctivitis+urethritis+polyarthritis)
- Fitz Hugh Curtis syndrome- perihepatitis
- Inclusion conjunctivitis – neonate (inclusion blenorrhea), adult (swimming pool conjunctivitis)

Trachoma-

- Chr conjunctivitis- follicular hypertrophy + papillary hyperplasia + pannus + cicatrization
- Stages: Trachoma dubium, protrachoma, established trachoma I –IV
- Inclusion body seen only in , established trachoma I –IV

LGV -

- MC by L2>L1,L3
- LGV serovars are more invasive than others
- MC LN involved- pararectal & intrapelvic nodes
- Pain less ulcer + painful LN↑
- Esthiomone (elephantiasis of vulva) rectal stricture
- Skin test → Frie test

Inclusion body :

- LCL body- Psittacosis
- Miyagawa corpuscle- LGV
- HP body- trachoma

Non gonococcal urethritis (NGU)

- *Chlamydia trachomatis* (MC)
- *Mycoplasma*
- *Ureoplasma urealyticum*
- *Herpes simplex*
- *Trichomonas vaginalis*
- *Candida albicans*

Comparison of the Genera Rickettsia, Chlamydia, and Mycoplasma

	Chlamydia	Rickettsia	Mycoplasma
Obligate intracellular parasite	Yes	Yes	No
Make ATP	No ATP	Limited ATP	Normal ATP
Peptidoglycan layer	Modified	Normal Peptidoglycan	No Peptidoglycan

MYCOPLASMA

- ***Mycoplasma pneumoniae*** → primary atypical pneumonia (PAP)
- Very small , filterable, Flagella ,pili –absent but some strains show gliding mobility
- Lack rigid cell wall. Need cholestrol as a growth factor
- Pleomorphism, poorly gram negative
- Stain with Dienes or Giemsa stain
- Fried egg colonies
- Culture medium ----- PPLO broth
- **Detection of Antibody**
  - Cold agglutinins
  - Streptococcus MG test
  - CFT (complement fixation test)
  - ELISA for IgM, IgG & IgA

MISCELLANEOUS BACTERIA

CAMPYLOBACTER JEJUNI

- Curved GN(gullwing shaped)
- single polar flagellum(darting motility),
- **Microaerophilic** – (5%O2 + 85%N2 + 10% CO2)
- Thermophilic (**42^0C)**
- Zoonotic
- Mode- ingestion of contaminated food(raw milk)
- Mechanism of diarrhea -LT resembling CT+ Invasion like Shigella+ Cytotoxin
- Culture- Skirrow's, Campy BAP, Butzler, charcoal based media

HELICOBACTER PYLORI

- Curved rod, motile unipolar tuft of lophotrichous flagella
- **Microaerophilic,** abundant **urease** production
- Prevalence- 30%(developed country), 80%(developing), 50%(world)
- Risk factor- MC in childhood(but immunity doesn't develop), low socioeconomic status, crwoding

Disease:

- Acute gastritis(Antrum MC), peptic ulcer disease(80% of duodenal ulcer & 60% of gastric ulcer),
- chronic atrophic gastritis, autoimmune gastritis
- Adenocarcinoma of stomach& esophagus & MALT
- Virulence - Cytotoxin associated gene, vacuolated cytotoxin gene, urease

Diagnosis:

Invasive

- Warthin starrey silver staining
- Culture – campylobacter media & chocolate agar – most specific, but not Sn
- Biopsy urease test (at least 3 biopsy) – most sensitive, quick, simple

Noninvasive :

Urea breath test-

- Most consistent & accurate test
- most sensitive, quick, simple
- monitoring of t/t(become-ve after t/t)

- Ag Detection in stool
- Serology : ELISA – not useful
- PCR

LEGIONELLA

- Pleomorphic rods ,poorly gram – negative
- Fastidious requiring iron and cysteine (**BCYE medium**, buffred charcoal Yeast Extract)

Reservoir –

- Natural water source- Rivers/streams/amoebae; Artificial Aquatic source- AC, water cooling tanks
- Transmission -Aerosols from contaminated AC
- No human – to human transmission
- Predisposing Factors -Smokers , alcohol, Immunosuppression, hairy cell leukemia

Legionnaires' disease ("Atypical Pneumonia") –

- CAP + Diarrhea + encephalitis
- MC extrapulmonary site – heart
- Numerous PMN, but no organism in sputum

Pontiac Fever –mild flu like

Treatment- macrolide, cipro, tetracycline

B lactamase & AMG- not affective

CALYMMATOBACTER GRANULOMATIS

- Present name Klebsiella granulomatis
- Produce ***granuloma inguinale, Granuloma venerum or Donovanosis***

- Bipolar staining, safety pin appearance
- Donovan bodies
- C/F → painless ulcer without Lymphadenopathy
- Culture – Embryonated hens egg
- Treatment → tetracycline ,EM, chloramphenicol

RAT BITE FEVER- *Streptobacillary moniliformis* & *Spirillum minus*

***Streptobacillary moniliformis*:**

- Gram negative, highly Pleomorphic bacillus
- **String of bead appearance**, non motile, fastidious
- Exists as L form also
- Haverhill fever – Erythema arthriticum epidemicum
- Lab diagnosis → Grow on culture media containing blood serum or ascitic fluid
- L forms – fried egg appearance

Spirillum minus*-** motile, ***causes Sudoku, not cultivated

GARDNERELLA VAGINALIS

- Causes bacterial vaginosis, Gram variable (mostly-GNCB)
- Other organisms implicated in BV- *Mycoplasma, Mobilincus, Prevotella*, Peptostreptococcus.
- BV is characterized by –
  - Thin profuse vaginal discharge,
  - pH > 4.5,
  - Fishy odour
  - Clue cells
- Nugent scoring used for diagnosis

	Syphilis	Chancroid	LGV	Donovanosis
Ulcer	Painless indurated single	Painful non indurated irregular	Painless	Painless
LN	Painless	Painful	Painful	No

Feature	Syphilis	Herpes	Chancroid	LGV	Donovanosis
Incubation period	9–90 days	2–7 days	1–14 days	3 days–6 weeks	1–4 weeks (up to 6 months)

GENERAL MICROBIOLOGY

HISTORY

History

Loius Pasteur –

- Father of Microbiology
- Proposed fermentation principle
- Devised - Autoclave, steam sterilizer, hot air oven & Pasteurization of milk
- Prepared the Vaccines for – Anthrax, Rabies, Cholera (CAR)

Robert Koch –

- Father of Medical Microbiology
- Proposed Koch Postulates
- Discovered – TB, cholera bacilli
- Aniline dye staining
- Solid culture media concept
- **Koch's postulates** –
    - The microorganism must be present in every case of the disease but absent from healthy organisms.
    - The suspected microorganism must be isolated and grown in a pure culture.
    - The same disease must result when the isolated microorganism is inoculated into a healthy host.
    - The same microorganism must be isolated again from the diseased host.

Paul Ehrlich-

- Proposed Ehrlich phenomena
- Detected- Ehrlichia (Bacteria)
- Founder- Acid fast stain
- Standardized toxin & antitoxin
- Proposed Side chain theory for Ab production

Other Important contributors:

- Joseph Lister – Antiseptic measures to prevent surgical sepsis
- Antony van Leeuwenhoek –Founder of Microscopy
- Karry B Mullis - PCR

Discoverers:

- *Kleb-Loeffler bacilli– C.diphtheriae*
- *Preisz Nocard bacilli – C.pseudotuberculosis*
- *Koch Week bacilli – H.aegypticis*
- *Whitmore bacilli – Burkholderia pseudomallei*
- *Pfeiffer's bacilli – Haemophillus infuenzae*

Morphology .

- Microorganisms - heterogeneous group of several distinct living structures of microscopic size, classified under the kingdom *Protista*
- Kingdom *Protista* - divided into following groups:
    - Prokaryotes – include bacteria and blue green algae
    - Eukaryotes – include fungi , algae (other than blue green) , protozoa and slime moulds

Characteristics	Prokaryote	Eukaryote
Nuclear membrane, nucleolus, Ribo Nucleo-protein	Absent	Present

Cell division	Binary fission	Mitosis , Meiosis
Chromosome	One, Circular	Many, liner
Cell wall	NAM /NAG	Sterols (also Mycoplasma)
Membrane bond Organelles	Absent	Present
Respiration	Mesosome	Mitochondria
Cytoskeleton	Absent	Present
Ribosome	70s	80s
Pinocytosis	Absent	Present

Size of bacteria

- The size of bacteria is measured in units of a micron (µm).
- Bacteria of medical importance measure 2-5 µm (length) X 0.2-1.5 µm (width).

Microscopy:

Principle of microscope depends on –

- *Magnification*- takes place at Objective lens & Eye piece
- *Contrast*- increases by staining
- *Resolution* –ability to distinguish two points separate
- Resolution power of –
    - Human eye-0.2mm
    - Light microscope -0.2µm
    - EM- 0.2nm

Staining methods -

Demonstrates structural details of the bacteria by producing colour contrast.

Staining techniques used –

- **Gram's stain**
    - It differentiates bacteria into two groups: Gram –positive and Gram -negative bacteria.
    - GPB appear violet- they have a relatively thick amorphous cell wall
    - Gram- negative bacteria take counter stain, appearing red.
- **Classification of bacteria according to Gram's staining-**
    - Gram positive cocci– *Staphylococcus, Streptococcus, Enterococcus, Pneumococcus*
    - Gram negve cocci – *Meningococcus, Gonococci, Veillonella, Moraxella*
    - Gram positve bacilli – *Corynebacterium, Clostridium, Bacillus, Listeria, Rodococcus, Actinomycetes/Nocardia, Mycobacteria, Erysopilothrix* etc.
    - Gram negative bacilli- *Enterobacteriaceae, Vibrio, Pseudomonas* etc.

Difference between Gram positive & Gram negative bacterial cell wall-

Characters	**Gram positive cell wall**	**Gram negative cell wall**
PG layer Thickness	100 layer thick	1-2 layer thick
Lipid content	2- 5% only	15 – 20%
Teichoic acid	Present	Absent
Variety of amino acid	Few	Several
Aromatic amino acid	Absent	Present
Lipopolysaccharide in outer membrane (endotoxin)	Absent	Present
NAM: N-acetyl muramic acid NAG : N- acetyl glucosamine	Each NAM molecule is attached with a tetrapeptide side chain which are cross linked through a pentapeptide cross bridge	Tetrapeptide side chains are directly liked to each other No pentapeptide bridge

Acid-fast stain

- Ziehl-Nielsen (ZN) staining method.
- Acid-fast bacilli (AFB) -appears bright red in stained smears

- Used for staining following acid-fast micro-organisms-
- *M. tuberculosis* (20% sulfuric acid), *M. leprae (*5% sulfuric acid), *Nocardia* (1% sulfuric acid), *& spores* (0.5% sulfuric acid) & *Rodococcus*
- Coccidian parasites such as *Cryptosporidium, Cyclospora, Isopsora, Tinea scolex*
- Acid fastness is due to the high content of mycolic acid and higher alcohols found in the cell wall & depends on integrity of the cell wall.

Albert's stain/ Neisser's stain/ Ponder's stain-

- Used for staining the volutin granules (metachromatic granules) of *Corynebacterium diphtheriae*.

Shape of bacteria-

Classification of bacteria depending on their shape-

1. *Cocci*: oval or spherical cells. Arranged in-
   - Pairs - e.g. *Pneumococci* (lanceolate shaped), *Meningococcus, Gonococci* (kidney shaped) & *Enterococcus* (spectacle eyed shaped)
   - Tetrads-e.g.- *Micrococci*
   - Chains –e. g. –*Streptococci*
   - Octate- e.g.-*Sarcina*
   - Clusters –e.g.-*Staphylococci*
2. *Bacilli*: rod shaped.

Arrangement-

- *Coccobacilli*: E.g. *Brucella*.
- *Streptobacilli*: Arranged in chains. E.g. *Streptobacillus, B.anthracis* (Bamboo stick appearance)
- *Cuneiform pattern*: Chinese letter or cuneiform patterns arrangement. E.g. *Corynebacterium.*
- *Comma shaped*: Curved appearance E.g. *Vibrio.*
- *Spirilla:* Rigid spiral forms E.g. *Spirillum.*
- *Pleomorphic e.g- Hemophillus, Proteus*

3. *Spirochetes*: Slender, flexuous spiral forms .E.g. *Treponema*
4. *Actinomycetes*: Actinomycetes are branching filamentous bacteria resembling fungi. They possess a rigid cell wall.

Bacterial cell structure-

Capsule and slime layer

The capsule has various functions:

- It contributes to invasiveness of bacteria by protecting the bacteria from phagocytosis.
- It facilitates adherence of bacteria to surfaces.
- It plays a role in formation of biofilms

Demonstration of capsule

- Negative staining
- M'Faydean capsule stain-used for demonstration of capsule of *Bacillus anthracis* (polychrome methylene blue stain)
- Quellung's reaction – Since capsules are antigenic they can be demonstrated by serologic methods & useful for rapid identification of capsular serotypes of
  - *Streptococcus pneumoniae*
  - *Neisseria menigitidis*
  - *Haemophilus influenzae*

Chemical composition of capsules of various bacteria

Organism	Polymer
Pneumococcus	Polysaccharide
Meningococcus	Polysaccharide
H,influenzae	Polysaccharide
Bacillus anthracis	Polypeptide (glutamate)

Streptococcus pyogenes	Hyaluronic acid

- ***Presence of both capsule & Slime layer in Streptococcus salivarius***

Flagella

- Thread-like appendages made up of flagellin embedded in the cell envelope.
- Confers motility to the bacteria.
- Arrangement varies between different species bacteria.
- ***Classification of bacteria depending on the arrangement of flagella-***
  - Monotrichous (single polar flagellum) - e.g. *Vibrio cholerae*
  - Lophotrichous (multiple polar flagella) - e.g. *Spirilla*
  - Peritrichous (flagella distributed over the entire cell surface)- e.g. *Salmonella typhi, Escherichia coli*
  - Amphitrichous (single flagellum at both the ends- e.g. *Spirillum minus*
- **Demonstration by**
  - Tannic acid staining (Leifson method)
  - Dark ground/phase contrast/electron microscope
  - By demonstration of motility- Craige tube, hanging drop, semisolid medium
- **Various types of motility**
  - Tumbling – Listeria,
  - Gliding – *Mycoplasma,*
  - Stately – *Clostridium,*
  - Darting –*Vibrio/Campylobacter*
  - Corck Screw-T. pallidum
  - Lashing- Borrelia

Fimbriae or pilli.

- Hair-like filaments that extend from cell surface.
- Composed of structural protein subunits termed pilins.
- Adhesins are located at the tips of pili and are responsible for the attachment properties.
- Sex pili helps in conjugation
- They are antigenic
- Form surface pellicle in liquid culture

Sporulation

- Bacterial spores ***are resistant*** to ordinary boiling, disinfectants and heating.
- Highly resistant ***resting form***
  - Bacillus, Clostridium, Sarcina, Coxiella
  - Terminal, Spherical – C.tetani
  - Terminal ,oval – C.tertium, B.anthrecis
  - Sub terminal – C.perfringens
  - Central- B.cereus,,C.bifermentans
- Favours –↑O2, oxalate agar,2%NaCl , & ↓ in N2,C, Zn, Fe, Ca, Mg
- Sporicidal –
  - EFGH- Ethylene oxide, Formal dehyde, Glutaral dehyde, Hypochlorite, H2O2
  - 2P- Phthalic acid, Peracetic acid
  - Autoclave, Hot air oven, Plasma sterilization,

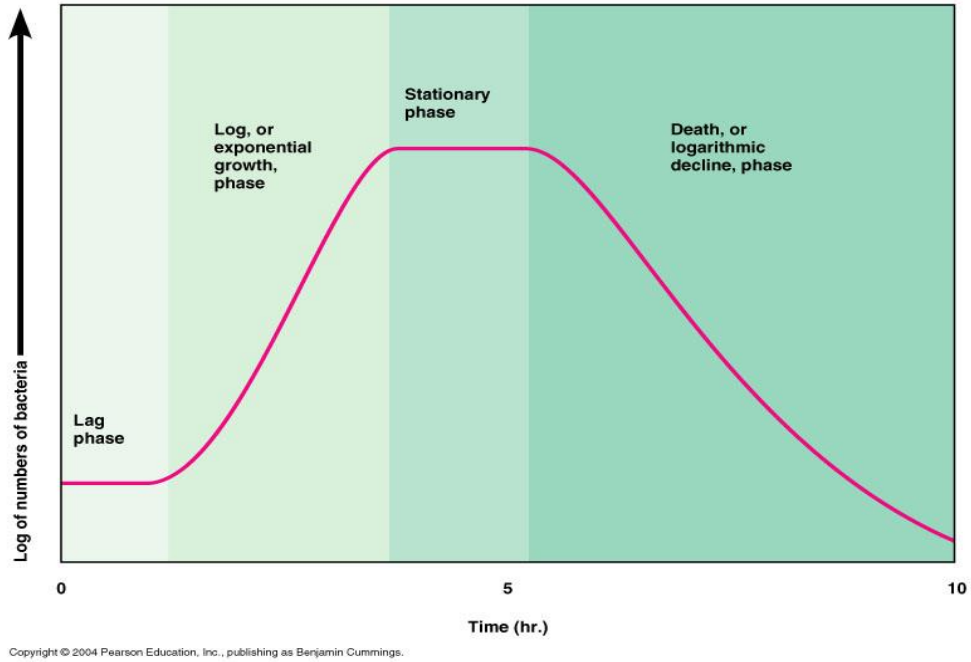
L Form-

- Cell wall free form, Spherical
- Klinberger
- Lister INSTITUTE, London
- Streptobacillus moniliformis
- Mycoplamsa- Unstable
- Resistant to cellwall acting drug

- Pyelonephritis
- Protoplast(Gm+ve), Sphaeroplast(Gm-ve)

Phases of Growth curve-

- Lag phase – enzyme & metabolites builds up, increase size(maximum at end of lag phase)
- Log- divides max, active, smaller, uniformly stained, biochemically active
- Stationary phase – Gram variable due to storage granules, sporulation, exotoxin, antibiotics
- Decline phase- Involution form



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Bipoar staing – safty pin appearance

- Y. pestis
- H. ducreryi
- V. parahemolyticus
- Calymatobacterium granulomatis
- Pseudomonas mellei
- Pseudomonas pseudomallei

Pigment producing bacteria

- Pseudomonas- Green
- Staph. Aureus – Golden yellow
- Rhodococcus- Red
- Bacteroides melanogenicus- Black
- Serratia marcescens – red

Sterilisation & Disinfection

Sterilization

Defined as a process by which an article, surface or medium is freed of all living microorganisms ***either in the vegetative or spore state.***

Germicide /microbicide

Chemical agent that kills pathogenic microorganisms, used on inanimate materials or on living tissue, but it ordinarily ***cannot kill spores.***

Disinfection

- Physical process or a chemical agent that destroys or removes all pathogenic organisms or organisms capable of giving rise to infection.
- This process destroys vegetative pathogens but ***not bacterial endospores.***
- Used only **on inanimate objects** because, in the concentrations required to be effective, they can be toxic to human and other animal tissue.

Methods of sterilization classified as:

- Physical methods of sterilization
- Chemical methods of sterilization.

Physical methods of sterilization

- Sunlight.
- Heat- Dry heat & Moist heat
- Filtration.
- Radiation.
- Sound (sonic) waves.

Heat Sterilization-

Dry Heat

- The dry heat kills microorganisms by **protein denaturation, oxidative damage** and the toxic effect of ***increased level of electrolytes***.
- ***Sunlight*** **-**Direct sun light is a natural method of sterilization of water in tanks, rivers and lakes.
- **Flaming, Incineration**
- **Hot air oven**-
    - **160°C for 1hr**
    - Materials sterilized – glass ware, forceps, liquid paraffin, grease, fat ,glycerol, dust powder
    - Control- ***Clostridium tetani*** (non toxigenic strain), Browns tube, Thermocouple

Moist heat-

- ***Mechanism- Coagulation & denaturation of protein***–
    1. Sterilisation at a temperature below 100 º C- eg
        - Pasteurisation,
        - Vaccine bath
        - Inspissation
    2. Sterilisation at a temperature of 100 º C- eg
        - boiling ,Koch's or Arnold"s steam sterilizer
        - Tyndallization
    3. Sterilisation at a temperature above 100 ºC- **autoclaves**
        - **121°C for 15min (15 psi)**
        - Control – *B.stearothermophillus* & Browns tube
    4. Intermittent sterilization-100c for 20min for 3 days- eg- tyndalisation
        - Used for Sugar solution, gelatin

Filtration

Excellent way to reduce the microbial population in solutions of **heat-labile material**

Filters types:

- Candle filters -
- Asbestos filters *(*Seitz and Sterimat filters),
- Sintered glass filters
- ***Membrane filters:*** Average pore diameter- 0.22µ

Radiation-

Types	Uses
Ionizing radiation-	For sterilization of antibiotics, hormones, sutures and

Cobalt 60 - X-rays, gamma rays and cosmic rays	disposable items such as syringes, infusion sets, catheters etc. **(COLD sterilization)**
Non-ionizing radiation - infra red and ultraviolet radiations	Disinfection of clear surfaces in OTs, laminar flow hoods

Disinfection

Types of disinfectants

- Phenolic compounds -(eg phenol)- active in presence of organic matter
- Diphenyl compounds- (eg hexachlorophene and chlorheximide),
- Halogens - (eg fluorine, bromine, chlorine, and iodine)
- Alcohols -Ethyl alcohol and isopropyl alcohol
- Aldehydes -Formaldehyde and gluteraldehyde
- Gaseous agents -Ethylene oxide, formaldehyde gas and betapropiolactone
- Surface active agents - detergents, wetting agents and emulsifiers.
- Oxidising agents - halogens, hydrogen peroxide, potassium permanganate
- Acridine dyes- (e.g acriflavine, euflavine, proflavine and aminacrine)
- Aniline dyes -(eg gentian violent, crystal violet and malachite green)

Classification of disinfectant based on efficacy-

- **Low level-** Kill vegetative bacteria + enveloped /medium-large virus
- **Intermediate level-** Low level + fungi
- **High level-** Intermediate level + MTB + Non env & small sized virus
- **Chemical sterilant** – High level + spore + Cryptosporidium cyst
- **Decreasing order of resistance**– Prion > Cryptosporidium cyst > spore > Non env & small sized virus> MTB > fungi > vegetative bacteria + enveloped /medium-large virus
- **Prions are sterilized** by : Hypochlorite for 1hr or NaOH for 1hr or Autoclave for 134°Cfor 1hr)

Testing of disinfectants

- Phenol coefficient (Rideal Walker) test,
- Chick Martin test,
- Capacity (Kelsey-Sykes) test,
- In-use (Kelsey and Maurer) test.

Sporicidal agents– EFGH + 2P-

- Ethylene oxide, Formaldehyde, Glutaral dehyde, Hypochlorite, H2O2
- O- Phthalic acid, Peracetic acid
- Autoclave, Hot air oven, Plasma sterilization

Biological Sterilization Indicator:

Hot air oven	*Clostridium tetani non toxigenic strain, B.subtilis subsp niger*
Autoclave	*B.stearothermophillus, B.subtilis subsp niger*
Filtration	*Brivundimonas dimunata, Serratia*
Ionizing radiation	*B.pumilus*
Ethylne oxide	*B.globigi*
Plasma sterilization	*B.stearothermophillus, B.subtilis subsp niger*

Various methods of sterilization

Material	Method of sterilization
Clinical thermometer	Isopropyl alcohol
Paraffin, glass syringe, flask, slide, oil, grease, fat, glycerol	Hot air oven
OT , entryway, ward, lab fumigation	Formal dehyde > UV > BPL
Cystoscope, bronchoscope	Orthophthaldehyde > glutaraldehyde 0.2% (cidex)
Heart lung machine, respirator, dental equipments	Ethylene oxide
Vaccine, sera, antibiotic , sugar	filtration
Sharp instrument	Cresol
Milk	Pasteurization

Plastic syringe, cadgut, swab, catheter	Ionizing radiation
Culture media , all instrument except sharp & glass, all suture except cadgut	Autoclave

Spaulding`s criteria of devices

Clinical device	Definition	Example	Infectious risk
Critical device	Medical device intended to enter a normally sterile environment, tissue or vasculature	Surgical Instruments, cardiac and urinary catheters, Implants,	High (to be sterilized)
Semi-critical device	Medical device intended to come in contact with mucous membranes or minor skin breaches	Respiratory therapy & anaesthesia equipments, endoscopes, laryngoscope, tonometers, rectal/vaginal /esophageal probes etc.	High, Intermediate (Sterilized/HLD)
Non-critical devices	Medical devices that comes in contact with intact skin	BP cuff, ECG electrodes, bedpans, crutches.	Low (ILD/LLD)
Medical equipments	Device or component of a device that does not typically come in direct contact with patient	Examination table, computers	Low (LLD)

Culture Medias & Methods .

Culture Media

- **Basic Ingradients** :
  - Agar or Agar agar-
    - long chain polysaccharide
    - It has no nutritive value
  - Peptone
  - Meat extract
- **Simple/ basal media-**
  - Peptone water- Peptone + NaCl
  - Nutrient broth - Peptone + meat extract + NaCl
  - Nutrient agar – Nutrient broth + 2% agar
- **Enriched media** contains- blood, serum, egg
  - Eg- Blood agar, Chocolate agar, Loeffler serum slope
- **Enrichment broth (liquid media)** – Selectively allows certain organism to grow & inhibit others
  - Eg- Tetrathionate(S.typhi), APW- Vibrio, Selenite F- Shigella
- **Selective media (solid media)**– Selectively allows certain organism to grow & inhibit others
  - Lowestein Jensen media-Mycobacterium tuberculosis
  - Crystal violet blood agar- Streptococcus
  - Mannitol salt agar- Staphylococcus
  - PTA- Corynebacterium diphtheriae
  - Wilson blair bismuth sulfite medium– Salmonella typhi
  - Thiosulphate Citrate Bilesalt Sucrose agar- Vibrio

- Salmonella Shigella agar- Enteric pathogens like Shigella, Salmonella
- Modified NewYork, Thayer martin medium - Gonococcus

- **Transport media-**
  - Bacteria doesn't multiplication
  - Stuart (charcoal swab), Amies medium -*Gonococcus*
  - Pikes - *Streptococcus*
  - Venkatraman Ramakrishnan medium, autoclaved sea water, Alkaline salt water- Vibrio
  - Buffered glycerol saline – Typhoid bacilli
  - Carry Blair medium – Enteric pathogen
- **Differential media –**
  - Mac Conkey agar
  - Cystein Lysine Electrolyte Deficient agar (CLED)

Special Media (E-enriched, En-enrichment, S-selective, D-differential media)
Transport media- bacteria doesn't multiply, only maintains viability of desired pathogenic bacteria

Streptococcus	Pike's media
Neisseria	Amies, Stuart's
Vibrio	VR, Autoclaved sea water, Carry Blair
Shigella	Buffered glycerol saline
Bordetella	Modified Stuart's(with casmino acid) Mischulow's charcoal agar Dacron or calcium alginate swab used

Uses of some commonly used solid media

Medium	**Uses**
Nutrient agar	Routine culture
MacConkey medium	Culture of GNB, Differential medium
Blood agar	Routine culture & culture of fastidious organisms such as Streptococcus species
Chocolate agar	Culture of Haemophilus influenzae, Neisseria
Deoxycholate citrate agar	Culture of Shigella , Salmonella (stool sample)
Thiosulfate citrate bile salt sucrose agar	Culture of Vibrio cholerae
Loeffler's serum slope	Culture of Corynebacterium diptheriae for metachromatic granules
Lowenstein Jensen medium	Culture of Mycobacterium tuberculosis.

Bacterial Genetics & Molecular Diagnostic Methods .

Bacterial Genetics

Bacteria have haploid genome- one copy of their genome DNA.

Plasmids

- Extra chromosomal DNA substances.
- Circular and double stranded DNA molecules that encode traits that are not essential for bacterial viability.
- Plasmids are of different types:
  - F factor,
  - R factors
  - Col factor
- Plasmids confer new properties to recipient bacteria
  - Resistance to one or several antibiotics,
  - Production of toxins,
  - Synthesis of cell surface structures required for adherence or colonization
- Plasmids integrated with host chromosome is known as *episomes*.

Methods of Transfer of DNA between bacterial cells-

- Transformation,

- Transduction and
- Conjugation

Transformation - process of the transfer of DNA itself from bacterium to another.
When purified DNA is injected into the nucleus of a bacterial cell the process is called as *transfection*.

Transduction- transfer of a portion of the DNA from one bacterium to another mediated by a *bacteriophage.*

- Bacterophages encode –diphtheria toxin, botulin toxin, cholera toxin and erythrogenic toxin.
- Transduction is of two types: generalized and specialized.

Conjugation - process of transfer of DNA from the donor bacterium to the recipient bacterium.

- Occurs between two closely related species
- It occurs mostly in Gram negative bacteria
- Donor ability of the bacteria is determined by specific conjugative plasmids called fertility (F +) plasmids or sex plasmids.
- Pilus is one of the most important proteins that form the sex pilus or conjugation tube.
- The sex pilus produces a bridge between conjugating cells in Gram negative bacteria.
- The transfer of plasmids during conjugation is responsible for the spread of multiple drug resistance among bacteria.

Transposons-

- Jumping gene – b/t chromosome & extra chromosome DNA
- Barabara McClintok
- Hair pin like -Centre
- Carry drug resist gene
- Not replicative
- DNA homology not required for transfer

Polymerase chain reaction – PCR

- Requires :
  - Taq Polymerase- withstand the high temp
  - Primers
  - Nucleotide , Mg
- Steps :
  - DNA extraction from micro-organism
  - Amplifi cation of extracted DNA- 3 steps
    - Denaturation (95°C),
    - Primer annealing (55°C),
    - Extension (72°C)
  - Gel electrophoresis of amplifi ed product

Bacteriology of Water -

Indicator of feacal contamination of water-

- Thermotolerant E.coli (recent contamination)- most definite
- Coliform count
- Fecal streptococci
- Clostridium perfringens

Detection of coliform count-

- Multiple tube method
- Presmuptive coliform count- most probable number detected by
- McCrady table.
- MacConkey fluid medium is used

Differnetial coliform count-

- Eijkman test- to detect Thermotolerant E.coli grown at 44c with gas & indole +ve.

Biomedical wastes :

- A vast amount of waste is generated in the process of health care, research, testing, or related procedures on human beings or animals conducted in hospitals, clinics, labs.
- It can act as a source of infection to residents or visitors of hospitals

Option	Waste Category
Category No 1	Human anatomical waste (human tissues, organs and body parts).
Category No 2	Animal waste (animal tissues, organs, body parts carcasses, bleeding parts, fluid, blood and experimental animals used in research, waste generated by veterinary hospitals/ colleges, discharge from hospitals, animal houses).
Category No 3	Microbiology and biotechnology waste (wastes from laboratory cultures, stocks or specimens of micro-organisms live or attenuated vaccines, human and animal cell culture used in research and infectious agents from research and industrial laboratories, wastes from production of biological, toxins, dishes and devices used for transfer of cultures).
Category No 4	Waste sharps (needles, syringes, scalpels blades, glass etc. that may cause puncture and cuts. This includes both used and unused sharps).
Category No 5	Discarded medicines and cytotoxic drugs (wastes comprising of outdated contaminated and discarded medicines).
Category No 6	Solid waste (*items contaminated with blood and body fluids including cotton, dressings*, soiled plaster casts, line beddings, *other material contaminated with blood*).
Category No 7	Solid waste (waste generated from disposable items other than the waste sharps such as tubing, catheters, intravenous sets etc.).
Category No 8	Liquid waste (waste generated from laboratory and washing, cleaning , house-keeping and disinfecting activities).
Category No 9	Incineration ash (ash from incineration of any bio-medical waste).
Category No 10	Chemical waste (chemicals used in production of biological, chemicals, used in disinfect ion, as insecticides, etc).

Colour Coding	**Type of Containers**	**Waste Category**	**Treatment Options**
Yellow	Plastic bag	1,2,3,6	Incineration/deep burial
Red	Disinfected Container/ Plastic bag	3,6,7	Autoclaving/Micro waving/ Chemical Treatment
Blue/ White translucent	Plastic bag/puncture proof container	4,7	Autoclaving/Micro waving/ chemical treatment and destruction/shredding
Black	Plastic bag	5,9,10 (Solid)	Disposal in secured landfill

MYCOLOGY

Classification of Fungi- -

Morphological Classification:

- Yeast – Cryptococcus,
- Yeast like - Candida
- Mould –Dermatophyte, Aspergillus,Zygomycetes , Penicillium

- **Dimorphic –Mould at 25c, Yeast at 37 & body**
  - *1.Histoplasma, 2.Blastomyces,*
  - *3.Coccidiodes, 4.Paracoccidides,*
  - *5.Sporothrix, 6.Penicillium marneffi*

Classification Based on sexual spore:

- Phyco/zygomycetes- zygospore, broad aseptate hypahe eg Rhizopus, Mucor , Absidia
- Ascomycetes- Ascospores, Noarrow septate hypahe - e.g Aspergillus
- Basidiomycetes- Basidiospore- e.g Cryptococcus
- Fungi imperfectii- Deuteromycetes / hypomycetes
  - Sexual phase not found yet
  - Candida, Dermatophyte, Dimorphic group

Systemic classification:

- I.Superficial- Tinea versicolor, Tinea nigra, Dermatophytes
- II.Subcutaneous- Mycetoma, chromoblastomycosis, Sporothrix, Rhinosporidium,
- III.Systemic -1.Histoplasma, 2.Blastomyces, 3.Coccidiodes, 4.Paracoccidides, 5.Candida 6. Cryptococcus
- IV.Oppurtunistc fungi-
  - Aspergillus
  - Zygomycetes
  - Penicillium spp
  - Penicillium marneffi
  - Pneumocysts jerovecii
  - Fusarium
  - Candida
  - Cryptococcus
  - **Sexual spore- (ZAB) –**
    - Zygospore,
    - Ascospore
    - Basidiospore
  - **Asexual spore (ABC)-**
    - Arthrospore,
    - Blastospore,
    - Chlamydiospore (Candia- Cornmeal agar)

Fungal media -

- Sabaraud's Dextrose agar-
  - Antibiotic- Cycloheximide, Chloramphenicol & Gentamicin
  - pH- 5.6
- Niger seed Agar- For Cryptococcus
- BHI broth- Histoplasma & Cryptococcus

Fungal stains -

- Lactophenol cotton blue (LPCB)- demonstrate the hyphae

- PAS- widely used histopathological fungal stain
- Mucicarmine- Cryptococcus & Rhinosporidium
- India Ink & Nigrosin - Cryptococcus capsule
- Gomori Methinamine Silver- Pneumocystis

Not cultivable Fungi-

- Rhinosproridium
- Pneumocystis jerovecii
- Locazia

Superficial mycoses.

Tinea Versicolor –

- Malassezia furfur
- Chronic recurrent, Non inflammatory ,Non pruritic
- Hypo to hyper pigmentation
- Diagnosis-
  - Sphagetti & meat ball appearance
  - Lipoplihilc (SDA with olive oil overlay)
  - Fried egg colony
  - Urease +ve
  - Wood's lamp- Scaly lesion show golden yellow fluorescens
- ***Other infection by Malassezia furfur -***
  - Seborrheic dermatitis
  - Atopic dermatitis
  - Malassezia folliculitis
  - Systemic infections- In immunocompromised

Tinea nigra –

- Hortaea werneckii–Painless black non scaly on inflammatory patch on palm & sole

Piedra –

- White Piedra –
  - White nodule on hair shaft (less firmly attached) – Trichosporoon
  - Hyaline septate hyphae and rectangular arthrospores
- Black Piedra –
  - Black nodule on hair shaft (firmly attached) – Piedra hortae
  - Dark brown septate hyphae with ascus containing ascospores.

DERMATOPHYTE (TINEA OR RING WORM)

- Trichohyton- skin,nail, hair *(Tri- Three)*
- Microsporoon – skin, hair *(M not for N)*
- Epidermophyton- skin,nail

Clinical types-

- Tinea capitis
- Tinea corpris
- Tinea cruris
- Tinea barbae
- Tineapedis
- Tinea inguim

Tinea capitis-

Endothrix – arthrospore formations occurs within the hair completely filling hair shaft caused by - *T. tonsurans* & *T. violaceum*
Ectothrix – arthrospore on the surface of hair shaft caused by Example: *M audounii* , *M canis* , *T. mentagrophytes*
Kerion – Painful inflammatory reaction producing boggy lesions on scalp.→ *T. verricosum*

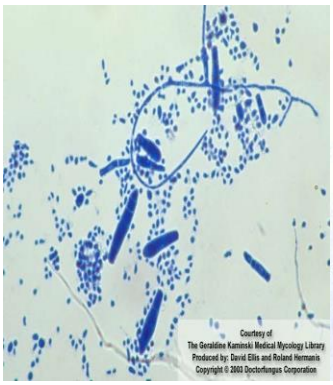
Favus-– cup like crust (scutula) forms around the infected hair follicle minimal hair shaft involvement Example: *T. schoenleinii*

Other dermatophyte-

- MC agent western world- T.rubrum, M canis
- MC type of dermatophytosis- T.pedis
- T.concentricum – T.imbricata

Lab diagnosis-

Dermatophytes	Macroconidia	Microconidia
Trichophyton	Rare, thin walled, smooth , Pencil shaped	Abundant
Microsporum	Numerous, thick walled, rough, Spindle shaped	Rare
Epidermophyton	Numerous, smooth walled ,Club shaped	Absent




- Wood lamp- For Microsporon spp
- Hair perforation test– T.mentagrophyte & M.canis
- Urease - T.mentagrophyte
- T/T- Gresiofulvin

Wood lamp examination-

Fungi	Fluorescence
Micosporum audouinii, M.canis and *M.ferrugineum*	Bright green
Trichophyton schoenleinii	Dull green
All other Dermatophytes	No fluorescence
Pityriasis versicolor	**Golden yellow**
Corynebacterium minutissimum	Coral-red

- **Dermatophytid or id reaction -** Hypersensitivity to fungus antigens may lead to secondary eruption in sensitized patients because of circulation of allergenic products.
- Occurs distal to primary site & culture negative
-

Sub cutaneous mycoses- -

Mycetoma

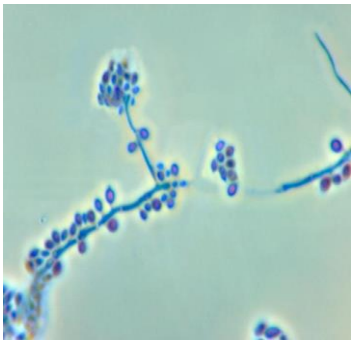
- ***SC infection - Triad- swelling, discharging sinus & granules***
- Affects extremities like hand and foot (bone), which are more prone for accidental trauma.
- Overall MC – Actinomycetoma
- Eumycetoma- More prevalent in North India, actinomycetoma more common in South India.

Eumycetoma	Actinomycetoma (white)
a. **Black Grain Eumycetoma**	*Actinomadura madurae*
Madurella mycetomatis	*Actinomadura pelletieri(red)*
Madurella grisea	*Nocardia brasiliensis*
Exophiala jeanselmei	*Nocardia caviae*
Curvularia spp.	*Nocardia asteroides*
b. **White Grain Eumycetoma**	*Nocardiopsis dassonvillei*
Aspergillus nidulans	*Streptomyces somaliensis*
Acremonium spp.	
Fusarium spp.	***Botryomycosis-*** MC agent-Staphylococcus
Pseudallescheria boydii	Others- Strepto ,E. coli ,Proteus species ,Pseudomonas

	ACTINOMYCOTIC	EUMYCOTIC
Tumor	Multiple tumour masses with ill defined margins	Single, well defined margins
Sinuses	Appear early, numerous, raised inflamed opening	Appear late, few in no.
Discharge	Purulent	Serous
Granule	White/ red	Black/white
Bone	Osteolytic lesions	Osteosclerotic lesions
Filament	< 2um (bacilli)	>2um (hyphae)

Sporothrix-

- Rose Gardner disease – gardner, carpenter, mine worker
- Chr SC pyo granulomatous nodulo ulcerative lesion
- Lymphatic spread
- Risk -Bare foot
- Sub Himalya
- Lab diagnosis : Cigar shaped asteroid body
- Hyphae with Flower like sporulation



Chromoblastomycosis

- Chronic localized infection of skin & subcutaneous tissue, most often involving limb with brown walled, globose bodies 5-13µ in size, called **sclerotic bodies or muriform cells/** Medlar body.
- ***Agents are- Phialophora , Fonsecacea, Cladosporium , Rhinocladiella***

Rhinospordiosis

- Chronic granulomatous disease by development of large polyps in the nose, conjunctiva & occasionally in ears, larynx, bronchus genitalia etc.
- India & Srilanka are endemic zones of Asia (Orissa, Kerala, Chennai)
- *Rhinosporidium seeberi* has not been cultured.
- Stagnant water is reservoir of infection.
- Spherules- Sporangia upto 350 µm contains endospores (6-9µ in size)
- Stains with mucicarmine stain
- Treatment →Radical surgery, dapsone

Systemic Mycoses- -

Candida-

- Commonest mycoses involving skin & its appendages, mucosa & internal organs
- Predisposing-
  - DM,
  - ↓ immunity,
  - Steroid,
  - Malignancy,
  - Febrile neutropenia,
- Pseudo hyphae- constricted wall near septa
- C.albicans- Most pathogenic species
- Diagnosed by-
  - Germ tube test(Renauld Braune Phenomena)+ve,
  - Chamydospore on cornmeal agar
  - C.dublinensis also shows +ve
- MC Non albican spp- C.parapsilosis >C.glabrata
- Disease produced-
  - Mucocutaneous,
  - Nail fold,
  - Systemic
- T/T-
  - Superficial- Nystatin, Mucocutaneous- Fluco,
  - Systemic- AmpB

Cryptococcus-

- True yeast
- Sexual spore- Basidiospore
- European Blastomycosis
- Source-Feces of Pigeon
- Mode of transmission- Inhalation >skin
- ***Pathogenesis*** – Virulence factors
  - Polysaccharide capsule – not immunogenic, No anti capsular ab formed.
  - Phenyloxidase enzyme responsible for production of melanin when grown on substrate like niger seed agar
- ***Clinical feature***- Pulmonary , Meningitis, Bone, Skin

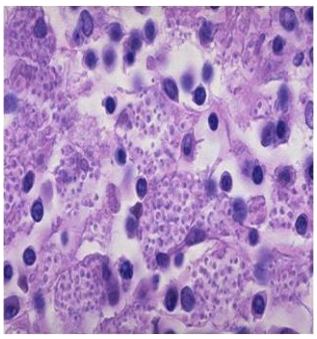
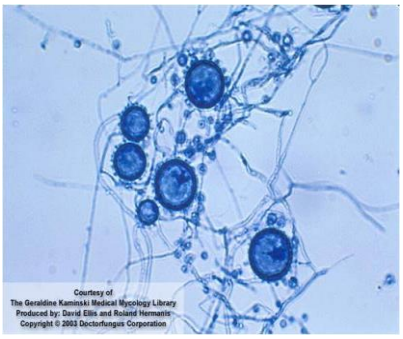
Lab diagnosis :

- India Ink / nigrosin– Capsule(Polysaccharide)
- Mucicarmine stain
- Latex agglutination test- Capsular Ag detection
- Culture-
  - SDA – smooth, mucoid, cream coloured colonies
  - Niger seed agar- Brown colony
- Urease+ve

Histoplasma capsulatum :

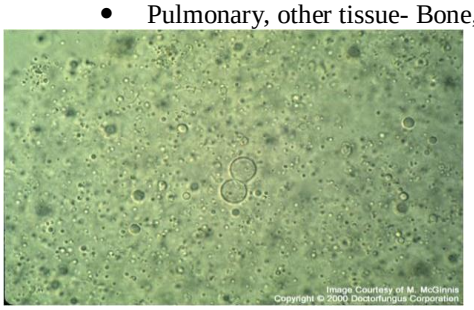
- Intracellular mycoses of Reticuloendothelial system.
- Darling Disease
- No true capsule
- **Clinical features -**
  - Pulmonary- Acute, Chronic (Histoplasmoma) .
  - Fever, wt. loss, hepatosplenomegaly and lymphadenopathy are the common
  - Skin(India)
- Presence of calcification and caseation necrosis , mimics Tuberculosis

- **Lab Diagnosis-**
  - Yeast at 37, Mould at 25
  - Narrow based budding YC 4-6µ
  - Tuberculate macroconidia
  - Ag detection in serum and urine
  - Histoplasmin skin test
  - Immunodiffusion for the detection of antibody (Exoantigen test)

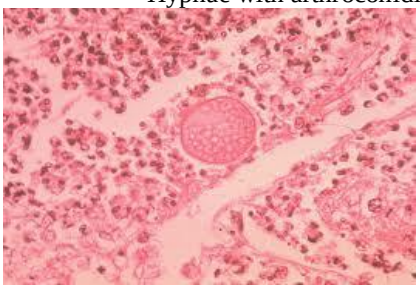
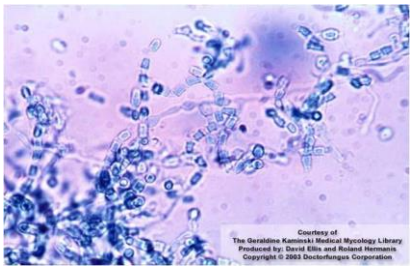
Blastomycosis-

- Chicago, North american Blastomycosis, Gilchrist disease
- Broad based budding YC 8-15µ
- Pulmonary, other tissue- Bone, skin



Coccidiodes :

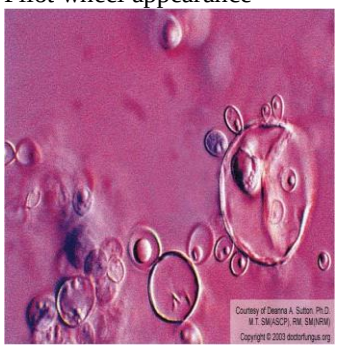
- Desert Rheumatism / Valley fever
- Endemic in South, North & Central USA & Mexico.
- Not reported from India
- Agent for Bioterrorism
- Pulm>bone
- Spherule filled withendospores at 37
- Hyphae with arthroconidia at 25

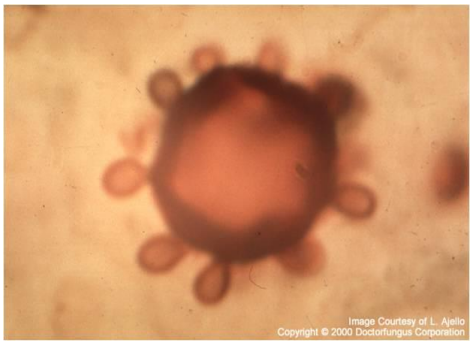



Paracoccidiodes -

- South american Blastomycosis
- Pulmonary infection
- Multiple budding yeast cell,

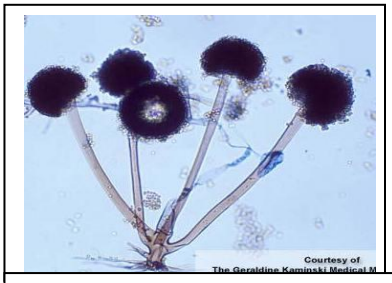
- Micky mouse appearance
- Pilot wheel appearance





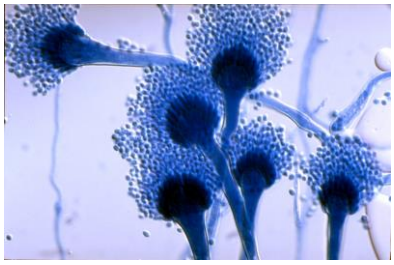
Opportunistic mycoses- **-**

Zygomycosis-



- Broad Aseptate wide angle hyphae
- Zygospore
- E.g – Rhizopus, Mucor, Absidia
- Risk- Diabetes(DKA), dialysis, iron overload
- Rhinocerebral, Pulmonary, Skin

Aspergillus-



- Narrow septate hyphae with acute angle branching
- ABPA
- Asthma
- Aspergilloma
- Invasive aspergillosis
- Otomycoses (MC)
- Oculomycoses
- Paranasal sinusitis
- Asp flavus- Aflatoxin- Ca Liver

Penicillium marneffi-

- Brick red pigment on SDA
- Isolated from Bamboo rat
- Shows thermal dimorphism
- Seen in S.E. Asia
- Produces wart like skin lesion, may spread systemically.
- Morphology -Sausage shaped cells with transverse septa which divide by ***binary fission.***

IMMUNOLOGY

IMMUNITY

Refers to resistance exhibited by host towards to injury caused by microorganism or its products

Innate immunity	**Acquired immunity**
• Resistance to infection that an individual possess from birth by its genetic or constitutional makeup. • Which can be- • Species or Racial or Individual specific	• Resistance to infection that an individual acquires during his life • Which can be- • active or passive • artificial or natural
• Occurs in Minute	• Occurs in Days
• Diversity limited	• Against Wide range of infection
• Non specific	• Specific
• No memory	• Memory Present
Components of Innate immunity • Phagocyte(monocyte, macrophage, neutrophils, dendritic cell), • Natural Killer cell, • Alternate complement pathway • Acute phase protein(CRP,MBP, serum amyloid protein), • Normal resident flora antagonism, • Inflammation, fever • Skin & mucosal barrier	
Components of Acquired immunity • T cell, • B cell, • Classical complement	

Active immunity	**Passive immunity**
Produced actively by host immune system	Received passively
Induced by infection or immunogen	Induced by readymade antibody transferred
Long lasting	Short
Lag period present	No Lag period
Memory present	No Memory
Booster doses-useful	Subsequent doses-Less affective
Negative phase may occur	No Negative phase
In Immunodeficiency- not useful	useful

Local immunity-

- Produced at mucosal surfaces- GIT or respiratory mucosa
- Provided by IgA antibody
- Induced by infection or by live vaccination

Herd immunity-

- Overall immunity of a community to a pathogen
- If Herd immunity is good- chance of epidemic is less
- Eradication of a communicable disease- depends on good Herd immunity
- Provided by mass vaccination by live vaccination to all individual at same time.

ANTIGEN, ANTIBODY AND ANTIGEN ANTIBODY REACTION

ANTIGEN

- Antigen- has to component
  - Immunogenicity
  - Immunological reactivity(antigenicity)
- Hapten – doesn't have Immunogenicity but retain immunological reactivity i.e antigenic
  - Simple hapten- Univalent, non precipitating with its antibody, only blocks the site on antibody
  - Complex hapten – Polyvalent, precipitating with its antibody.
  - Hapten becomes immunogenic by combing with carrier molecule.
- Epitope- Antigenic determinant

Antigenicity depends on:

- Size – Larger size, more antigenic
- Chemical – Protein> carbohydrate>lipid
- Susceptibility to Tissue enzyme- (latex & d amino acid are non antigenic)
- Foreignness
- Route of entry
- Genetic constituent

Heterophile specificity –

- ***Paul Bunnel*** – EBV with sheep RBC
- ***Weil felix***- Proteus OX2,OX19,OX K with Ricketssia alkali stable polysaccharide
- ***Cold Agglutination test*** – Mycoplasma with human O+ve RBC at 4c
- ***Streptococcus MG*** with Mycoplasma
- ***Forssman antigen*** – Lipid-CHO complex, present all except rabbit. So anti- Forssman anibody can be prepared in rabit
- ***Non treponemal test*** for syphilis like VDRL , RPR (Flocculation test)-
  - Treponemal antibody detected by using Cardiolipin antigen prepared from beef heart

T Independent Ag	T dependent Ag
Simple- LPS, capsule, flagella	Chemically Complex
Dose dependent Immunogenicity	Immunogenic over wide range of dose
Limited antibody response-IgM & IgG3	All Antibody can be raised
No memory	Memory present
No macrophage processing	Macrophage processing step is needed
Slowly metabolized	Rapidly metabolized

ANTIBODY

- Papain digestion produces-
  - 2 Fab portion- antigen binding site
  - 1 Fc portion- biological function like complement attachment site, adherence to monocyte
- 2 Heavy & 2Light chain
- Heavy chain - $\mu,\gamma,\alpha,E,\delta$
- Light chain – kappa/lambda
- Hinge region– rich in cysteine, prolin

IgM Antibody-

- *Possess highest MIS* –
  - Molecular weight (900,000),
  - Intravascular distribution (blood Antibody) (80%) ,
  - Sedimentation coefficient (19),
- Pentameric with 10 valency
- 1st to appear following infection, indicates recent infection
- 1st to appear in intrauterine life also ,(20wk),
- Responsible for-
  - Agglutination,
  - Haemolysis,
  - Opsonization,
  - Classical complement
- Example –
  - O Antibody in typhoid,
  - Reagin Antibody(syphilis),
  - Antibody of ABO), Rh system

IgG Antibody—

- *Possess highest DHS*-
  - Daily production,
  - Half life(23d),
  - Serum concerntration (*GAMDE*) ,
- Types- IgG1>2>3>4
- Responsible for-
  - Precipitation,
  - neutralization,
  - NK cell (ADCC)
  - Classical complement
- Appear late, indicates past / chronic infection
- IgG avidity increases with time – So, less avidity IgG indicates relatively recent infection
- Secreted in placenta, breast

IgE Antibody—

- Heat labile,
- Lowest *DHS*
- Responsible for- Type I HSN,
- Homocytotropic
- Reaginic antibody

IgA Antibody—

- 2nd most abundant
- Secretes in breast milk
- Types-
  - IgA2 – Surafce / mucosal IgA- Dimer joined by J chain & secretory piece (from epithelium)
  - IgA1 - Serum IgA – monomer, minor
- Responsible for-
  - Alternate Compliment
  - Mucosal /local immunity

Ig D Antibody –

- Surface Immunoglobulin
- Highest carbohydrate content

Abnormal Immunoglobulin

- Bence Jones Protein
  - Coagulate at 50c, redissolve at 70c
  - Elevated in Multiple myeloma
  - Light chain of immunoglobulin i.e. Kappa or lambda (but never both in same patient)
  - Unchecked proliferation of single clone of plasma chain
- Waldenstrom – IgM

Immunoglobulin specificity

- Idiotype – based on antigenic determinant in paratope known as idiotopes
- Isotype – difference b/t Immunoglobulin of different classes & subclasses present in all individual in a given species – IgG /A/M/D/E
- Allotype - difference between immunoglobulin of same classes between different individual in same species.

Antigen Antibody Reaction

Precipitation –

- Definition- *{Soluble Antigen + Antibody} → insoluble precipitate / floccules*
- Ring test –
  - Ascoli thermo precipitation test (anthrax) ,
  - Lancefield grouping (Streptococcus)
- Slide flocculation test – VDRL, RPR
- Tube flocculation test – Kahn test, standardization of toxin
- Immuno-diffusion (In gel) –
  - Produces visible band, so interpretation is easy
  - Can be preserved
  - Differentiate between antigens
- Example
  - Elek gel precipitation (C diphtheriae toxigenicity testing),
  - Eiken(E.coli)
- CIEP - αFP, Antigen of Cryptococcus & Meningococcus
- Rocket electrophoresis

Agglutination:

- Definition- *{Insoluble Antigen + Antibody)- clumps formation*
- **Example-**
- *Widal test*- Enteric fever
- *Standard agglutination* test - Brucella
- *Microscopic agglutination test* - Leptospira
- *Weil felix* - Rickettsia
- *Paul Bunuel* – EBV
- *Coomb test* for incomplete(IgG) Antibody
- *Passive agglutination test* (IHA)-
- Soluble antigen is coated on carrier particle like RBC, latex
  - So that a precipitation reaction can be converted to an agglutination reaction & hence better visualized.
  - E.g. -TPHA(syphilis), Rose Waaler test(Rheumatoid arthritis)
- *RPHA* (Reverse Passive Hemagglutination test)-
  - Antibody is coated on a surface of carrier particle like RBC to detect antigens
  - E.g. -HBsAg

CFT --

- Syphilis – Wasserman, TPI
- Sabin feldman test – Toxoplasma
- CFT for viral diseases

STRUCTURE OF IMMUNE SYSTEM AND IMMUNE RESPONSE

Lymphoid System – Lymphoid Organ + Lymphoid Cells

Lymphoid Organ -

- Central / Primary- Thymus & Bursa Of Fibricus(birds) / Bone Marrow(human)
- Peripheral/Secondary- Spleen, Lymph Node, MALT (GIT & Resp), Liver

LYMPHOID CELLS –

- T & B CELLS, NK CELL,
- Macrophage/ dendritic cell

THYMUS :

- 3rd / 4th pharyngeal pouch
- cortex – t lymphocytes, nurse cell
- medulla- epithelial cells , lymphocytes , Hassal's corpuscle
- Progenitor T cells passed through thymus
- 1% released out , rest destroyed(self reacting T cells)
- T cells are educated -Acquire Thy Ag- T dependent T cells
- Defect in thymus – CMI
- Runt disease- nude mice
- DiGeorge syndrome

Bursa of fabricus & bone marrow

- Bursa of fabricus- Birds
- bone marrow – humans
- All lymphocytes originates in BM
- T cell goes to thymus
- B cell proliferates in BM

Lymphocytes

- Short lived lymphocyte– life span 2week, effector cells
- Long lived lymphocyte – life span 2-3 yrs, memory cells
- Markers-
  - CD 10,19,22,23 – B cell markers
  - CD 3, 4,7,8 – T cell markers

T lymphocyte-

- Blast transformation occurs by –
  - Anti CD3,
  - Phytohemagglutinin,
  - Concovalin
- Formation starts from Yolk Sac → fetal liver → Bone marrow→ Thymus

In Thymus :

- CD7 +ve Pro T cell enter thymus
- Pre T cell (CD2 & CD3)
- Immature T cell – CD1,4,8 and T cell receptor
- TCR - $\alpha\beta$ or $\gamma\delta$
- TCR $\gamma\delta$ - IEL & intracellular organism like MTB
- Self reacting T cells are deleted

- Mature T cells – (loose CD1) – CD4 Tcells & CD8 T cells

CD4 T Cell

- Microbe→ APC → broken down to peptides → combined with MHCII → Presented to T cell with CD4

⇩

- 1st signal – Antigen-MHC complex to CD4 (CD3 also help)
- 2nd signal – B7(on APC) to CD28(on Tcell), can be blocked by CTLA4

⇩

- CD4 T cell activated – differentiated to Th1 or Th2

⇩

- TH1 – secrete IL2 & IFN ϒ
    - IFN ϒ– Activate macrophage , stimulate B cell & class switch to IgG2b
    - IL2 – T cell growth factor, activate DTH Tcell, convert NK cell → LAK cell
- TH2 – secrete IL4,5,6,10
    - IL4- Inhibit Th1, class switch to **IgE, IgG**
    - IL5- class switch to **IgA,** Chemoattractant to eosinophil

CD 8 expressing cells - MHC I restriction site (30%)

- T cytotoxic cells (Tc cells) – cause cytotoxic lysis of target cells
- T supressor cells (Ts cells) – supress immune response

B lymphocytes

- 10-20% of total lymphocyte
- Also act as APC - mediated by surface IgM receptors
- CD40 of B cell attach to CD40L of T cell – leads to B cell maturation
- Possess Fc Receptor
- CD21 – EBV receptor
- CD2 – measles receptor
- Microvilli present on surface
- Blast transformation occurs by –
    - Endotoxin,
    - Anti Ig, EBV,
    - Super Antigen

Macrophages

- Derived from the bone marrow
- Has role in :
    - phagocytosis
    - secretion of cytokines
    - antigen presentation
- Example-
    - Peripheral blood –***Monocytes***
    - Liver - ***Kupfer*** cells,
    - Brain - ***microglia***,
    - Kidney - ***mesangial cells***,
    - Bone - ***osteoclasts.***
    - Lung - ***Alveolar macrophage***
    - Any solid tissue- ***Histiocyte***
    - Inflammation site -***Multinucleated cell & epitheloid cell***

- Kill cell by –
    - Phagocytosis & phagolysosome fusion & lysosomal degranulation
    - Also by generating oxygen free radical

Dendritic cell

- Act as APC during primary immune response
- BM derived(separate lineage)
- Transport the presented Antigen to lymph node
- Possess MHCII > MHC I , also possess B7
- Non phagocytic
- Types-
    - Interdigitating
    - Interstitial
    - Langer Hans
    - Follicular –
        - Special type of dendritic cell
        - Doesn't possess any MHCII,
        - But have FcR & CR
        - Act on memory B cell

NK cell

- Natural killer cell / null cell / large granular (LGL)/ lymphokine activated cell
- NK cell→ LAK transformation is induced by IL2
- Constitutes 5-10% of total lymphocyte
- Possess indented nuclei & several granules
- NK cells are Not MHC restricted
- Possess CD16 & CD 56 markers
- Used in treatment of – renal cell Ca
- Mediates **ADCC** –Antibody dependent cytotoxicity
    - CD16(FcR like) recognized Fc portion of antibody bound to a target cell
    - Then, NK cell release perforins and granazyme which lyses the target cells
    - Cytotoxicity to virus infected cell & malignant cells, parasitic infection

HLA / MHC

- Transplantation antigen- determining histocompatibility
- HLA I, II,III
- Play a central role in antigen recognition
- MHC-short arm of chromosome 6 in humans
- Highly polymorphic – 24 alleles – HLA A, 50-HLA B
- Alleles – co dominantly expressed

- Class I MHC genes – glycoprotein-all nucleated cells
    - Present peptide antigen to Tc cells (CD8 T Cells)
    - Processing of virus infected cells & tumour cells

- Class II MHC genes- glycoprotein on all antigen presenting cells
    - Present peptide antigen to Th cells (CD4 T Cells)
    - Regulates immune response
    - Plays a central role in initiation of the immune response to transplantation antigens
- Class III MHC genes –genes coding C2,C4, properdin, factor B, C3 convertase, TNF, HSP

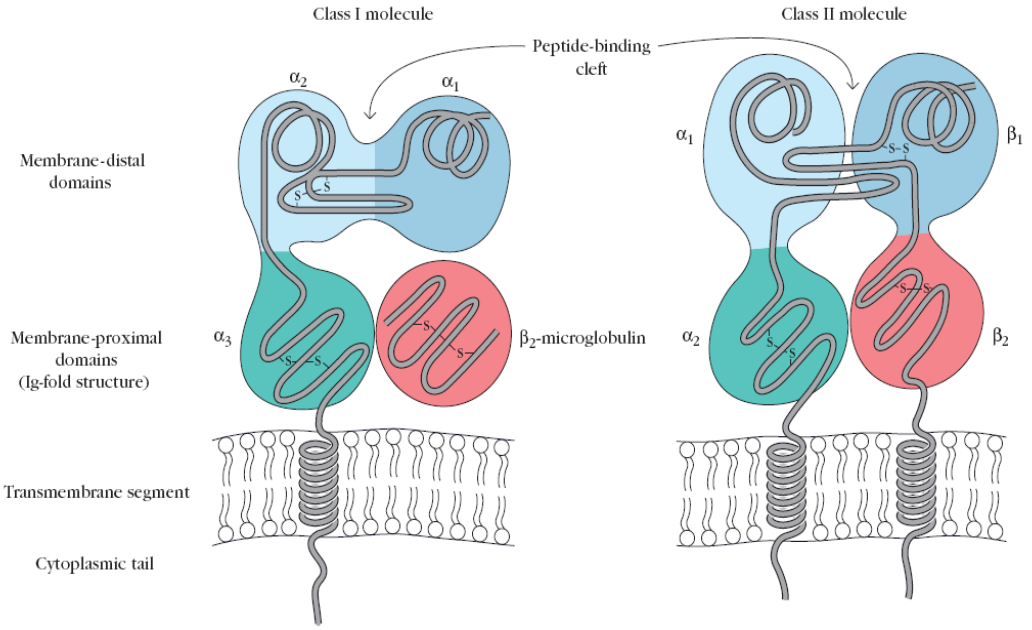


FIGURE 7-5 Schematic diagrams of a class I and a class II MHC molecule showing the external domains, transmembrane segment, and cytoplasmic tail. The peptide-binding cleft is formed by the membrane-distal domains in both class I and class II molecules. The membrane-proximal domains possess the basic immunoglobulin-fold structure; thus, class I and class II MHC molecules are classified as members of the immunoglobulin superfamily.

HYPERSENSITIVITY REACTION

Character	Immediate	Delayed
Type	I,II,III	IV
Time	minutes to hours	days
Mediator	antibodies	T cells
Route	any route	intradermal

TYPE I HSN :

- Sensitization phase-
  Priming Ag→ APC → T cell → Th 2 → B cell →IgE → Mast cell coated with IgE (Fc)
- Effector phase-
  Shocking dose → IgE (Fab) binds to Ag → mast cell degranulation
- Mast cell mediators-
  - Primary mediator(Hist, Serotonin, E&N chemotactic fact)
  - 2ndary factor – PG & cytokines
- Examples-
- Atopy – Asthma, allerigic rhinitis (hay fever) , food aller, atopic eczema
- Anaphylaxis
- Theobald smith phenomena , Schultz Dale phenomena
- Prausnitz Kustner reaction, Casoni test
- Cercarial dermatitis

TYPE II HSN :

- Compliment depd cytolysis – Transfusion reaction, erythroblastosis fetalis, AI hemolytic anemia
- Compliment depd inflammation (C3a,C5a) – GoodPasteur syndrome, Pemphigus vulgaris, Bullous pemphigous, rheumatic fever , vasculitis
- Compliment depd phagocytosis
- ADCC – (TypeVI)- by NK cell / eosinophil / neutrophil – parasite removal, graft rejection
- Ab depd cellular dysfunction – (TypeV)
    - Stimulation- Grave's disase (LATS)
    - Inhibition – Myasthenia gravis (Anti Ach receptor Ab)

Type III

- Immune complex mediated – deposited in tissue
- Usually complement bound to complex – so serum level falls
- Neutrophil attracted & release enzymes
- Examples-
    - Arthus Vs serum sickness
    - Hypersensitivity Pneumonitis(farmer's lung)
    - PSGN
    - Sub acute bacterial endocarditis
    - Microbial antigen – Str pyogenes, M.leprae, Trepanema, Plasmodium, Trypanosoma, HBV, HCV , EBV, Dengue
    - SLE , Rh A, PAN, Schick test
    - Hyper acute graft rejection
    - Katayama fever
    - Lepra reaction type II

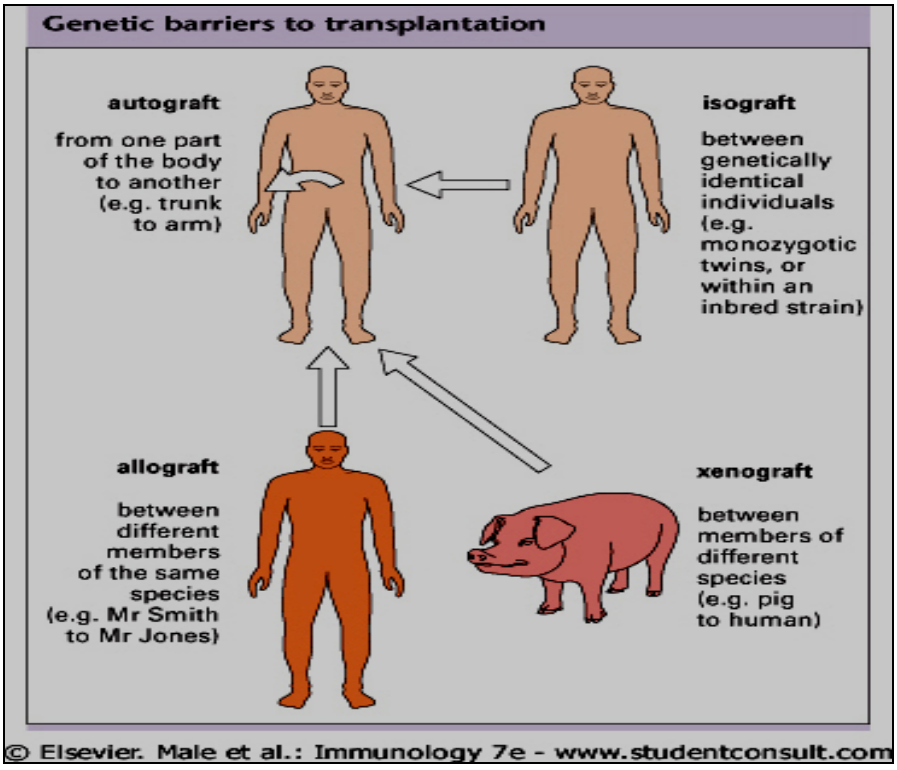
Type IV HSN

- Delayed type by CD4 Th1 cell
- IL2, monocyte chemotactic factor, Migration inhibitory factor
- IFNγ & TNF
- Examples-
    - Manotoux/Heaf,
    - Lepra reaction type I,
    - Contact dermatitis,
    - Frie test

Immunomodulator like –

- Monoclonal Ab- against tumor antigen
- Tumor vaccine
- Corynebacterium parvum , BCG
- Thalidomide, Levamisole

TRANSPLANT IMMUNOLOGY



GVH :

- Occurs when Graft mounts an immune response against host tissue.
- GVHR – Occurs when
    - graft contains immunocompetent cells
    - Recipient has transpalnt Ag that is absent in graft
    - Recipient has not rejected graft
    - Recipient cannot respond immunologically
- Embryos or neonates, adults in immunosuppression
- Runt disease
- Skin, liver and GI tract - main target organs of GVHD.

VIROLOGY

General properties of Virus

Virus Vs Bacteria –

Property	Virus	Bacteria
Nucleic acid	DNA or RNA	Both
Binary fission	No	Yes
Cellular organelle	absent	Present
Cellular organization	No	Present
Location	Intracellular	Intra / Extra
Resistant to	Antibiotics (exception- Rifampicin to Pox)	Sensitive
Culture in artificial media	No	Can be grown (except Rickettsia Chlamydia)
Ribosome	Absent	Present

Size of viruses

- Size – determines by
  - Ultrafiltration in gradcol membrane of graded porosity
  - Ultracentrifugation
  - Electron microscopy
- Largest – Pox(300nm) – Possess Elementary body
- Smallest – Parvo(20nm)

Shape of viruses

- Structure – consists of nucleocapsid (Nucleic acid & protein layer capsid)
- Capsid is made up capsomere units
- Most of the viruses are roughly spherical except-
  - Rabies –Bullet
  - Pox virus- Brick
  - Ebola virus- Filamentous
  - Tobaco mosaic virus– Rod
  - Space vehicle – Adenovirus

Nucleic acid-

- Made up either DNA or RNA
- DNA viruses- Herpes, HBV, Adeno, Papova, Parvo & Pox
- RNA viruses- others
- All the RNA viruses are single stranded except Reoviruses (double stranded RNA)
- All the DNA viruses are double stranded except Parvoviruses (single stranded RNA)

Symmetry –

- Icosahedron – All DNA, most of the RNA virus possess icosahedron symmetry
- Helical – Few RNA viruses (Bunya, Myxo, Rhabdo, Filoviridae)
- Pox – complex symmetry

Envelop:

Enveloped Virus:

- Made up lipoprotein subunits called peplomere
- Lipid part is host cell membrane derived & protein part is virus derived ,
- Envelop provides chemical, physical & biological properties to cell.
- Ether sensitive, heat labile, pleomorphic
- Example – All, other than nonenveloped virus are enveloped virus (See below)

Non-Enveloped virus:

- Ether resistant, heat stable & non-pleomorphic
- DNA- Parvo,Adeno,Papova ***(PAP)***
- RNA- Picorna, Astrovirus, Calcivirus, Reovirus ***(PARC)***

Segmented RNA (*BIRA*)-

- Bunya,
- Influenza,
- Rota,
- Arena(LCM)

Replication

1. **Adsorption-**
   - Most specific step requires respective receptors
   - If bypassed then any virus can attack any cell.
2. **Penetration**
3. **Uncoating-**
4. **Biosynthesis-**
   - *DNA Viruses* –Replicates in nucleus (except-Pox)
   - *RNA Virus*-Replicates in Cytoplasm (except Myxo & Retro virus)
   - Viral protein is synthesized only in cytoplasm
   - *+ve sense RNA virus*-
     - Viral RNA itself acts as mRNA
     - Infectious & translated directly to protein
     - E.g. –Picorna , Togaviruses
   - *-ve Sense RNA virus*-
     - Have polarity opposite to mRNA
     - Non infectious & possess their own RNA polymerase for transcription to form mRNA.
     - E.g. -Myxo, Rabies
   - *Retroviruses* -
     - Viral reverse transcriptase converts viral ssRNA to dsDNA
     - Then dsDNA integrates with host DNA.
5. **Maturation**
6. **Assembly**
7. **Release-**
   - Bacteriophage- by host cell lysis
   - Animal virus- usually without lysis (Myxo – by budding)
   - Exception- Picorna- by host cell lysis

Viral Cultivation

Animal inoculation

- Coxsackie – Mice- A-flacid paralysis, B- spastic paralysis
- Arbovirus

Egg inoculation-

- Chorioamniotic membrane – Produce pocks . E.g. Vaccinia, Variola , HSV
- Yolk sac- Arbovirus, chlamydia, Rickettsia
- Amniotic membrane- Influenza culture
- Allantoic cavity- vaccine preparation for Influenza , Yellow(17D), Rabies(Flury)

Tissue culture

- **Organ culture-** tracheal ring (corona)
- **Explant culture-** adenoid (Adeno)
- **Cell line –**

- ***Primary cell line*** – Undergo limited division(5-10) , diploid karyosome
  - E.g- Rhesus Kidney cell line, Human amniotic cell line, chick embryo fibroblast
- ***Secondary cell line*** – Undergo moderate cell division(10-50) , diploid karyosome
  - E.g.- Human fibroblast used for CMV
- ***Continuous cell line*** – indefinite divisions, haploid karyosome
  - E.g.- HeLa, Hep2, BHK
- **Cytopathic effect**
  - Crenation of cells & degeneration of entire cell sheet : Enterovirus
  - Syncytium formation: Measles virus , RSV, Herpes, Parainfluenza
  - Large granular clumps like grapes: Adenovirus
  - Cytoplasmic vacuolation- SV40
  - Diffuse roundening of cells- Herpes

Inclusion bodies

Intracytoplasmic –

- Negri body - Rabies
- Guarnier body – Vaccinia,
- Paschen body - Variola
- Bollinger body- Fowl pox
- Molluscum body – Molluscum contagiosum virus

Intranuclear -

- Cowdry A (*HAY*) -Herpes *(Lipschultz* body), Yellow fever (*Torres body*)
- Cowdry B (*BAP*) - Adeno , Polio
- Both – Measles, CMV

Viral interference –

- When two viruses infect a cell, one inhibits the multiplication of the other virus.
- Seen in Rubella, Polio

Viral hemagglutnation-

- Myxovirus, Rabies, Arbo, Pox
- Elution (d/t neuraminidase)– only in Myxovirus (except RSV & Measles)
- Hemadsorption- RBC adsorbed onto virus infected cell surface
- Mumps, Influenza, Parainfluenza

Latent virus

- Herpes – HSV I,II, VZV - nerve
- CMV – kidney, secretory gland
- EBV -lymphoid
- HIV – CD4 T cell
- Slow virus – neuron

Teratogenic virus

- CMV
- Rubella
- Herpes
- VZV

Transfer through Placenta -

- Coxsackie B
- Hepatitis B, C
- Parvo B19
- HIV
- Measles, Mumps

VIRAL VACCINE

Inactivated Vaccine –

Characters	Alpha	Beta	Gamma
Replicative cycle	fast (12-18hrs)	Slow (>24hrs)	Slow
Host range	Wide	Narrow	Narrow

- Salk polio -formalinized MKD
- Influenza
- Japanese B –(Nakayama) formalinized mouse brain
- Hepatitis B (subunit –HBsAg cloned in yeast)
- Rabies – BPL, Semple and non neural (PVC, PCEC, HDC)

Live Vaccine -

- Sabin Polio – Avirulent MKD
- Influenza - egg
- Japanese B- (14-14-2)
- MMR
- Yellow (17D) – chick embryo
- Small Pox
- Oka Strain – Varicella
- Towne & AD – CMV
- Jerryl Lynn- Mumps
- Edmonston – Zagreb : Measles
- RA 27/3 - Rubella

HERPES

HSV 1 & 2 – differentiated by

- HSV1 : lesion around mouth,
- HSV2 : lesion around genital infection,
- Transmission- HSV1- contact droplet inhalation, HSV2- sexual mode
- HSV2 larger pocks in Chick embryo
- HSV 2 replicates well Chick embryo fibroblast
- HSV 2 more temperature sensitive
- HSV 2- more neurovirulent
- HSV 2- more drug resistance
- Antigen detection & PCR also can differentiate.

Latency	Sensory ganglia	Salivary gland	Lymphoid tissue.
Cytopathology	Rapid, Cytolytic	Slow, Cytomegaly	Lympho-proliferative
Virus	HSV1,2, VZV	CMV, HHV6,7	EBV, HHV8
Latency	Neuron	CMV-Secretary glands, kidney, organs& tissues HHV6,7-Lymphoid tissue	Lymphoid tissue

Clinical feature

Mucosal –

- MC site – buccal mucosa
- Most frequent primary lesion– gingivostomatitis, pharyngitis
- Most frequent recurrent lesion - herpes labialis
- MC cause of ulcerative stomatitis- HSV
- 2ndrary bacterial infection is common with — Streptococcus, Pneumococcus

CNS-

- MC sporadic acute viral encephalitis
- Molaret meningitis
- MC site- temporal lobe
- Most imp diagnosis – brain biopsy
- Transverse Myeltis, GBS

Cutaneous-

- MC site- Face
- Herpetic whitlow- seen in doctor, nurse
- Erythema multiforme- MC cause –HSV

Ophthalmic-

- Acute keratoconjunctivitis
- Dendritic ulcer – Rx- Topical acyclovir, IFN C/I-steroid

Genital –

- HSV II MC than HSV I

Diagnosis-

- TZANCK smear of keratinocyte-
- Toludine blue staining of base of vesicle reveals multinucleated giant cell with faceted nuclei & homogenously stained ground glass appearance.
- Type A intranuclear inclusion body in Giemsa stain
- Isolation- CAM, primary human kidney cell line

Varicella Zoster-

- MC site- Spinal cord-D3 to L2(chicken pox) or Trigeminal N-Ophthalmic branch (Zoster)
- Contact with either chicken pox or zoster patient leads to only chicken pox but not zoster.
- One attack gives life long immunity
- Source- patients
- Portal of entry- respiratory tract or conjunctiva
- Incubation period- 2 weeks
- Infectious during initial stage- -2 to +5 days of onset of rash.
- Rash- usually start in trunk, rapid evolution, appears in crops, centripetal distribution
- Chicken pox is a disease of childhood
- When occur in adult, it is more severe with bullous & hemorrhagic rash
- Complication-
    - Varicella pneumonia
    - Myocarditis, nephritis, encephalitis, cerebellar ataxia
    - Reye's syndrome- fatty liver after salicylate

- Vaccine- Oka strain (live attenuated) & VZIG(immunoglobulin)
- Treatment- acyclovir, Steroid is contraindicated.
- Zoster-
    - Reactivation of latent virus
    - Trigeminal N (Ophthalmic branch)
    - Segmented & unilateral.
    - Affect Geniculate ganglia – ***Ramsay hunt syndrome*** characterized by-
    - Tetrad- *facial N palsy + vesicle on tympanic membrane, External auditory meatus & tongue*

Chicken Pox in pregnancy-

- For mother- high risk for pneumonia
- 1st half of pregnancy- asymptomatic
- Fetal varicella syndrome- skin lesion, limf hypoplasia, chorioretinitis & CNS defects
- Infection near delivery- congenital/neonatal Varicella
- If mother gets infection >1 week before delivery - then baby is mostly asymptomatic due to maternal antibody
- If mother gets infection within 2days of delivery - disseminated infection in baby

Chicken pox	**Herpes Zoster (Shingles or Zona)**
Primary infection	Reactivation of latent virus
MC site- Spinal cord-D3 to L2	Trigeminal N (Ophthalmic branch)
Generalized & bilateral	Segmented & unilateral.
Child > adult(severe)	Old age
Localization in skin capillary endothelial cells	Confined to segment of sensory nerve
Persist and reactivate as zoster	Act as source of chicken pox

CMV

- Largest member of Herpes virus family
- Enlargement of the virus infected cell
- Intranuclear & cytoplasmic inclusion body – owl's eye
- Spread slowly & requires close contact
- Route- via secretions, sexual, Blood transfusion
- Exhibit strict host specificity

Congenital – Cytomegalic inclusion disease

- Hepatosplenomegaly (MC),
- Microcephaly,
- Mental retardation
- 3Cs- chorioretinitis, cerebral calcification, convulsion
- Infants are -highly infectious
- Transmit the virus in urine for 3-5 year

Acquired :

- Mononucleosis like syndrome –
    - Occurs in adult (following Blood Transfusion)
    - Atypical lymphocytosis seen
    - Paul bunnel test (heterophile antibody) is negative
- Post kidney transplant infection

Lab diagnosis –

- Specimen – urine, saliva, cervix secretion, semen
- Culture- human fibroblast cell line,
- Growth occurs in 2-3wk, can be improved by shell vial technique
- IgM or fourfold rise of IgG
- pp65 antigenemia
- DNA PCR
- Transplacental CMV infection –
    - At Birth- IgG antibody +ve, IgM just appearing
    - At 1month – IgG antibody +ve, IgM peak
- Post natal CMV infection –
    - Birth- IgG antibody +ve, IgM -ve
    - 1month – IgG antibody +ve, IgM just appearing
    - 3month – IgG+, IgM peak

Treatment-

- Gancyclovir,
- Foscarnet,
- Valgancyclovir,
- Cidofovir,
- Miribaviris

EBV

- Attach to CD21/CR2 receptor on B cell
- But atypical lymphocytosis occurs with T cell
- B cell become immortalized, polyclonally activated leading to hypergammaglobulinemia
- Not highly contagious, Spread slowly
- Intimate oral contact required
- Source- Saliva (Kissing disease)
- Commonly found in hyperendemic malaria areas
- Disease –
    - Hodgkin lymphoma,
    - Burkit lymphoma
    - Nasopharyngeal Ca- Risk factor- genetic, salted fish(nitrosamine), herbal snuff (phorbolester)
    - Duncan syndrome- X linked lymphoproliferative disorder
    - Infectious Mononucleosis

Infectious Mononucleosis

- Glandular fever
- Seen in nonimmune young adults following primary infection with EBV
- Incubation period- 4-8weeks
- Characterized by-
    - atypical T cell (IM) ,
    - LN ↑ ,
    - Hepatosplenomegaly &
    - Rash(after ampicillin)
- Heterophile Antibody to sheep RBC – Paul bunnel test
- Confirmed by monospot test / differential absorption test

HHV 6-

- Sixth disease
- Exanthem subitum , Roseola infantum

HHV 8 –

- Kaposi sarcoma,
- Primary effusion lymphoma

OTHER DNA VIRUSES .

Small pox

- Variola- largest virus, possess ds DNA,
- Brick shaped,
- Only DNA virus which replicates in cytoplasm
- Small pox-Eradicated from world- 1983
- Still can be a potential agent of bioterrorism
- Paschen body (Variola) , Guarneri body(Vaccinia)
- Vaccinia pocks on CAM- larger necrotic & hemorrhagic than Variola
- Ceiling temperature- Vaccinia (41c), Variola(38c)
- Live vaccine using Vaccinia virus
- Vaccinia but not Variola can produce plaques on chick embryo

Small pox Eradication is successful because-

- Exclusively human pathogen, no reservoir
- Source- patient only, No carriers
- Highly affective Live vaccine-
    - Prepared from Vaccinia
    - Freeze dried vaccine (↑stability) & multiple puncture technique

Small Pox	**Chicken pox**
Rash- palm &sole & extensor surface	Rash- Axilla & flexor surface
Rash- deep seated & appear in 1 stage evolution is slow	Rash- superficial & pleomorphic (appear in crops) evolution is rapid
Fever subsides with appearance of rash	Fever rises with each crop of rash

Molluscum contageosum

- Seen in Children & young adult
- Pearly white wart like nodule on skin composed of eosinophilic inclusion bodies.
- Human are the only host.
- Cannot be grown in egg or tissue culture & animal.
- Sexually transmitted.

Adenovirus

- DNA, non enveloped, space vehicle shaped
- Manifestation-
    - *Hemorrahgic cystis* – Adenovirus type 11 & 21 (also by cyclophophamide)
    - *Infant diarrhoea*- Adeno 40,41
    - STD- Adenovirus type 37
    - Epidemic conjuntivitis – Adenovirus type 8,19,37 (shepard eye, industrial worker)
    - *Swimming pool conjunctivitis*- Adenovirus type 3,7,14
    - Pharyngitis & Pneumonia- Adenovirus type 3&7
- *Adeno-associated* virus – defective virus – requires Adenovirus for multiplication

Parvovirus

- Smallest, ss DNA
- ***5th*** disease
- Causes ***Erythema infectiosum***- characterized by rash, arthralgia , lymphadenopathy
- ***Slapped cheek*** appearance – rash 1st on cheek
- Causes ***aplastic crisis*** in sickle cell anemia patients
- Pregnancy- causes nonimmune fetal hydrops
- Transmission- respiratory route / blood

Papovavirus

Human Papillomavirus-

- Ca cervix –
    - Low risk – type 6,11 - CIN
    - High risk – type 16,18,31,33 – Ca Cx
    - Risk factor- early sex, multiple sex, multiparous, OCP
- Condyloma acuminata /genital wart- by Type 6,11
- Common wart /verruca vulgaris- by Type 1,2,3,4
- Epidermodysplasia verrucoplasia
- Vaccine –
    - Prophylactic – using HPV 16/18 late structural protein L1
    - Therapeutic – using HPV 16/18 Early non structural protein E6/E7

Other Papova viruses-

- JC virus- Hodgkin disease & PML (progressive multifocal leukoencephalopathy)
- BK virus- renal infection
- Polyoma virus
- Simian Vacuolating virus

Bacteriophage

- Viruses that attack bacteria
- dsDNA surrounded by protein coat
- Bacteriophage with ssDNA or RNA -also identified
- Consists of head, neck & tail
- 2 cycles- Lytic & lysogenic phage
- Uses:
    - ***Phage typing***- Staphylococcus, Vi antigen typing of S.Typhi, V.cholerae
    - ***Phage assay***- depending on the no, size, shape & nature of the plaque produced by the bacteriophage on a lawn culture of bacteria
    - ***Transduction***
    - Used as a ***cloning vector***
    - Used in diagnosis- e.g. *Mycobacteriophage*
    - ***Codes for Toxin***- Cholera toxin, VT of EHEC, Botulinum toxin C,D, Diphtheria toxin & Streptococcal pyrogenic toxin A,C

MYXO VIRUS .

Orthomyxo –

- Influenza A,B,C
- Segmented RNA, unstable
- Both hemagglutinin(HA) & Neuraminidase(NA) spikes present, So Hemagglutination is reversible(Elution)

Paramyxo-

- Para influenza, Measles, Mumps, RSV, Metapnemuovirus
- HA spike present in - Parainfuenza, Mumps ,Measles,
- NA spike present in - - Parainfuenza, Mumps
- Single RNA, stable
- Inclusion body- intracytoplasmic (only for measles it is both intracytoplasmic & intranuclear)

INFLUENZA

- Influenza – A(MC), B, C
- Antigenic Shift – Results in Pandemic – MC seen in Type A
- Antigenic drift – Results in Epidemic – MC seen in A,B

- HA Ag- H1 to H15- Protective
- NA antigen – N1 to N9, less protective
- MC manifestation- URTI
- MC complication-
    - Bacterial pneumonia > viral pneumonia
    - Reye's syndrome- with ***Type B*** (following aspirin)

Diagnosis-

- Egg inoculation– Amniotic cavity- by A,B,C Allanotic cavity -by only A
- Hemagglutination with Fowl & Guinea pig RBC
  Type A- agglutinates with Guinea pig, B-both, C- agglutinates with fowl RBC at 4c
- Ag detection from nasopharyngeal cells by Immunfluroscence
- Four fold rise of Antibody by – Hemagglutination inhibition test (HAI)
- Vaccine- Killed, Live, Recombinant

Avian Flu

- H5N1
- Seen from 2003 onwards
- Only bird to human transmission seen, but no human-human transmission seen
- Highly virulent (as PB1F2 targets host mitochondria, induces apoptosis)

H1N1 2009 Flu

- April 2009
- Pandemic – Including India
- Recombination of 4strain- (1 Human + 2 Swine + 1 Avian)
- Swine influenza strain causing pandemics so far-
  H1N1classical (1918), H1N2, H3,N2, currently 2009 H1N1recombinant strain
- Human to human transmission seen
- Less virulent (as it lacks PB1F2 gene)
- Diagnosis – by RT PCR detecting HA & NA genes
- Treatment- NA Inhibitor – Oseltamivir (Tamiflu) , Zanamivir
- Resistant to Amantadine
- Vaccine-
    - Injectable killed – HA Protein
    - Live nasal spray – HA protein

MUMPS

- MC cause of Parotitis (Non suppurative enlargement)
- Complication-
    - Epididymo-Orchitis (U/L > B/L)- seen in 1/3rd of post pubertal male patients
    - Aseptic meningitis
    - Pancreatitis
    - Diabetes- Mumps, Rubella, CoxSackie B4
- Resolve except deafness
- 2^{ndary} attack – 85%
- Human – only host,
- Source –only patients, no carriers
- MC seen in Children
- Once infected, gives lifelong immunity
- Transmission- Droplet, saliva, Direct contact, Fomite borne, urine
- Saliva is infectious from -1 to +2 wk of parotitis
- Specimen- urine, saliva, CSF
- Lab diagnosis-
    - Isolation- Egg, MKC,
    - IgM Antibody by ELISA
    - CFT using S Antigen

  - Antibody to S antigen (internal Ag) appear early, goes early – indicates acute infection
  - Antibody to V antigen (surface Ag) appear late, goes late
- Vaccine- Jeryl Lynn strain (at 9months) & MMR

MEASLES

- Rubeola
- MC childhood rash
- Source – cases only, no subclinical/carrier stage
- Highest 2ndary attack rate- 90%
- Period of communicability- -4 to +5 d of rash
- Incubation Period- 10days
- Koplik's spot – pathognomic, appear before rash
- Rash – appear at 4th day fades after 4day, MC site is face & neck
- Diagnosis-
  - Antigen detection by IF (also +ve before rash)
  - Warthin finkeldy giant cell
  - Isolation from nose, throat, conjunctiva & blood- Amniotic route & PKM cell line
- Complication-
  - Diarrhea
  - Pneumonia
  - Otitis media
  - Encephalitis,
  - ***SSPE*** – (subacute sclerosing panencephalitis)-high titer antibody in CSF is diagnostic
  - Suppressed delayed hypersensitivity (false –ve Mantoux test & worsening of TB)
  - Recovery from measles leads to- recovery from asthma, lipoid nephrosis
- Vaccine-
  - Prepared- chick embryo or human diploid cell line (no egg vaccine available)
  - Age- given at 9 months (maternal antibody disappears)
  - Can be given at 6 months if measles outbreak seen (2nd dose to be given at 9 month)
  - Type- Live attenuated- Edmonston-Zagreb strain or combined (MMR, MR, MMRV)
  - Side effect- toxic shock syndrome(d/t contamination of vial), mild measles like illness
  - Vaccination to contacts- given with in 3days is affective
    (IP of vaccine strain is 7days & wild strain is 10days)
  - Immunoglobulin- can also be given 3days (in this case live vaccine is given after 8-12wk)

RESPIRATORY SYNCYTIAL VIRUS

- Only HA, No NA spike
- MC cause of bronchilitis in children
- Also causes pneumonia & otitis media
- Age- ***6wk-6months***, new borne are protected due to maternal antibody
- RSV- MC precipitating factor for Acute asthma
- Rhino- MC precipitating factor for Ac exacerbation of chronic asthma
- Reinfection- milder illness
- Causes epidemics in winter(temperate) & rainy (tropics)
- Culture- giant cell & Syncytial formation
- T/T- Rivabirin

PARA INFLUENZA VIRUS

- Para influenza type 1& 2- causes ***Croup*** (acute laryngotracheobronchiolitis)
- Para influenza type 3 – LRTI
- Type 3- more Endemic & affects <1yr, causes shipping fever in cattle
- Type 1 & 2 – affect preschool children

RUBELLA

- German measles,
- Belongs to family Togaviridae, Not a Myxovirus
- Identified by interference with Echovirus
- Source- cases
- Period - -1wk to +1wk
- IP-2-3wk
- Transmission- droplet, contact, sexual
- Rash on day1 (face), Lymphadenopathy
- **Forchheimer spots** seen

- ***Congenital Rubella-***
  - 1st Trim –risk is maximum , after 5th month– risk negligible
  - Classical Congenital Rubella syndrome- Triad- cataract(MC), deafness, cardiac(PDA)
  - Expanded Congenital Rubella syndrome – myocarditis, hepatosplenomegaly, bone lesion

- **Vaccination – *RA 27/3live attenuated***
  - Prepared from Human diploid cell line
  - Given aft 1 yr,
  - If later then, pregnancy should be avoided for 3mnoths

Rash :

- 1st – Rubella
- 2nd – Chicken pox
- 3rd –Small pox
- 4th- Measles
- 5th- Parvo B19 – Exanthem infectiosum
- 6th- HHV6- Exanthem subitum / Roseola infantum

Vaccine storage :

- Deep Freezer –Polio, Measles
- Vaccine stored at 4c : DPT, Typhoid, TT, DT, BCG diluent

Incubation Period-

- 1wk- Diphtheria(2-6d)
- 1-2wk –Measles(10d), small Pox-(12d), Pertussis(7-14), Polio(7-14) ,Tetanus (6-10d)
- 2-3wk – chickenpox(14-16d), Mumps(18d), Rubella(18d)

PICORNAVIRIDAE .

Include two major groups of human pathogens:

- Enteroviruses - Polio & Coxsackie
- Rhinoviruses

Poliovirus

- 3 subtypes-
  - Type1- MC wild type
  - Type2
  - Type3 – MC cause of vaccine associated paralysis
- CFT detects-
  - C Ag (coreless),
  - D Ag(dense Ag, type specific)
- Risk factors –
  - Following Tonsillectomy,
  - Pregnancy

- IM injection
- Muscular activity,
- Coxsackie A,
- Genetic predisposition

- Pathogenesis-
  - Mode if transmission- Faeco oral > Inhalation > conjunctiva
  - Spread- hematogenous spread(MC), also direct neural spread(Following Tonsillectomy)
  - Site of action- Anterior horn of spinal cord
  - Earliest change in neuron ***-Nissl body*** degeneration
  - Pathological changes always more extensive than distribution of paralysis
- Clinical types-
  - In apparent infection- 90-95%,
  - Abortive infection- 5-10%
  - Aseptic meningitis (non paralytic) – 0.1%
  - Paralytic (<0.1%) - Ascending flaccid paralysis (AFP)

Lab diagnosis-

- Blood, throat swab, CSF, feces(till 6wk)
- Isolation- Monkey kidney tissue culture followed by Neutrilization test.
- Neutralizing Antibody- comes early, stay long
- CFT Antibody- Anti C- comes early, goes early, Anti D- comes late, goes late
- OPV- Type1-1L, 2-2L, 3-3L TCID50, MgCl2, pH<7, 20c
  wild strain-

Polio vaccine	**Salk (Injectable)**	**Sabin(Oral)**
Preparation	Formalin killed preparation of all 3 types in MKC	Each dose contains- • Type 1- 10lakh, Type 2- 2lakh • Type 3- 3lakh of TCID50, • Stabilized in *MgCl2, pH<7*
Safety	Relatively more safer	Safer except in immunocompromised pt
Efficiency	80-90% by full course	90-100% by 1 or 2 doses Efficacy decreases by • Interference by other enteroviruses • Frequent diarrheal disease • Breast feeding
Economy	Relatively expensive	Economical
Duration of protection	Need booster doses periodically	Long lasting
In epidemics	Cannot be used	Can be used
Herd immunity	Not provided	Provided
Local mucosal immunity	Not provided	Provided (IgA antibody)

Coxsackie virus

Group A Coxsackie virus	**Group B Coxsackie virus**
Suckling mouse inoculation- • Flaccid paralysis • Generalized myositis	**Suckling mouse inoculation-** • Spastic paralysis • Focal myositis • Necrosis of brown fat • Pancreatitis, hepatitis, myocarditis, encephalitis

Furious Rabies	Dumb Rabies
80% of all cases	20% of all cases
Hydrophobia,	No hydrophobia
Absent- fever, Fasciculation ,Percussion edema	Present –Fever, Fasciculation ,Percussion edema
Paralytic, Proximal muscle	Paralytic, Proximal muscle
Seen in unvaccinated individuals	Associated with partial vaccine course

• Herpangina (vesicular Pharyngitis) • Hand-foot-and-mouth disease • (Also by Enterovirus-71) • *Acute hemorrhagic conjunctivitis* • (Cox-A24 &Enterovirus 70) • Pneumonitis of infants • Diarrhea • URTI • Fever with rash	• Pleurodynia (epidemic myalgia)/ Bornholm disease- B1,B5 • Myocarditis, pericarditis • Diabetes mellitus – B4 • Pneumonia **Both Cox A & B – can cause** • Aseptic meningitis (MC-A7), • encephalitis, • cold, • hepatitis

RABIES VIRUS .

- Bullet shaped, -ve sense RNA virus
- Reservoir –
  - Urban Rabies- Dog 99%, cat
  - Wild life Rabies- Fox, Jackal, Wolf
- Source – Saliva of Rabid animal
- Mode of transmission- Bite(MC), Lick on abrasion, corneal transplant, air borne
- Speed of Rabies progress in sensory nerve – ***3mm/hr***
- Earliest symptom – Neuritic pain at bite site
- Sensory N → UMN → sympathetic → mental system
- Incubation period- 1-3 month,
- IP is shorter in children & upper limb bite (than leg bite)
- Mechanism-Neural apoptosis, ↓Acetyl choline

Street virus-	Fixed virus
• Freshly isolated,	• After serial passage
• Produce Negri body ,	• Doesn't Produce Negri body ,
• Affect salivary gland	• Doesn't affect salivary gland
• Incubation Period-1-12wk	• IP-5-6days • Used for vaccine

Antemortem diagnosis:

- Direct Florescent Antibody test-
  - corneal impression smear (30%Sn, in late stage) ,
  - hair follicle of nape of neck
- Isolation – mice, cell line followed by IF
- Antibody in CSF – MNT, RFFIT, FAVN, IFA
- RNA detection by RT-PCR

Post mortem diagnosis :

- Negri body-
  - Pathognomic – 3-27µ
  - Hippocampus MC site (next-cerebellum)

- Absent in 20% cases
- Seller technique – methylene blue & basic fuchsin

- Mouse inoculation,
- Isolation
- DFA on brain smear

Vaccine –

- Neural –
  - Semple, BPL (Konnor), Infant brain
  - Poor immunogenic, encephalogenic
- Non neural –
  - Purified chick embryo cell (PCEC)
  - Purified vero cell (PVC)
  - Human diploid cell (HDC)
  - Protection- for 6m
- Pre Exposure – 3dose- at day 0,7,28
- Post exposure – 6 dose, at day 0,3,7,14,28 & 90 days

ARBOVIRUSES

Arboviruses common in India –

- Hemorrhagic - Dengue, Chikungunya, KFD
- Encephalitis- Japanese B ,Westnile, Sindbis
- Rare- Ganjam, Vellore, Chandipura, Bhanja
- Yellow fever NOT found in India

Animal Reservoirs

- In many cases, the actual reservoir is not known.
- Birds: Japanese encephalitis, St Louis encephalitis, EEE, WEE
- Pigs: Japanese encephalitis (Amplifier host)
- Monkeys: Yellow Fever
- Rodents: VEE, Russian Spring-Summer encephalitis

Clinical features

- Fever and rash with athralgia- non-specific.
- Encephalitis - e.g. EEE, WEE, St Louis encephalitis, Japanese encephalitis.
- Hemorrhagic fever - e.g. yellow fever, dengue, Crimean-Congo hemorrhagic fever, Chikungunya, Hanta
- Hemorrhagic fever with shock - Dengue

Lab Diagnosis

- Serology –
  - HAI Antibody, CFT Antibody, Neutralizing Antibody
  - ELISA – MAC ELISA(IgM), IgG ELISA
  - Indicators:
    - IgM detection
    - Seroconversion of IgG,
    - Four fold rise in titer of IgG of paired sera
- Isolation-Suckling mice brain ,Mosquito inoculation
- Culture -mosquito cell lines : C3/36 cell line
- Detection of antigen – NS1 Antigen (ELISA & ICT)
- Detection of RNA (rt-PCR)

Japanese Encephalitis

- 1st seen in Japan as "Summer encephalitis" epidemics – but now uncommon in Japan
- Called 'B' – to distinguish from encephalitis A (encephalitis lethargica / von economo disease)

- Affect from Korea to India & Malayasia
- Vector : *C. tritaeniorhynchus,* C.vishnui(India)
- India- vellore, TN-AP-KA border, now world wide
- Reservoir: Ardeid (wading) birds
- Amplifying hosts- Pigs, bats
- Incidental hosts-Horses, humans, others
- The most common cause of epidemic encephalitis.
- Seasonal variation-
    - Temperate area (June –September) &
    - Subtropical areas (March- October)
- Live attenuated vaccine (14-14-2)
- Inactivated vaccine (Nakayama strain)

Dengue

- DEN 4 serotypes -1,2,3,4 – Type3 MC
- Vector -Aedes aegypti mosquito
- Mixed infection : Antibody dependent enhancement (ADE)
- Clinical feature-
    - Dengue fever: (DF) : Biphasic (Saddle back), Break bone fever, LN↑, Maculopapular rash
    - Dengue Hemorrhagic Fever (DHF)
    - Dengue Shock Syndrome (DSS)

Chikungunya

- Fever, rash, LN↑, arthralgia (name derived -doubled up due to joint pain)
- Differ from Dengue-
    - Hemorrhagic manifestations rare,
    - Chikungunya outbreaks are shorter
- Vector- Aedes aegypti
- 1964 outbreak- Africa, India (vellore,pondicherry,chennai)
- 1973-2005 – No outbreaks
- Re-emerged in 2005 – Hyderabad, Karnataka, Maharastra
- Reason of re-emergence-
    - New vector- Aedes albopictus
    - Viral factor- E1 glycoprotein mutation of virus ,
    - More rural involvement

Yellow fever virus

- Endemic- West Africa and Central South America.
- Reservoir-
    - Forest- Monkey & forest mosquito
    - Urban- cases urban mosquito
- 2 major forms:
    - Jungle YF -cycle involving primates and forest mosquitoes.
    - Urban YF is - cycle involving human to human by Aedes aegypti mosquito.
- ***Don't Exist In India***- because
    - Aedes aegypti is present in East cost area in India (where as YF is endemic in West Africa)
    - Strict vigilance & Quarantine for the travelers
    - Cross reacting Dengue antibody provides protection
    - But YF immunization doesn't protect from Dengue
- Incubation period- ***3-6days***
- Clinical feature-Hemorrhages, Fever, Platelet dysfunction, Relative bradycardia, Jaundice
- Liver -midzonal necrosis, Councilman bodies
- ***Torres*** bodies(intranuclear inclusion body)
- Darkar vaccine – mouse brain vaccine, risk of encephalitis
- 17D Live attenuated vaccine
    - Prepared in India(CRI, Kasuli)

- Chick embryo, no risk of encephalitis
- Single dose given sc
- 95% effective within 10 days of inoculation
- Reimmunization required every 10 years for travelers
- Cholera & YF vaccine shouldn't be given together
- Measures in airport-
    - Unprotected travelers - 6days quarantine
    - Aedes aegypti index <1 400mt surrounding airport

Kyasanur Forest Disease

- Hemorrhagic fever
- Tick borne
- Seen in Kyasanur Forest in Shimoga District, Karnataka

Incubation Period –

- Dengue-5-6days,
- Chikungunya-5-6d days,
- Japanese B-5-15 days,
- Yellow Fevr-3-6 days,
- KFD-4-8 days

Hemorrhagic virus (Non Arthropod borne)

Hantavirus :

- HF with renal syndrome –
- Rodent borne (MC route- inhalation from rodent excreta)
- Hanta pulmonary syndrome – by Sin Nombre virus

Others- Marbug , Ebola, Lassa

HEPATITIS VIRUSES .

Viral causes of hepatitis

- Hepatitis A,B,C,D,E,G
- Yellow fever virus
- Cytomegalovirus
- Epstein bar virus
- Herpes simplex virus
- Rubella virus
- Entero virus
- Others - Leptospira, Toxoplasma, Coxiella

	HAV	HBC	HCV	HDV	HEV
Common name	Infectious	Serum	Non A non B Post transfusion	Delta agent	Non A non B Enteric transmitted
Family	Entero 72	Hepadna	Flavi	Viroid like	Calci
Onset	Abrupt	Insidious	Insidious	Abrupt	Abrupt
Age	Children	Any	Adult	Any	Young adult
Route	Feaco-oral	Blood, sexual, vertical	Blood, sexual, vertical	Blood, sexual	Feaco-oral
I.P (days)	15-50	50-150	15-150	15-50	15-50
Mortality	<0.5%	1-2%	0.5 – 1%	High	Usually 1-2% & Pregnancy -20-40%
Chronic carrier	No	yes	yes	Yes	No
Oncogenic	Nil	Present(neonate)	Present	Nil	Nil
Associated Other feature	Secondary attack rate 10-20%	HCC, cirrhosis, Autoimmune disorder like AGN, arthritis, PAN	HCC, cirrhosis, Autoimmune-AGN, arthrits, cryo-globulinemia	HCC, cirrhosis, fuminant hepatitis	Secondary attack rate 1-2% Not seen in western countries

Laboratory diagnosis of Hepatitis

HAV	HBV	HCV	HDV	HEV
• **IgM HAV** • **HAV RNA**	• HBsAg- Acute, chronic, carrier • ***HBeAg- active infection*** • Anti HBc – ○ IgM- Acute , ***window period*** ○ IgG- chronic infection • Only Anti HBs – vaccination • HBV DNA - active infection, viral load (monitoring infection) • Anti HBc – epidemiological marker	• Antibody by 3rd generation ELISA using **NS5**Antigen • HCV RNA • Genotyping- • 7 sub types	• HBsAg • **Anti HBc** ○ IgM-Coinfection ○ IgG-Superinfection • HDV Ag • HDV RNA • Anti HD IgM	• Anti HBE Antibody • EM of stool • HEV RNA

HBV

- HBV – ds DNA virus (double strand is incomplete)
- DNA polymerase has double action – DNA depd DNA polymerase + RT activity
- Has 3 form- spherical(MC), tubular , Dane particle(complete)
- > 1/3rd of world population are infected with HBV
- Pathogenesis- immune mediated
- Hepatocyte carrying viral antigen subjected to Antibody dependent NK cell / CD8 T cell cytotoxicity
- In absence of affective immune system- leads to carrier state (infants)
- Animal exp – chimpanzee
- HBV doesn't grow in conventional cell line

HBV Gene-

- S gene (HBsAg),
- Core gene- HBcAg,
- Pre core- HBeAg,
- P gene-DNA polymerase,
- X gene –Regulatory gene

Mutants-

- Precore mutant – unable to form HBeAg, medeterrian
- Escape mutant- unable to form HBsAg-ve (S gene mutation)
    - Seen in Infant borne to HBeAg +ve mother
    - Seen in Liver transplant recepient receiving combined HBV Vaccine + Immunoglobulin

HBV Carrier:

- >6 months
- India ranks 2nd (China 1st)
- Populations can be divided to-
    - Low endemicity – carrier rate - <2% (North India)
    - Intermediate – 2-8% - India
    - High endemicity- >8%(south India)
- ***Super carriers*** **-** High HBsAg , HBeAg, HBV DNA, DNA Polymerase
- ***Simple carrier*** **–** Low HBsAg, No HBeAg
- ***Carrier rate*** : Following infection-
    - 5-10% of adult becomes carrier
    - 50% of children becomes carrier
    - 90% of neonate becomes carrier

Transmission-

- Blood(MC route in developing)- highly infectious than HIV
- Vertical-
    - During delivery(MC)
    - In-utero
    - Breast feeding
    - HBeAg +ve mother – high risk
- Sexual(MC route in developed country)
- Direct skin contact with open skin lesion
- High risk occupation- paramedical workers, sex workers
- Though HBV can survive in Mosquito, but no transmission seen.
- Age- Developed country(Young adults), Developing country(younger age)
- No seasonal variation

Vaccine-

- HBsAg subunit – prepared in Baker's yeast
- 3 dose – 0,1,5 months
- Booster after 5yrs if *Anti HBS <10 IU/ml*
- Non responder – 5-10%
- Neonate born to HBV mother – ***HBIG + Vaccine*** (*with in 12hr*)
- Newer- containing whole HBsAg (i.e. *Pre S1 + Pre S2 + S)*

HCV

- Belongs to Flaviviridae,
- ss RNA, enveloped
- 50-80% of patients develops - chronic infection
- Carrier rate- 1-20%
- Affect Only human
- MC cause of post transfusion hepatitis
- Mode of transmission – BT,IV drug abuser, sexual, vertical
- Antigenic Diversity- 6 genotype (quasispecies)
- Hence, affective vaccination is difficult

- 3rd generation ELISA detecting antibody against NS5 Ag
- RNA PCR

HGV

- Flavivirus
- Also known as GB virus
- Ss RNA
- Mode of transmission – BT, sexual, vertical
- Associated with – Acute, chronic, fulminant hepatitis

HIV .

Antigen-

- Envelope antigens
  - Spike antigen-gp120(principal antigen)
  - Transmembrane pedicle protein-gp41
- Shell antigen
  - Nucleocapsid protein-p18
  - Core antigen
  - Principal core antigen-p24
  - Other core antigens-p15, p55
- Polymerase antigens
  - p31, p51, p66

Types of HIV

- HIV- I & HIV- 2
- HIV- 1- divided to 3 groups – M (major), O (outlier) , N (new)
- M group -10 subtypes or clades (A to J)
- India- Type 1-clade C more common and both type 1 & 2 are seen
- 90 %HIV cases in AP, TN, Karnataka, Maharashtra, Nagaland and Manipur

Mode of transmission

Type of exposure	risk %
Sexual	**0.1-1%**
Blood transfusion	**>90%**
Tissue or donation of organ	**50 -90%**
Injection and injuries	**0.5 – 1%**
Mother to baby	**30%**

High viral load found in – blood, genital secretion, CSF

- **Receptors** -binds to gp120 of HIV
  - All cells expressing CD4 molecules like T helper cells(T tropic strains),
  - Macrophages(M tropic strains)
  - Dendritic cells and
  - Glial cells
- **Co-Receptors**- binds to gp -41 of HIV
  - CXCR4- T tropic strains
  - CCR5-M tropic strains

ASSOCIATION BETWEEN OPPORTUNISTIC INFECTIONS AND CD4+-LYMPHOCYTE COUNT

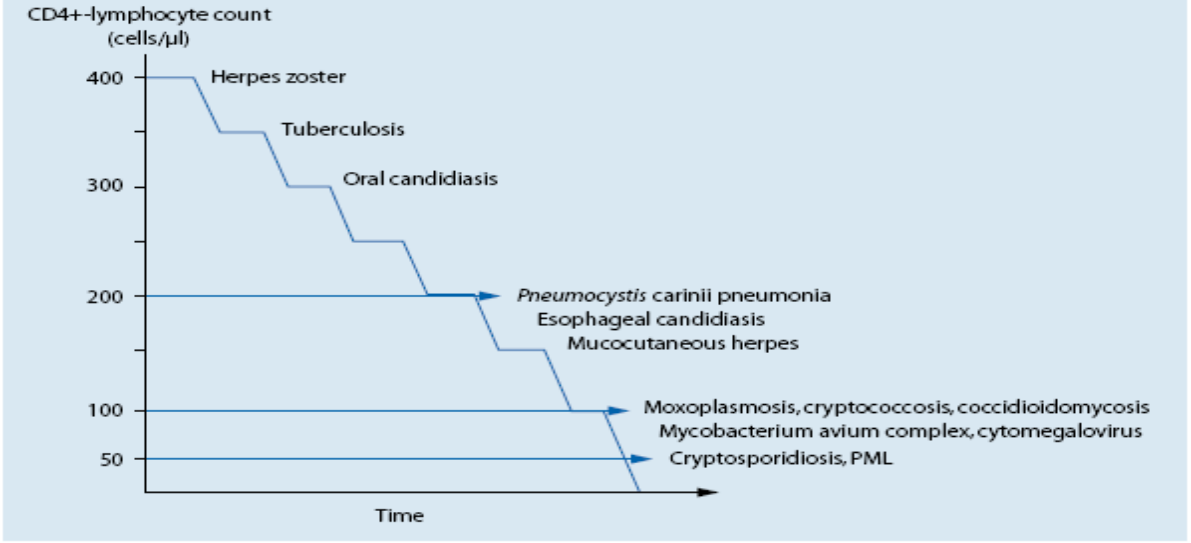


Table 1.1. HIV-related infections most frequently encountered in India

Bacterial	Viral	Fungal	Parasitic	Other illnesses
Tuberculosis	Herpes simplex virus infection	Candidiasis	Cryptosporidiosis	AIDS dementia complex
Bacterial respiratory infections	Oral hairy leukoplakia	Cryptococcosis	Microsporidiosis	Invasive cervical cancer
	Varicella zoster virus disease	*Pneumocystis* jiroveci pneumonia	Isosporiasis	Non-hodgkin lymphoma
Salmonella infection	Cytomegalovirus disease	Penicilliosis	Giardiasisww Stongyloides	
	Human papillomavirus infections		Toxoplasmosis	

* Rare infections include those due to *Bartonella henselae*, *Rhodococcus equi*, atypical mycobacterioses and human herpesvirus (HHS)-8 infections

Lab Diagnosis of HIV-

- ***Screening –*** (E/R/S)
  - ELISA,
  - Rapid ,
  - Simple

  ELISA-
  - 1st generation ELISA - crude preparation of HIV antigen is used
  - 2nd generation ELISA - recombinant Ag
  - 3rd generation ELISA - synthetic oligopeptide
  - 4th generation ELISA - Combination of recombinant. & synthetic Peptide and detect both HIV antgen (p24) & Antibody
- ***Confirmatory*** –
  - Antibody detection-
    - Western blot- uses whole virus lysates
    - Immunofluorescence assay
    - Radioimmunoprecipitation assay (RIPA)
    - Line immunoassay (LIA)- recombinant / synthetic Antigen used- More specific
  - HIV RNA,
  - Viral culture
- ***Surrogate markers –***
  - CD4 count
  - Hypergammaglobulinemia
  - Altered CD4 : CD8 ratio
- ***Criteria used for HIV 1 by Western Blot-***
  - WHO – Two envelop bands gp41, gp120/160 with or without gag or pol bands
  - CDC- Any two – p24, gp41, gp120/160
- ***NACO Strategy*** of HIV diagnosis-
  - Strategy I – done for screening for Donor/Blood bank – 1 test
  - Strategy IIa - done for Seroprevalance / epidemiological purpose – 2 test
  - Strategy IIb – done for HIV symptomatic patients– 2 test
  - Strategy III – done for Asymptomatic HIV patients – 3 test
- ***Prognosis / monitoring-***
  - CD4 T cell count – most commonly used
  - HIV RNA – Most consistent
  - P24 antigen detection
  - Neopterin
  - β2 macroglobulin
- ***Pediatric HIV –***
  - HIV DNA – Most recommended
  - IgM antibody
  - p24 antigen detection
  - IgG ELISA aft 18 months only
- ***NACO Guidelines to prevent neonatal HIV:***

  Single dose NVP to mother during labor and to the baby within 72 hours after birth.
- ***Diagnosis in window period-***
  - Initial time when the antibody detection methods are negative
  - p24 antigen detection (30% sensitive)
  - HIV RNA- best
- Guidelines for post exposure prophylaxis (PEP)
  - Body fluid considered at risk- blood, genital secretion, CSF & other body fluid
  - Body fluid not considered at risk- tear, sweat, saliva, feces
  - PEP should be started within 2hr.

Basic regimen – Zidovudine or Lamivudine for 4 weeks
Expanded regimen- Basic + Indinavir for 4 weeks

MISCELLANEOUS VIRUSES .

ROTAVIRUS

- Belong to family Reoviridae
- Double walled virus- looks like a wheel with short spokes
- Segmented ds RNA virus (11 segments)
- Serologic types – A to G (MC –group A, Adult rotavirus strains belongs to Group B)
- Commonest cause of diarrhea in infant & children (6-24months)
- Seasonal variation- MC in Winter
- Route- Faeco-oral
- Incubation period- 2-3days
- Lab diagnosis-
    - Electron microscopy- Detection limit 10^6 particle /ml
    - ELISA detecting antigen in stool
    - Culture- difficult, rolling of tissue culture facilitates growth
    - RT-PCR

Causes of Viral Gastroenteritis

- Rotavirus
- Enteric adenovirus, type 40,41
- Calcivirus-
    - e.g. Norwalk,
    - Outbreaks associated with uncooked ***shellfish*** in older children
    - Calci- means 32 cup shaped depression on virus surface
- Astroviruses
- Coronavirus
- H1N1

SLOW VIRUS DISEASES

- Definition: group of viruses which cause slow, progressive, neuro degenerative disease of CNS with long incubation period and high mortality rate.
- Character-
    - Long Incubation periods ranging from months to years
    - Predilection to CNS
    - Immune response is either absent or contributes to pathogenesis
    - Fatal termination.
    - Slow growth rate
    - Genetic predisposition
- Group A Slow viral disease
    - Slowly progressive infections of sheep,
    - Caused by lentivirus
    - Visna - Demyelinating disease of sheep
    - Maedi- hemorrhagicpneumonia of sheep,
- **Group B -Prion disease of the CNS**
- Discoverer- Stanley B Prusiner
- Prions are proteins not virus (protein without nucleic acid)
- The pathogenic mechanism -
- Normal prion protein PrPc present on chromosome 20 gets mutated to abnormal prion protein (PrPsc) which acumulates-- Amyloid Plaques gets deposited in CNS

- ***Pathology-***
    - Progressive vacuolation in dendritic & axonal process of neuron
    - Astroglial hypertrophy
    - Spongiform degeneration
- ***Animal-***
    - Scrapie (sheep)
    - Mink encephalopathy,
    - Bovine spongiform encephalopathy (BSE)(Mad Cow Disease)
- ***Human Prion disease***
    - Kuru – tremor, due to cannabalism
    - Gerstmann-Straussler-Scheinker(GSS)syndrome,
    - Creutzfeldt-Jakob disease
    - Fatal familial insomnia
- ***Symptoms are related to site-***
    - Cerebral cortex – Loss of memory and mental acuity, and visual imparement (CJD).
    - Thalamus - Insomnia (FFI)
    - Cerebellum - Problems to coordinate body movements and difficulties to walk (kuru, GSS).
- **Group C Slow viral disease**
    - Two unrelated CNS disease of human
    - Subacute sclerosing panencephalitis (defective measles virus)
    - Progressive multifocal leucoencephalopathy (JC virus)

PARASITOLOGY

Entamoeba

Life cycle

- Definitive host- Man
- Infective form – Mature quadrinucleate cyst
- Mode of infection- food or water contaminated with cyst. (Resistant to chlorination)
- Mode of transmission- **faeco-oral route** & Sexual transmission (20-30% in homosexuals)
- Reservoir of infection- Asymptomatic carriers

Culture Media

- Polyaxenic- Bacterial supplement (for diagnosis)
  - Boeck & Drbohlav's Media
  - Locke's egg serum / egg albumin
  - Balamuth, Nelson's Medium
- Axenic Medium-Diamond Medium
- For study of pathogenesis, anti amoebic susceptibility, Preparation of antigen

Virulence factors

- Lectin antigen (galactose inhibitable adherence lectin)
- Cysteine proteinase

Clinical picture-

- Amoebic dysentery-
  - Flask shaped ulcer
  - MC site- Coecum
- Amoebic Liver Abscess –
  - 10% of Amoebic dysentery
  - Ancovy sauce pus (chocolate syrup)
- Extra intestinal- brain, Lung etc

Diagnosis-

- Stool examination- E.h Vs E.coli
- lectin antigen in Stool
- Serology – Imp for ALA
- PCR

Characteristics	E. histolytica/E. dispar	E. coli
Trophozoites	Actively motile with finger shaped pseudopodia Cytoplasm is - presence of RBCs	Sluggishly motile, Blunt pseudopodia
Cysts	10–20 µm, Nucleus- Four nuclei Fine uniform granules, evenly distributed	20–25 µm, Nucleus- Eight nuclei Coarsely granular -unevenly arranged

E.histolytica *and* E.dispar

- **Similarities -**
  - Spread through ingestion of infectious cysts.
  - Cysts -morphologically identical.
  - Both species colonize the large intestine.
- **Differences**
  - Only *E. histolytica* –Causes invasive disease
  - Differentiated by -
    - Gal/N Acetyl Gal lectin
    - PCR
    - Distinct surface antigens and isoenzyme markers.

Free living Amoebae

	Naegleria fowleri	Acanthamoeba
Disease	Primary amoebic meningo-encephalitis	Granulomatous amoebic Meningoencephalitis Ulcerative keratitis
Portal of entry	Nose – olfactory N route	Upper respiratory tract – hematogenous route
Predisposing factor	Swimming in contaminated water	Immunodeficiency
Clinical course	Acute	Subacute- Chronic
Pathology	Acute Suppurative changes	Granulomatous inflammation
Morphological form	Trophozoite, cyst, flagellated form	Cyst & trophozoites
Culture	Frequently positive	Negative
Trophozoites	Single pseudopodium	**Thorn like Pseudopodium**
Leukocytes in CSF	Neutrophils	Lymphocytes

- Other free living amoeba-
  - Balamuthia
  - Sappinia

Giardia Lamblia

- **Habitat**- Mucosa of duodenum and upper ileum
- **Morphology- 2 forms**
  - **Trophozoites**-
    - Tear-drop shaped with **2 nuclei**,
    - 4 pairs of flagella,
    - Axostyles, parabasal body and Ventral sucking disk
  - **Cyst**- Mature cyst consists of **4 nuclei** and cysts
- **Life cycle**
  - Infective stage- Cyst
  - Route of infection- Faeco-oral route
  - Trophozoite has ***falling –leaf like motility***
- **Susceptibility to infection**
  - Children & Immunocompromised individual
  - Individuals with Achlorhydria
  - Antigenic variation
- **Clinical Disease**
  - Chronic diarrhea with malabsorption
  - Steatorrhoea-fats
- **Lab diagnosis**
  - Stool Microscopy- for cyst and trophozoites
  - Demonstrates either trophozoites or cyst
  - **String test/ Entero test**-Duodenal contents
  - Serology

Trichomonas Vaginalis –

- Twitching motility
- Only Trophozoites – Pear shaped, 5 flagella
- stains – Paps, Giemsa, PAS, Leishmania, Fl. ab
- Most common Parasitic cause of STD
- *Foul smelling discharge, strawberry appearance*

Haemoflagellates .

Parasite	Epidemiology	Location	Mode	Symptoms
Leishmania tropica	Mediterranean area, Asia	Skin	Sandfly (Phlebotomus)	Skin lesion (Oriental sore)
Leishmania brasiliensis	Central and south America	Skin & muco-cutaneous	Sandfly (Lutzomyia)	Skin lesions, espundia
Leishmania donovani	Asia, Africa,	Skin and somatic organs	Sandfly Phlebotomus	Skin lesions, liver and spleen
L.mexicana	America	muco- cutaneous		Chiclero ulcer

- **Leishmania-**
  - Amastigote form -human (LD body)- diagnostic from
  - Promastigote- infective form (by bite of sandfly)
- **Trypanosoma-**
  - Human-Non multiplying Trypomastigote (Infective form & diagnostic from)
  - Released with faeces while insect is taking blood meal & then faeces is rubbed into the bite wound site.
  - Insect- Epimastigote & multiplying Trypomastigote
- **Kalazar-**
  - Anemia , leucopenia, thrombocytopenia
  - Hyper gammaglobulinemia
  - Spleen ↑ , Liver ↑
  - Fever
  - Hyper pigmentation (Indian)
  - LN↑ (African but not in Indian cases)
- **Kalazar with HIV-**
  - Absence of hepatosplenomegaly
  - GIT & resp. symptoms
- **PKDL-**
  - 2 year after t/t
  - 3%(African), 10% (Indian)
  - Non ulcerative hypopigmented lesions

Laboratory diagnosis

- Microscopy- stained peripheral blood smear examination
- Sample-Spleen (most Sn)/ Bone marrow aspiration(MC preferred)
- Lymphnode aspirate(Not useful in Indian cases)
- Blood culture- NNN medium (Promastigotes grown)
- Serological tests-
  - Napier's aldehyde test - +ve aft 3months and also false +ve seen
  - Chopra's antimony test
  - ICT- Ab to K39 antigen
  - CFT- using WKK antigen
- Leishmanin (Montenegro) skin test- negative in acute case of Kala azar
- Molecular diagnosis-Kinetoplast DNA
- Animal inoculation- Chinese and golden Hamsters.

Parasite	Epidemiology	Location in Host	Mode	Symptoms of Infection
T. cruzi (Chagas')	South America	Cardiac muscle, blood and other tissues	Reduviid bugs	Chagoma Muscle pain, LN, Myocarditis, Meningoencephalitis, Romana's sign- (eye edema)

T. brucei

Comparison	West African	East African sleeping sickness
Organism	*T. b. gambiense*	*T. b. rhodesiense*
Vectors	Tsetse flies (palpalis group)	Tsetse flies (morsitans group)
Primary reservoir	Humans	Antelope and cattle
Human illness	Chronic CNS disease	Acute (early CNS disease)- < 9m
Lymphadenopathy	Prominent Cervical LN(Winter bottom sign)	Minimal Axially & Inguinal
Parasitemia	Low	High

Coccidian parasites.

Property	*Cryptosporidium*	*Cyclospora*	*Isospora-*
Size	4-6µ size	8-10 µ	10-20 µ
shape	Round	Round	Oval
Cyst contains-	4 sporozoites	2 sporoblast, each having 2 sporozoites	2 sporoblast, each having 4 sporozoites
Acid fast	Uniformly acid fast,	Variable acid fast	Uniformly acid fast,
Autoflouroscence	No, but can be stained with fluorescent dye	Autoflouroscence ++	Autoflouroscence +/-
Treatment	Co-trimoxazole	co-trimoxazole	Spiramycin, Nitazoxanide

Toxoplasmosis-

- Cat- definite host- producing oocyst
- Man- intermediate host
- All 3 morphological forms are infective- Oocyst, tissue cyst, tachyzoites
- Congenital toxoplasmosis-
  - 1st Trimester- More sever infection
  - 3rd Trimester- More chance of infection
  - If Mother is previously infected- Asymptomatic
  - Featured by - 3C + 2M
  - IgM detection , IgA can also be used (experimental but better sensitivity)
- Sabin Feldman test
- Most common manifestation of congenital toxoplasmosis – chorioretinitis
- Most common manifestation of toxplasmosis in HIV pt. – encephalitis
- Most common manifestation of Acquired Toxoplasmosis. – Asympt. & if sympt – Cx LN↑.
- ***Treatment-***
  - Immunocompetent hosts- No treatment is typically indicated
  - Congenital toxoplasmosis- Combination of pyrimethamine and sulfadiazine
  - Toxoplasmosis during pregnancy- Spiramycin is typically used

- Prophylaxis in HIV patients- Trimethoprim-sulfamethoxazole

Balantidium coli –

- Largest protozoa
- Habitat – large int. of man + Pigs
- Morphology – Troph. And cyst
- Troph. – Revolving motility
  - Two nuclei → Macronucleus & Micronucleus
- Cyst - Infective form. Two nuclei +
- Diagnosis – Dysentery
- In large intestine → ulcers mimic amoebic ulcers but never invade muscular layer.
- Treatment – Tetracycline – DOC

Plasmodium- -

Species	Disease	Periodicity(hours)
Plasmodium vivax	Beningn tertian	48
Plasmodium falciparum	Malignant tertian	24-48
Plasmodium ovale	Ovale tertian	48
Plasmodium malariae	Quartan	72
Plasmodium knowlesi		24-48

Life cycle-

- Man – Intermediate host.
- Mosquito – Definitive host
- Sporozoites are infective forms
- Present in the salivary gland of female anopheles mosquito
- After bite of infected mosquito sporozoites are introduced into blood circulation.
- Other modes of transmission include blood transfusion and transplacentally. (Infective stage- Merozoites and No EE cycle)
- Africa (sub saharan)
- India (Orissa & north east)

Recrudescence Vs Relapse-

- Recrudescence- d/t treatment failure
- Occurs after 2-3 weeks of t/t
- Occurs in all spp (MC- P.falciparum)
- Relapse- d/t hypnozoites
- Seen in P.vivax & ovale

Pathogenesis

- Sequestration (binding of RBC to endothelium) – Pf emp
- Rossetting- unparasitized RBC clump together with parasitized RBC
- Cytokines – d/t GPI- glucosyl phosphatidyl Inositol
- Antigenic diversity

Immunity

- Nature of Haemoglobin-
  - Sickle cell anemia trait protective
  - Thalassemia trait protective
  - Fetal Hb- protective
- Nature of RBC-
  - Pf- All RBC,
  - P.malariae- old RBC,
  - P.ovale/vivax- Young RBC
- Nature of Enzyme- G6PD deficiency -protection.

Clinical features- Triad

- Malarial paroxysm – fever, chill and rigor
- Anemia

- Hepatosplenomegaly

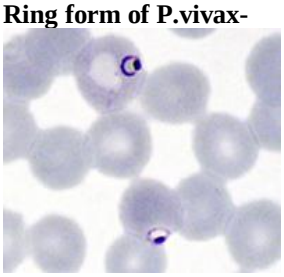
Complications of severe falciparum malaria

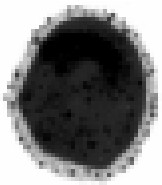
- Recrudescence Vs Relapse
- Black water fever
- Cerebral malaria
- Tropical splenomegaly syndrome
- Pulmonary edema
- Renal failure
- Hypoglycaemia with lactic acidosis

Laboratory diagnosis-

- Microscopy – for demonstration of parasites & for speciation
- Stained peripheral blood smear examination
- (thick & thin smears)- Gold standard method
- Thick smear- for identification, quantification
- Thin smears- for speciation
- Quantitative Buffy Coat examination- Rapid method for detection of parasites

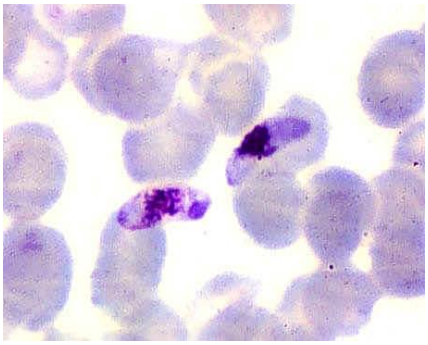
Ring form of P.vivax-





Microgametocyte

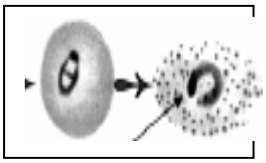
Gametocyte of P.falciparum-



- **Ring form of P.falciparum**

Multiple ring form **Accole /Applique form** **Double dot or (head phone shaped)ring form**

- Band form- P.malariae
- Enlarged RBC and fimbrianated - P.ovale



Points to remember-

- In **vivax infections**, gametocytes appear in blood **4-5 days** after appearance of the asexual parasites.
- In **falciparum infections**, they do not appear until **10-12 days** after the first appearance of asexual parasites.
- Gametocytes are most **numerous during the early stages** of infection and their density may exceed **1,000 per cubic mm of blood**".
- Gametocytes are numerous during early stages and do not increase in blood with time.

Species	Colour of pigment	No. of merozoites / mature Schizont
P.falciparum	Dark brown	18-24
P.vivax	Yellowish-brown	12-24
P.ovale	Dark Yellowish-brown	8
P.malariae	Dark -brown	8

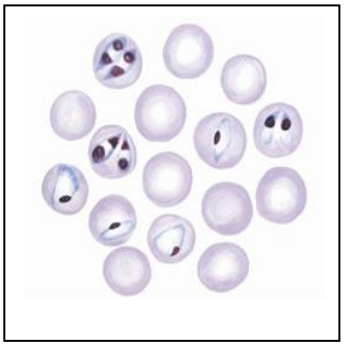
Other test-

- Antigen Detection - Immunochromatographic tests –
- Rapid & simple
- pLDH – Pan malarial
- HRP-2 Ag detection- for P.f
- Serology - antibodies against malaria parasites
- Molecular Diagnosis- PCR

Characteristic	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
Red cell preference	All age RBC	Reticulocytes and young RBC	Reticulocytes and young RBC	Older cells
Morphology	**Ring forms'** • Multiple • Double dot • **Accolle form** **Banana-shaped** gametocytes	Irregularly shaped large rings and trophozoites; Schizont & late trophozoites also seen	Erythrocytes enlarged and fimbrinated Schizont & late trophozoites also seen	Band form Schizont & late trophozoites also seen
Relapses	No	Yes	Yes	No
Recrudescence	Yes	No	No	Yes

Babesia

- Intra erythrocytic protozoa
- Not found in India
- Tick borne malaria like illness in animal
- Zoonotic- opportunistic to human
- Treatment -Clindamycin with oral quinine
- Differ from Plasmodium-
    - Hemozoin absent
    - Gametocyte absent
    - Maltese cross form seen- Ring in tetrad in merozoites
    - Vermicule in Tick



Species	*B.microti*	*B.bovis/divergens*
Distribution	North America	Europe
Host	Rodent	Cattle
Immunity of host	Spleen is usually normal	Seen in splenectomised and immunocompromised patient
Clinical feature	Asymptomatic /mild fever	Severe, but no cerebral involvement
Clinical Course	Self limiting	Severe, fulminant

CESTODE-

Intestina Cestodes – Humans are definitive host

- *Taenia saginata* & *T.solium* – causing intestinal taeniasis
- *Diphyllobothrium,*
- *Hymenolepis,*
- *Dipylidium caninum*

Tissue cestodes (Larval-stage)- Humans are intermediate host

- Echinococcus granulosus
- ***Taenia solium*- causing cystcercosis**
- Sparganum
- Multiceps spp.

Characters	*Taenia saginata*	*Taenia solium*
Length	5- 10 metres	2-3 metres
Scolex	Large & quadrate	Small & globular with hooklets
Neck	Short	Long
Proglottid	1000-2000	Less than 1000
Larva	Cysticercus bovis	Cysticercus cellulosae

Characters	Intestinal taeniasis	Cysticercosis
Infective stage	Cysticercus cellulosae	*Taenia solium* egg
Clinical symptoms	Intestinal symptoms only	Depending on the location of the cyst
Diagnosis	Demonstration of eggs in the faeces	Demonstration of larvae in tissues. Serological tests are of great value.

Cysticercosis

- Potentially dangerous systemic disease.
- Most commonly found in subcutaneous and **intermuscular tissues** followed by the **eye and then the brain.**
- Lab diagnosis
  - ELISA
  - Western blot
  - CT/MRI

Echinococcus

- **Life cycle-**
  - Definitive host – Dog and wild carnivores
  - Intermediate hosts – man and other herbivorous animals.
  - Man is an accidental host.
  - Eggs – infective stage of the parasite.
- **Clinical features-**
  - Hepatomegaly with or without palpable abdominal mass.
  - Bone- rapid erosion leading to fractures.
  - Other sites - tumor like condition or an abscess.
  - *Echinococcus multilocularis – Lungs- mistaken asmalignant tumor. Has ability to metastasize*
- **Parasitic diagnosis**
  - Hydatid fluid microscopy – on wet mount examination protoscolices
  - **Acid fast staining of centrifuged deposit –**
  - **Casoni's skin test – immediate hypersensitivity reaction.**
  - IHA, CIEP, ELISA and Western Blot used for the detection of antibodies.
  - Detection of antigen –
  - Imaging methods like USG, MRI and x ray are of immense importance to show the size and condition and to pinpoint the exact location of the cysts
  - Water lily sign in X ray

- **Treatment-**
  - Surgery is the mainstay of this condition.
  - Albendazole and mebendazole are the only antihelminthics useful in cystic echinococcosis.
  - Percutaneous Aspiration Injection Reaspiration (PAIR) of the cyst

***Diphyllobothrium latum* (fish tape worm)**

- Definitive host- man,
- Intermediate host-
- 1st intermediate host- Cyclops/ diaptomus
- *2nd intermediate host- fresh water fish*
- Megaloblastic anemia

***H. nana* →**

- Egg is infectious to man
- Only one host involved
- Dwarf tapeworm

H.dimmunita → Rat tapeworm

TREMATODES .

- ***Agents-***
  - *Schistosoma (blood fluke)*
  - *Fasciola (liver /intestinal fluke)*
  - *Paragonimus (lung fluke)*
  - *Clonorchis /Opisthorchis*
- ***Infective form-*** *Metacercaria (Cercaria-Schistosoma)*
- ***Host-***
  - *Definite host- Man*
  - *Intermediate host-*
    - *1st – Snail*
    - *2nd –Aquatic plants (Fasciola) Crey /crab Fish (Paragon.., Clon..)*
    - *No 2nd intermediate host for Schistosoma*
- Eggs of trematodes-
  - Oviparous (lays eggs)
  - Eggs- operculated (except Schistosoma)-Diagnostic form
- All are Hermaphrodite (except Schistosoma)

Schistosoma hematobium –

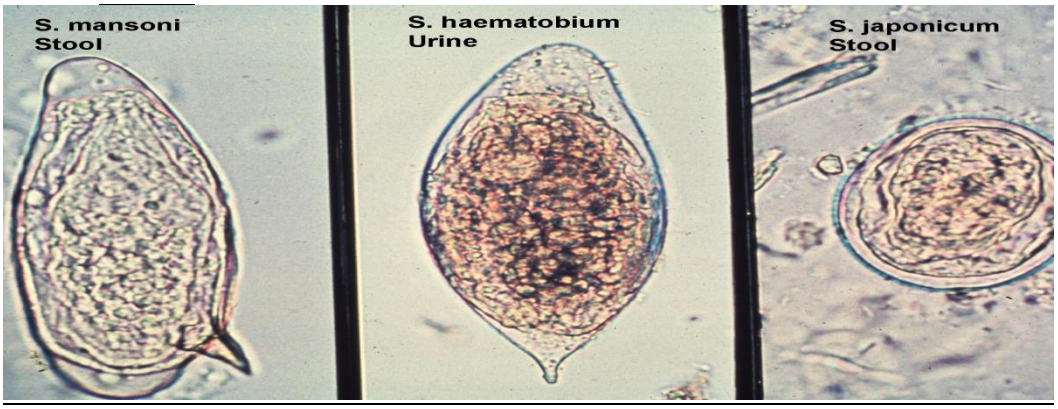
- Vesical and pelvic venous plexus
- Associated with bladder CA.
- Egg has terminal spine
- Mode of transmission - Bathing in contaminated water where cercaria larva peneterates skin
- Intermediate host- Snail(infected by miracidium larva)
- Sign & symptoms – hematuria , dysurea , urgency and Squamus cell carcinoma
- Treatment – Praziquintal

S. Mansoni -

- Sigmoidorectal plexus(inferior mesenteric vein)
- Egg has lateral spine
- **Dysentery**
- Cercarial dermatitis
- Katayama disease

***S. japonicum* -**

- ilio caecal plexus
- Eosinophilic diarrhoea
- Egg has central spine



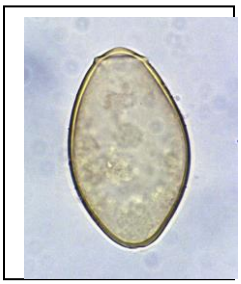
Liver flukes

Fasciola hepatica - Sheep Liver fluke

- Sheep is def host
- Snail & water cress (1^{st} + 2^{nd}) intermediate – host.
- Mode of transmission - Man get accidental infection by ingestion of aquatic vegetation contaminated with encysted metacercaria.

***Fasciola buski* -** Intestinal fluke → Largest fluke

- Lung fluke – *Paragonimus westemanii*,
- Definite host man; Intermediate host- pig



***Paragonimus westemanii*, - (Lung fluke) –**

- Def host man; Intermediate host- crey /crab fish
- Mode of transmission- ingestion of raw , undercookes crab/ crayfish
- Causes endemic hemoptysis
- Operculated eggs

Clonorchis sinensis- Oriental / Chinese liver fluke –

NEMATODE

Small Intestine

- *Ascaris lumbricoides/ round worm*
- *Ancylostoma duodenale/ hook worm*
- *Necator americanus/ American hook worm*
- *Strongyloides stercoralis*
- *Trichinella spiralis*
- *Capillaria philippinensis*

Large Intestine (vermiform appendix & caecum)

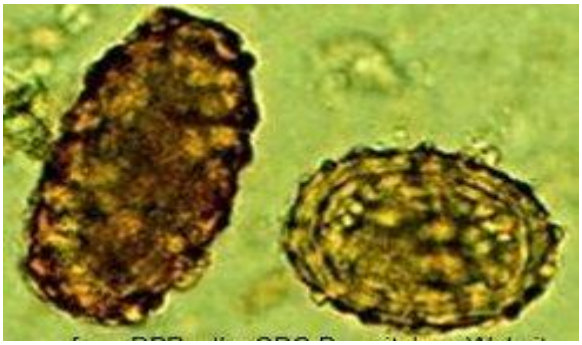
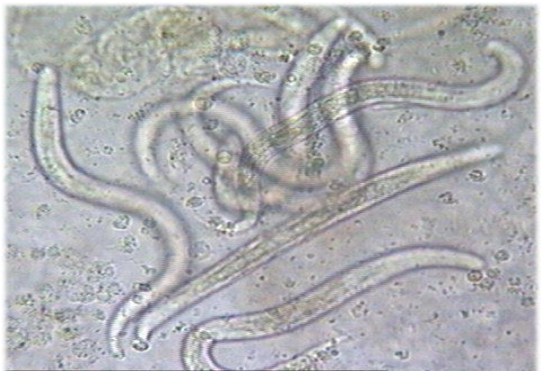
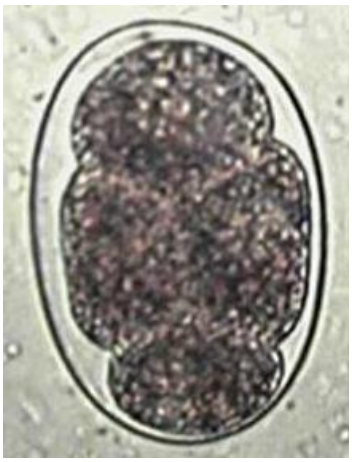
- Enterobius vermicularis / pin worm
- Trichuris trichuira/ whip worm

Lymphatic system

- Wuchereria bancrofti
- Brugia malayi

Subcutaneous tissues

- Loa loa / African eye worm
- Onchocerca volvulus
- Dracunculus medinensis/ Guinea worm
- Mansonella perstans
- Mansonella ozzardi

Feature	***Ascaris lumbricoides* (Roundworm)**	***Necator, Ancylostoma* (Hookworm)**	***Strongyloides stercoralis***	***Trichuris trichiura* (Whipworm)**	***Enterobius vermicularis* (Pinworm)**
Infective stage	Egg	Filariform larva	Filariform larva	Egg	Egg
Route of infection	Oral	Percutaneous	Percutaneous or autoinfection	Oral	Oral
Gastrointestinal location of worms	Jejunal lumen	Jejunal mucosa	Small-bowel mucosa	Cecum, colonic mucosa	Cecum, appendix
Pulmonary passage of larvae	Yes	Yes	Yes	No	No
Principal symptoms	Rarely gastrointestinal or biliary obstruction	Iron-deficiency anemia in heavy infection	Gastrointestinal symptoms; malabsorption or sepsis in hyperinfection	Gastrointestinal symptoms, anemia	Perianal pruritus
Diagnostic stage	Eggs in stool	Eggs in fresh	Larvae in stool or	Eggs in stool	Eggs from

		stool, larvae in old stool	duodenal aspirate; sputum in hyperinfection		perianal skin on cellulose acetate tape

Filariasis-

Organism	Periodicity	Vector	Location of Adult	Microfilarial Location	Sheath
Wuchereria bancrofti	Nocturnal (world) Subperiodic (pacific, andaman)	*Culex Anopheles* *Aedes* *Aedes*	Lymphatic tissue Lymphatic tissue	Blood Blood	+ +
Brugia malayi	Nocturnal Subperiodic	*Mansonia, Anopheles* *Coquillettidia, Mansonia*	Lymphatic tissue Lymphatic tissue	Blood Blood	+ +
B. timori	Nocturnal	*Anopheles*	Lymphatic tissue	Blood	+
Loa loa	Diurnal	*Chrysops* (deerflies)	Subcutaneous tissue	Blood	+
Onchocerca volvulus	None	*Simulium* (blackflies)	Subcutaneous tissue	Skin, eye	-
Mansonella ozzardi	None	*Culicoides* (midges)	Undetermined site	Blood	-
M. perstans	None	*Culicoides* (midges)	Body cavities, mesentery, perirenal tissue	Blood	-
M. streptocerca	None	*Culicoides* (midges)	Subcutaneous tissue	Skin	-

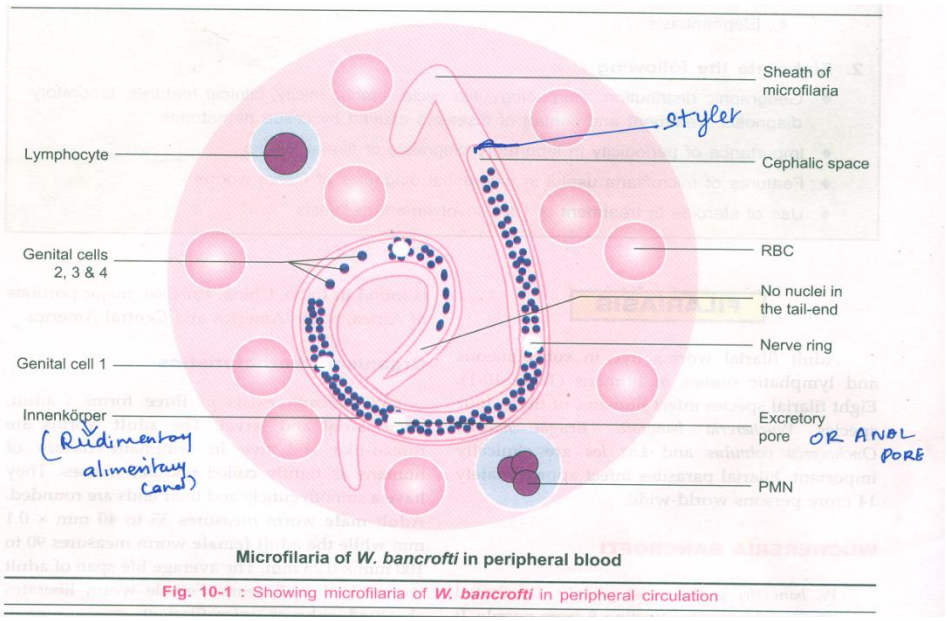
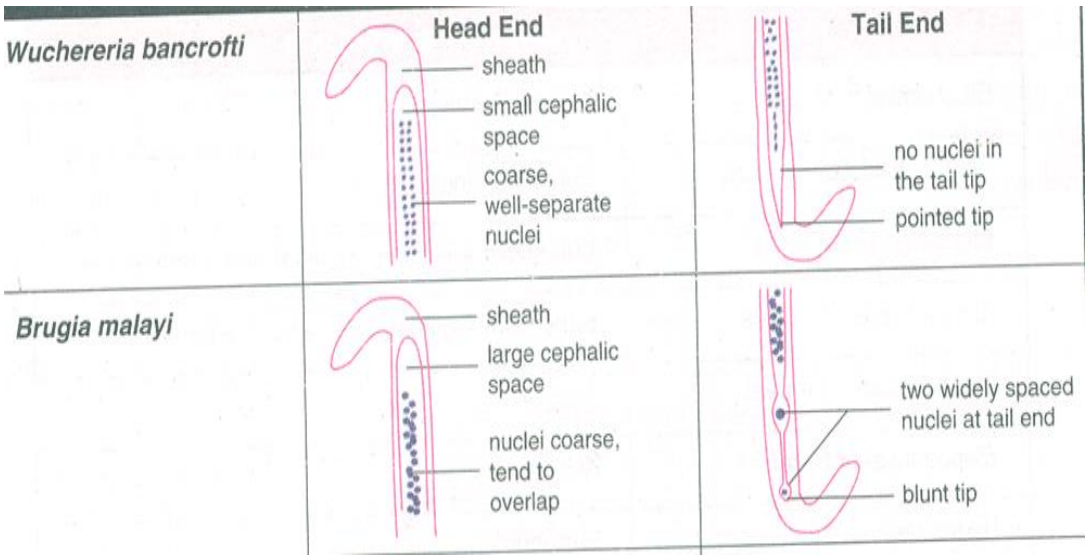


Fig. 10-1 : Showing microfilaria of *W. bancrofti* in peripheral circulation



Pathogenesis

- Infective form-third-stage filarial larvae (L3)onto the skin of the human
- Transform to adult worm- then to microfilaria
- Microfilaria are not pathogenic
- Adult worm- crucial role
- Triad of -
    - Dilatation of lymphatic vessel
    - Lymphadenitis
    - Obstruction to lymphnode- fibrotic degeneration of lymph vessel

Clinical feature- Lymphatic & Occult

Lymphatic filariasis

Asymptomatic Stage-

- Microfilaria present in blood, but no cl.feature
- Th1 is down regulated but Th2 is high (IL4↑)
- After several years, hypo responsiveness breaks and inflammatory reaction occurs

Acute Filariasis-

- d/t antigens released from female adult worm
- Tetrad of -
    - Fever,
    - Lymphoedema- adult worm in lymph channel
    - Lymphadenitis – LN inflammation
    - Adenolymphangitis – Lymph vessel inflammation

Chronic Filariasis-

- 10-15 years after acute phase
- Fibrotic changes occurs (obstructive phase) in lymph vessels
- Featured by-
    - Lymph varices
    - Hydrocele
    - Elephantiasis- of scrotum, leg, arms, breast & vulva (non pitting edema)
    - Granuloma of female breast
    - Chyluria- chylus in urine (d/t obstruction of lymph vessels of kidney & abdomen)

	Classical filariasis	Occult filariasis(Tropical Pulm Eosinophilia)
Causative Agent	Adult	Microfilaria
Diagnostic form	Microfilaria in blood & in fluid	Microfilaria absent
Pathology	LN & vessels	Lungs, liver, spleen
	Lymphangitis & lymphadenitis	Eosinophilic granuloma Hypersensitivity reaction
Antibody	Ab not diagnostic	Antibody – diagnostic

Brugiyan filariasis

- B.malayi-
  - Nocturnal- Man-Man- by Anopheles
  - Sub periodic- Zoonotic (monkey)- by Mansonia
  - Leg Below knee -ONLY affected (Contour of knee- normal)
  - Genital & chyluria NOT marked
- B.timori- Timor island of Indonesia, Anopheles barbirostris

Parasitic diagnosis-

- Blood microscopy-
- Blood is collected during-
  - Nocturnal- 10pm to 4am
  - Sub periodic Nocturnal- 8pm -10pm
  - Sub periodic Diurnal- 2pm -6pm
- Direct wet mount- to see serpentine movement of microfilaria
- Staining- Thick blood smear stained with Leishman/giemsa
- Concentration methods
- DEC provocation test-
- QBC- Quantitative Buffy Coat
- Microfilaria NOT found in peripheral blood-
  - OCCULT filariasis
  - Chronic filariasis (some cases)
  - Wrong time
- Other samples-
  - Urine microscopy- 10-20ml early morning Chylous urine
  - Hydrocele fluid & LN aspirate microscopy
- **Serology**
- **Demonstration of antibody-**
  - Methods- IHA , IFA, ELISA, RIA
  - Disadvantage-
    - Cross reactivity
    - Unable to discriminate b/t recent & past infection
- **Demonstration of antigen-**
  - Indicates recent infection
  - Day time- Ag can be detected
  - ELISA- using monoclonal antibody against AD12 antigen- detects adult worm only

- ELISA- using monoclonal antibody against Og4C3 antigen- detects adult worm & microfilaria
- Molecular methods- PCR detecting as low as 1pg of filarial DNA
- Imaging methods-
  - X Ray- Dead & calcified worm in LN & chest X ray shows Pulmonary infiltrate in TPE,
  - Ultrasound- of scrotum
  - Live adult worms with serpentine movement (Filarial Dance sign)

MISCELLANEOUS

Larva migrans

Agents causing Visceral larva migrans-	Agents causing Cutaneous larva migrans
Angiostrongylus cantonensis	*Ancylostoma braziliensis*
Angiostrongylus costaricensis	*Ancylostoma caninum*
Toxocara canis	*Strongyloides stercoralis (Larva currens)*
Toxocara catis	*Necator americanus*
Aisakine spp.	*Acylostoma duodenale*
Gnathostoma spinigerum	*Gnathostoma spinigerum*

Important Point

- Largest protozoa – B.coli
- Largest helminth- D.latum

- **No intermediate host required for-**
- PROTOZOA
  - E. histolytica
  - Giardia lamblia
  - Trichomonas vaginalis
  - Balantidium coli
- HELMINITHS
  - Enterobius vermicularis
  - Trichuris trichura
  - Ascaris lumbricoides
  - Ancylostoma duodenale
  - Necator americanus
  - H. nana

- **Two Intermediate Hosts required for-**

  Intermediate hosts -Parasites
  - Cyclops, fish -D.latum
  - Snail, fish - Paragonimus, Clonorchis sinensis
  - Snail, plant -Fasciola spp.

- **Parasites entering through skin penetration**
  - A. duodenale Necator americanus
  - Strongyloides stercoralis
  - Schistosoma spp

- **Parasites transmitted by sexual contact**
  - Trichomonas vaginalis
  - Entamoeba histolytica
  - Giardia lamblia

- **Parasites transmitted congenitally**
  - Toxoplasma gondii
  - Plasmodium Spp.
  - Microsporidia
  - Trypanosoma cruzi

PARASITES INFECTING DIFFERENT TISSUES

- **Parasites infecting intestines**
  - Entamoeba hystolytica
  - Giardia lamblia
  - Balantidium coli
  - Cryptosora
  - Isospora belli

- **Parasites infecting Liver**
  - Entamoeba hystolytica
  - Echinococcus granulosus
  - Fasciola hepatica

- **Parasites infecting brain**
  - Plasmodium falciparum
  - Neglaria foeleri
  - Acanthamoeba
  - Trypanosoma sp.
  - Toxoplasma gondi
  - Entamoeba hystolytica
  - Echinococcus granulosus
  - Tenia solium

- **Parasites infecting lung**
  - Paragonimus westermani
- **Parasites infecting Lymphatic System**
  - Wuchereria bancrofti
  - Brugia malayi

Non-bile stained eggs (NEHA)-

- Necator americana
- Enterobius vermicularis
- Hymenolepis nana
- Ancylostoma

Doesn't float in saturated salt solution- (ULTO)-

- Unfertilized egg of ascariasis
- Larva of Strongyloides
- Tinea egg
- Operculated egg of trematodes

Parasites causing Malignancy

- *Schistosoma haematobium*
  - Squamous cell carcinoma of urinary bladder
- *Clonorchis sinensis*
  - Cholangiocarcinoma of liver, bile duct & Adenocarcinoma of pancreas
- *Opisthorchis viverrini*
  - Cholangiocarcinoma of bile duct

Auto infection is seen in-

- Cryptosporidium
- H nana
- Ecchinococus
- Tenia solium
- *S.sterecoralis*

Obligate intracellular parasite

- *Plasmodium spp.*
- *Babesia spp.*
- *Leishmania spp.*
- *Toxoplasma spp.*
- *Trypanosoma cruzi*
- *Microsporidia*

Parasites associated with anemia-

- Hookworms → Iron deficiency anemia
- *Diphylobothrium latum* → Megaloblastic anemia
- Haemolytic anemia → malaria
- *Trichuris trichiura* → Prolonged massive infection leads to iron deficiency anemia
- Leishmania donovani