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Study materials for FMGE

www.indiaconnectingcontinents.com
indiaconnectingcontinents@gmail.com
(+86)15069629250 , (+91)93441 60131

MICROBIOLOGY

GENERAL BACTERIOLOGY

* Father of Microbiology
Louis Pasteur.

① * He prepounded the "germ theory"
② * He coined "vaccine" & "microbiology".
③ * He laid down the principles for sterilization & dis-infection.
④ * He is the first to introduce live attenuated vaccines → for
- Rabies
- Anthrax
- Chicken cholera (Pasteurella)

Robert Koch

* Father of Bacteriology
* He introduced staining methods
* He introduced solid culture media
* Discovered causative organisms of
- TB
- Cholera
- Pink eye (Hemophilus aegyptius)

* To check motility of bacteria, he introduced the "hanging drop preparation" method.

* Established growth requirements of bacteria. (By Louis pasteur)

Resolving Power (RP)

* Human unaided eye (RP is) 0.2 mm / 200 µm
* RP of compound microscope (Light / Bright field) 0.2 µm / 200nm (0.3µm > 0.2)
* RP of electron microscope is 0.2 - 0.5 nm.
* Light microscope is introduced by (or Compound M)
Anton Van Leewen Hook
* Electron microscope by
Knoll & Ruska
* RP of Interference microscope → estimation of chemical constituents of a cell (proteins, nucleic acid)

Gram Staining

* First introduced by Danish bacteriologist (1884)
Hans Christian Gram
* It differentiates bacteria in to 2 large groups:
→ Gram positive
→ Gram negative

Steps MCQ

① * 1° stain → with Crystal violet or Gentian violet or Methyl violet

* Then drain the 1° stain

② * After that ~~dxxx~~ add mordant (fixer) → Iodine (which fixes the 1° stain in to cytoplasm)

* Then drain again

③ * Add decoloriser → Alcohol/ Acetone/ Alcohol-Acetone.

④ * Then add counterstain → Safranine

⇒ Gram +ve → Blue

⇒ Gram -ve → Decolourise, so appears pink.

Principle of gram stain

* G+ve cell wall is thicker → so retains 1° stain better & hence decolourisation is slower.

* G+ve cytoplasm is more acidic and this acidic cytoplasm binds the basic 1° stain better → so decolourisation is again slower.

* G+ve cell wall has more lipids → gets dissolved by alcohol/acetone → pore formation → rapid decolourisation

Exceptions to gram stain

1) Spirochaets
   - Treponema pallidum
   - Leptospira
   - Borrelia
   ↓
   Uses negative staining (India ink or Nigrosine) or silver impregnation stains

2) Mycobacteria (lipid rich cell wall)
   ↓
   Uses acid fast stain (ZN stain)

3) Mycoplasma → smallest pathogenic bacteria.
   ↓
   Uses Giemsa staining

4) Chlamydia & Rickettsia
   ↓
   Giemsa stain / Castaneda stain

5) Polysaccharide granules by
~~Sudan B~~ Iodine

6) Lipid granules by
Sudan Black B.

7) Spores are demonstrated by
Ashby stain

Gram Positive bacilli (rod shape)

* Corynebacterium
* Mycobacterium
* Bacillus } spore bearing bacteria
* Clostridium }
* Actinomyces } GPR, slender, branching
* Nocardia }
* Listeria
* Erysipelothrix

(GPR → Gram +ve rods)

Gram Positive cocci (rounded)

* Staphylococcus
* Streptococcus
* Enterococcus

Gram Negative bacilli

* Pseudomonas
* Enterobacteriaceae
* Vibrio (G-ve, curved rod, comma shaped)
* Bacteroides (Anaerobes)
  ↓
  MC commensal in the human colon.

Gram Negative cocci

* Neisseria
* Moraxella catarrhalis
  (causes respiratory tract infections → Otitis media, sinusitis, acute exacerbations of COPD)

• Neisseria gonorrhoeae
  - Kidney/bean / coffee bean shaped diplococci (3D)

• Neisseria meningitis
  - Half moon shaped (3D) diplococci

Gram negative spirals

* Spirochetes → Treponema
  Leptospira
  Borrelia
* Campylobacter (causes invasive diarrhea)
  Blood & mucus in stool
  (pus cells & RBC +ve)

* Helicobacter → causes gastritis, peptic ulcers, gastric adeno carcinoma, MALTomas

Helicobacter pylori (H. pylori)

* Spirillum
  = S. minus → causes rat bite fever or Sodoku fever.

Gram negative coccobacilli

- Pleomorphic G-ve bacilli
  * Chlamydia
  * Rickettsia
  * Hemophilus
  * Brucella
  * Bordetella
  * Pasteurella
  * Francisella
  * Legionella

Cultivation of bacteria

* Louis Pasteur → established that all bacteria requires a minimally a source of carbon & nitrogen
* Grew bacteria for the 1st time on liquid media.
* Robert Koch → introduced solid media

Solidifying agents (by Robert Koch)

* Gelatin → from animal bone & hide (animal skin)
  ① * chemically gelatin is protein in nature.
  * Needs to be added in 15% concentration to solidify the culture media
  * 2 disadvantages:
    1) Proteolysed by several bacteria.
    2) Solid only at temp < 24°C

* Agar-agar
  ② - Derived from sea weeds & red algae.
  - Polysaccharide in nature
    ↓
    30% is Agaropectin
    70% is Agarose
  - Absolutely inert substance ie, not hydrolyzed by any bacteria
    Also contains no growth promoters or inhibitors.
  - Provides no nutrition
  - 1.2 – 2% concentration (2% > 1.2–2)

- If ↓ agar conc. to 0.2-0.5% → it becomes semisolid in nature → k/a soft agar medium

↓

Used in checking motility of the bacteria

eg: Motility Test agar.

- Semisolid medium → transparent → if nonmotile bacteria → it grows only along line of insertion of rod → if motile → spreads all over & opaque completely.

- If ↑ agar conc. to 5-6% → k/a firm agar

↓

Used in inhibition of swarming of bacteria (spread across medium)

Swarming bacterias

G+ve
- Clostridium tetani
- Bacillus cereus

G-ve
- Proteus vulgaris
- Proteus mirabilis
- Vibrio alginolyticus
- Vibrio para-haemolyticus

⇒ Agar solidify < 42°C

(N) incubation temp. 37°C)

Other solidifying agents

3) Egg or Serum

Types of culture media

100% mcq

A) Simple or basal medium

* Provides source of C & N
* ONLY non-fastidious bacterium grows (no need of other nutrients other than C & N here)

- Pseudomonas
- Staphylococci
- Vibrio
- Bacillus
- Enterobacteriaceae.

eg: • Peptone in H_2O → Peptone water
• Meat extract + Peptone water → Nutrient broth
• Meat extract + Peptone water + Agar → Nutrient agar

B) Enriched medium

* Contains egg or serum or blood to provide extra nutrition to bacteria

eg: • Blood Agar ⇒ 5 or 10ml sterile sheep blood + autoclaved nutrient agar cooled to 50-55°C (90/95 ml)

Depending on blood added,
5% or 10%
↓
Preferred is 5% sheep blood agar.

- Chocolate agar:
  5 or 10 ml sterile sheep blood + 90/95 ml nutrient agar but cooled to 70–75°C

- Egg containing enriched medium
  eg: Lowenstein Jensen (LJ) medium, Dorset Egg medium
  ↓
  Only need for mycobacterium
  - LJ medium kept in Mc Cartneys bottle (green coloured)
  - Mycobacterium need 3–8 wks incubation.

- Serum containing enriched medium
  Loeffler's serum slope
  ↓
  used for Corynebacterium diphtheriae

* PPLO medium (Pleuro pneumonia like organism)
  ↓
  For mycoplasmas (also k/a PPLO)

© Selective medium.
Medium in ⊆ a selective agent is added ⊆ inhibits unwanted bacteria & favours growth of wanted bacteria.

(i) Change the pH
- For all pathogenic bacteria, optimum pH = 7.2 – 7.4
- For Vibrio, optimum pH is 8.2 – 8.4
  eg: TCBS

(ii) By addition of antibiotics
eg: Thayer Martin medium
Modified "
- Thayer Martin medium contains Vanco + Colistin + Nystatin
- In modified thayer martin V + C + N + Trimethoprim

⇓
Both are selective for Neisseria.

- Other eg: PPLO medium
  ↓
  for mycoplasma (lacks cell wall)
  ↓
  So can add cell wall acting antibiotic like penicillin

(iii) Adding chemicals

eg • Bile salts (inhibits G+ve)
↓
Mac Conkey medium
which is selective for G-ve

• TCBS medium
Selective for Vibrio

• XLD medium.
Selective for Salmonella & Shigella

• DCA medium
Salmonella & Shigella

• Potassium tellurite (KT)
All Corynebacteria are resistant to 0.3–0.4 % of KT. ↓
eg: TINSDALE medium
or cystine tellurite

• Cetrimide blood agar
Selective for Pseudomonas

• 7–10 % salt concentration (NaCl)
All Staphylococci species are resistant to it.
eg: Salt Agar / Salt milk agar ↓
Selective for Staphylococci

(iv) Adding dyes

eg: Eosin-methylene blue agar (EMB Agar)
↓
Inhibits G+ve (GP)
↓
≈ Mac Conkey → selective for G-ve (GN)

- LJ medium
Selective for Mycobacterium
MALACHITE GREEN

- Crystal violet BA (Blood agar)
Selective for Streptococcus pyogenes (sore throat)

- Mc Conkey medium
Selective for GN
dye: Crystal violet
(other was bile salt)

(2) Enrichment medium.

* Liquid & selective medium

eg: Alkaline peptone water
→ for Vibrios

Selenite F broth, GN broth } for Salmonella & Shigella

Tetrathionate broth
(for Salmonella)

(E) Differential medium

* Colony colour / appearance → differentiates b/w groups of bacteria.

eg: Blood Agar:

↓

Helps to diff. b/w ∝-hemolytic (green color / partial hemolysis) from β & γ-hemolytic

- β → clear/yellow, complete hemolysis
- γ → no hemolysis, complete color of the colonies & media.

Imp Mc Konkey Agar (Mac)

- Pink colour
- Contains Lactose
- Forms either lactose fermenting colonies or non lactose F.C.
- LFC → Bright pink
- NLFC → Pale

Imp TCBS medium

- Sucrose fermentes → Yellow
- Non " → Pale
- Both for Vibrio colonies

Mannitol salt agar (MSA)

- Selective for Staph
- Mannitol ferments → Yellow
- Non mannitol " → Pale

(whenever sugars used → acids formed → changes pH) (color change)

(F) Indicator Medium:

Indicator agent added & changes colour when certain bacteria grow.

eg: Mc Conkey agar

Added neutral red.

TCBS medium

Bromothymal blue.

MSA (mannitol salt agar)

Phenol red

KTBA (Pottasium tellurite blood agar)

Corynebacterium converts KT in to metallic tellurium (black color)

(G) Transport media.

Used for transporting clinical specimen to microbiology lab to maintain original count of bacteria in clinical specimen.

MCQ

eg: • Stuart's medium } for Neisseria
• Amie's " }

• VR medium (Venkat raman Ramakrishnan) → for Vibrio

• Thioglycollate medium → for Anaerobes

MCQ • Pike's medium → for Streptococcus pyogens

• Cary Blair medium
Universal stool transport

↓

This turbidity 0.5 corresponds to 1.5×10^8 bacteria/ml

eg: UTI → urine → extract E.coli → then do sensitivity testing → take peptone water + 4-5 colonies → measure turbid of this suspension in spectrophotometer → make turbidity 0.5.

Antibiotic susceptibility or sensitivity test.

MCQ * Mc Farland's is used for expressing the turbidity

+ which antibiotic is most effective in Rx →

(C) * Temperature of incubation of tubes → 37°C

(D) * Time of interpreting results 16-18 hrs

(A) Recommended media:

MCQ i) Mueller Hinton agar or broth (MHA/B) (for non fastidious bacteria)

ii) MHA/B + 5% blood (for fastidious bacteria)

(B) standard inoculum of test bacterium / test isolate

Special suspension in peptone H_2O or nutrient broth ≤ has a turbidity of 0.5 Mac Farlands (McF)

(E) Methods of testing

• Best / Reference method is dilution method →
Agar dilution / Broth dilution

• Broth dilution
- Macro-broth dilution → done in test tubes
- Micro broth dilution → done in micro titer plates

- Prepare serial concentrations of antibiotics (AB) in MHA / MHB.

+

Fixed amount of standard inoculum to each tubes / plate / wells

↓

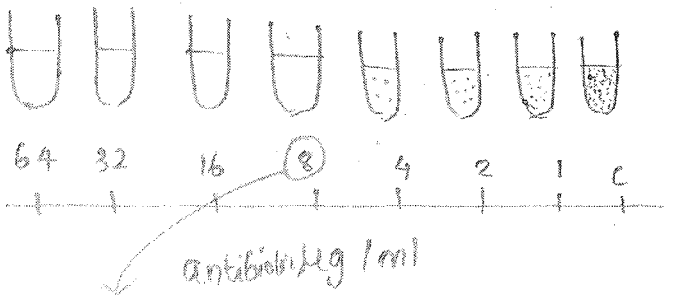
Incubate at 37°C for 16-18 hrs

↓

Find out lowest concentration of antibiotic ⊆ just inhibits the growth of bacteria.

k/a minimum inhibitory concentration (MIC)

- MIC changes for every bacteria
- Control test tube have no antibiotic
- Take test tubes → add MHB → 1ml of standard inoculum is added → incubate 37°C, 16-18hrs
- Control has max. turbidity → ie, max. growth of bacteria.

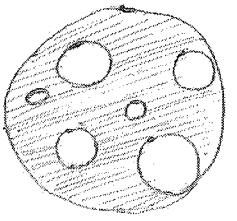


No bacterial growth → MIC is 8 µg/ml

② Disc diffusion – Kirby Bauer method

⇓

- MC used routine laboratories
- Here only use MHA
- 0.5 McF → std. inoculum
- Temp : 37°C
- Time : 16-18 hrs.
- Take sterile MHA → swab inoculate the std. inoculum to get a lawn culture (semi congruent growth)
- Antibiotic disc (impregnated ⊆ single standarized conc^n of antibiotic) are placed over the swab inoculated MHA.
- Can see zones of inhibition of growth → 37°C, after 16-18 hrs → measure the diameter of zone of inhibition → compare with standardised tables.
- By this method → can categorize bacteria → in to → sensitive / intermediate / resistant.



* Disc diffusion method is a qualitative test not quantitative

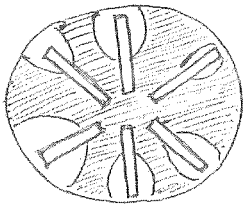
mea

↓

cannot measure MIC

(3) • Latest test : Epsilometer test / E-test

* It is a combination of dilution of disc diffusion method.
* MHA → 0.5 McF → 37°C, 16-18 hr
* Here instead of disc, have 6 plastic strips which have graded concentration of antibiotics



* Here can calculate MIC with the readings on strip.
* Medium recommended
  Muller hinton agar & broth
* So E-test is quantitative test

Bacterial Growth Curve

* All bacteria → 2 types → Photrophs & Chemotrophs

↓

those bacteria which derives all energy from sunlight for binary fission

* Chemotrophs → Those bacteria ⊆ need to undergo chemical reactions for synthesis of energy

Chemotrophs

↙ ↘

Autotrophs

↓

bacteria ⊆ can utilize atm. gases for undergoing chemical reactions

Heterotrophs

↓

bacteria ⊆ needs preformed organic compound (glu, aa) for chemical reacts

↓

Also k/a Chemo-organotrophs.

mea

* Most human commensals & pathogens are chemoorganotrophs

———×———

Generation time

Time required for 1 binary fission
Also k/a population doubling time

- GT of E. coli is 20 min
- M. tuberculosis → 20 hr
- M. leprae → 20 days

Viable count

No: of live bacteria

Total count

Live + Dead bacteria

———×———

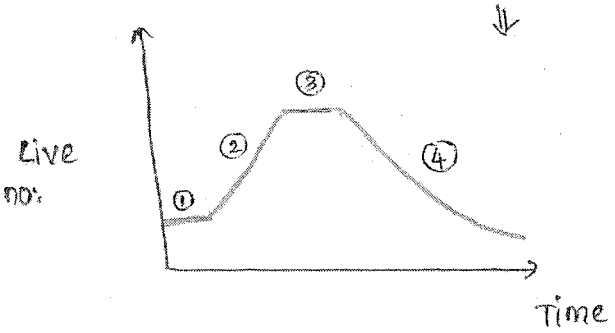
Peptone water

→ Count the no: of live bacteria regularly

↓

Bacterial growth curve

⇓



① → Lag phase

* Stage of adaptation of the bacterium.
* Varies for different bacteria
* Metabolically active stage
* NO replication
* No viable count is constant (VC)
  Total count → constant (TC)
* At the end of lag phase, the size of bacteria is maximum.

② → Log phase

* Stage of active replication of bacteria.
* VC ↑
* TC ↑
* Size of bacteria is small

[Maximum effect on antibiotics seen here]

③ → Stationary phase:

* Stage of gradual nutrient depletion.
* No: of bacteria multiplying = no: of bacteria dying
* VC → constant
* TC → ↑↑
* Toxin production & spore formation (sporulation) occurs.

④ Death phase / Declining phase

* Total nutrient depletion
* Toxic metabolites ↑↑
* No further replication
* Most bacteria are dying.
* VC ↓
* TC constant.

× ——— ×

Batch culture

* A closed system with fixed amounts of nutrients in which bacterial growth curve is followed.

Continous culture

* Maintainence of the log phase
* By adding fresh nutrients at regular intervals
* Removing toxic metabolites @ regular intervals

mcq

* Done in a Chemostat & Turbidistat

KINGDOM MONERA

* Includes prokaryotes
* All eubacteria & cyanobacteria (Blue green algae)

* MCQ

Prokaryotes	Eukaryotes
• Nuclear membrane -nt Nucleolus -nt Histone protein -nt	All present
• Cytoplasmic membrane bound organelle -nt (mitochondria, golgi body, ER, lysozyme)	Present
• Single, circular chromosome	Multiple, linear chromosome
• Mesosomes +nt	Absent
• Extrachromosomal DNA +nt in plasmids	• Extrachromosomal DNA is +nt in mitochondria
• Sterols -nt in cell membrane.	• Present
• Muramic acid +nt in cell wall	• Do not have cell wall / lacks muramic acid
• 70S ribosome (30S + 50S)	• 80S ribosome (40S + 60S)

• Bacteria → outer well defined capsule / not well defined slime layer.
• Invagination of cell membrane with respiratory enzymes
Mesosome.

Special exceptions

* Only prokaryote lacking cell wall (OR)
* Only prokaryote having sterols in cell membrane

⇓

MYCOPLASMA / PPLO

* Hence k/a joker of microbiological park.
* PPLO : Pleuro Pneumonia like organisms.
* Only prokaryote lacking muramic acid
Chlamydia
* Only eukaryotic having plasmids
Fungi

Glycocalyx

* Layer (outermost) outside cell wall
* 2 parts → Slime & Capsule

* Slime: Loose ill defined polysaccharide layer around CW

* Group of bacteria growing together producing slime
Biofilm

* Biofilm ⟹ Role in virulence of bacteria
1) Helps in adhesion
2) Antiphagocytosis
3) ↓ Entry of abs antibiotics

* eg: of Slime producing bacteria
✓ - Strep. mutans
✓ - Staph. epidermidis
✓ - Pseudomonas aeruginosa
(Not a normal flora in healthy → but resp. tract commensals in cystic fibrosis
→ occasionally mutates giving them ability to produce excessive slime
→ now k/a mucoid P. aeruginosa)

Causes of Pulmonary disease in cystic fibrosis

* 70-75% due to mucoid P. aeroginosa
* 20-25% due to small colony variants of Staph. aureus
* 5-10% due to Burkholderia cepacia
* 1% → Hemophilus influenza

* Capsule
- Well defined layer around the cell wall
- Polysaccharide in nature
- Antiphagocytic
- Protection from lytic enzymes & bacteriophages
- Viruses infecting bacteria is k/a bacteriophage.
- Most important virulence factor.
- Serial subculture of a capsulated bacteria leads to loss of capsule → loss of virulence → k/a smooth to rough variation.
- Has no net charge → hence cannot be stained
- So demonstrated by negative staining → can use India ink or Nigrosine.
- Capsule is antigenic → going to generate anticapsular Ab
- Capsule + specific anticapsular Ab's → swelling of capsule
↓
k/a Quellung reaction
- Streptococcus pneumoniae ← capsulated bacteria.

Capsulated Bacteria

(Yes Some Bacteria Have Killer And mean Capsules)

- Y. pestis (Yersinia)
- Streptococcus pneumoniae
- Bacteroides fragitis
- Bordetella pertussis
- Hemophilus influenza
- Kleibsella pneumoniae
- Bacillus anthracis
- Meningococcus
- Clostridium perfringens & Clostridium neoformans (fungus)

⇒ Bordetella pertussis → Non antigenic

⇒ Bacillus anthracis → Polypeptide capsule.

⇒ Bacteroides fragitis → Zwitter ionic capsule.

⇒ C. neoformans → Cryptococcus.

Cell Wall

G+ve CW

* Thicker (30-80nm)
* 50-100 layers of murein monomers cross linked by peptides
* Aromatic & sulphur containing aa -nt
* Teichoic acids are +nt in CW

G-ve CW

* 10-25 nm thick
* 2 layers of murein cross linked by peptides
* All varieties of aa +nt
* Teichoic acid are -nt
* Outer membrane periplasmic space + Lipopolysaccharide (LPS) or endotoxin +nt

Gram positive cell wall

* Murein monomer → 2 carbohydrate
  - NAG (N-Acetyl glucosamine)
  - NAMA (N-Acetyl muramic acid)
* Both are arranged alternatively

NAG - NAMA - NAG - NAMA -

NAMA - NAG - NAMA - NAG -

* Tetrapeptide side chains in all NAMA.
* Each cross linked by ~~tetra~~ penta peptide side chains

* Cross linking of murein monomer is mediated by
  - Carboxy peptidases
  - Trans peptidases

* All β-lactam antibiotics (Peninullin, Cephalosporin etc) bind transpeptidase & inhibits them.

* so trans peptidases is also k/a penincillin binding proteins.

● Teichoic acids

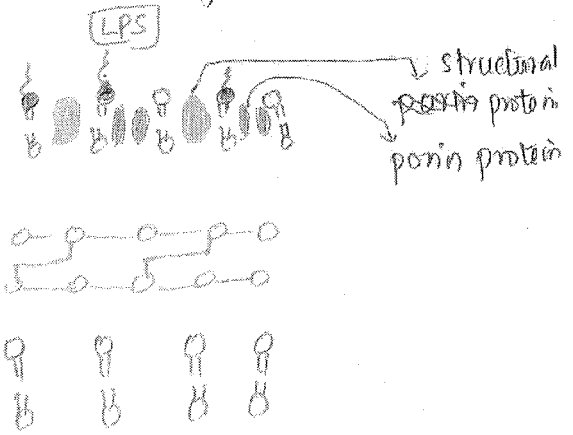
* 2 types
  1) Cell wall teichoic acids
  2) Cell membrane teichoic acids

* Cell wall TA are polymers of ribitol PO_4

* Cell membrane TA are polymers of glycerol PO_4.

Gram Negative cellwall

* Cell membrane → phospholipid bilayer → periplasmic space outside CM → contains 2 murein monomers → they are crosslinked



→ outer membrane is phospholipid bilayer → with porin proteins & structural proteins / integral protein ~~bfw them~~ in them → outside has LPS / Endotoxin

* Murein is also k/a peptidoglycan or murein mucopeptide.

● LPS / Endotoxin

* Unique in G-ve bacteria
* 3 parts
  1) LIPID-A (embedded in outer membrane) ↓ have actual endotoxin activity
  2) Core polysaccharide short chain of 6-10 carbohydrates.
  3) 'O' or somatic Ag it is polysaccharide, outermost part of LPS

MOA of LPS in humans

* GN (-ve) infection → GN lysis → endotoxin / LPS released → lipid-A is recognized by Toll like receptor-4 (TLR-4) located on surface of MΦ & dendritic cells (APC) → binding → ~~releases cytokine~~ dcy

activation of nuclear transcription factor - NF κB → Releases cytokines → IL-1, IL-6, TNF-α, IL-8, IL-12 released.

MCQ
⇒ LPS acts by releasing cytokines

Pharmacological effects of LPS

- Fever, ↓BP
- ↑ vasc. permeability
- Intravascular coagulation (DIC)
- Shock & multi organ failure

All these effects are only seen with large amounts of LPS

MCQ
Endotoxin

- Present only in CW of G-ve
- Released only on lysis
- LPS in nature (Lipo poly saccharide)
- Heat stable
- Low antigenicity (ability to induce Ab almost absent)
- Cannot be toxoided
- Low toxicity (large amt needed)
- Constant effect on body ~~pharmacological~~ action

Exotoxin

- Both G+ve & G-ve
- Actively secreted
- Polypeptides
- Heat labile
- High antigenicity
- Very well toxoided
- Highly toxic (small amounts shows pharmacological actions)
- Variable effect ~~pharmacological~~ action

ability to cause disease

Toxoid → Toxin loses its virulence but has antigenicity (to produce Ab)

eg: Tetanus toxoid
Diphtheria toxoid.

~~Also has low toxicity (large amount required)~~

Special exceptions

MCQs
* Only G+ve having LPS

Listeria

MCQ
* Botulinum toxin (exotoxin) is not secreted but released on lysis

LAL Assay

- Limulus Amebocyte Lysate assay
- Can detect small amounts of endotoxin in clinical specimen.
- Amoebocyte & lysate
  ↓
  In tube + Clinical specimen (of LPS)
  ↓
  Lysate coagulation

(only)
- Endotoxin → in cell wall of G-ve bacteria

* Horse ~~crab~~ shoe crab → its body cavity contains amoebocyte

L-forms

* Cell wall deficient bacteria
* Either arise spontaneously or in the presence of cell wall inhibitors like penicillin
* 1st shown on bacteria Streptobacillus moniliformis at LISTER institute in London
* Can arise from any G+ve / G-ve bacterium
  → highly pleomorphic
* On culture medium → forms tiny colonies → not visible to the naked eye → best seen directly under the microscope.
* Since they loss cell wall → non pathogenic in lab animals (unable to cause disease)
* But proposed to play a role in development of antibiotic resistance & persistence of infections.

Flagella

* Organs of locomotion
* 3 - 20 μ long
* broad - 0.01 μ
* Made up of protein-repeating subunits of flagellin.
* Each flagella has 3 parts
  - Filament
  - Hook
  - Basal body.
* Basal body is made up of rings rotated by a proton dependent pump (originates here)
* Rotation is transmitted to the filament
* On G-ve bacteria, basal body has 4 rings ~~(BB pump)~~
  M, S, P, L
* G+ve bacteria, BB has 2 rings
  M, S
* Flagella can present only on poles or all over the body
* Monotrichous (one flagella @ one pole)
  eg: Vibrio
  Pseudomonas aeruginosa
* >1 flagella @ single pole
  - Lophotrichous
  eg: Helicobacter pylori
* >1 flagella @ both poles
  - Amphitrichous
  eg: ~~Campylo~~ Campylobacter, Spirillum.
* All over → Peritrichous
  eg: Bacillus, Listeria, Clostridium, Enterobacteriace family

* Peritrichous flagella of Listeria is formed @ only 25-28°C
Not @ 37°C (room temp)

Flagella demonstration

Direct (seeing them)
↓
- Electron microscope
- Leifson & Ryu impregnation stain

Indirect (motility)
↓
1) Hanging drop preparation
2) Soft agar medium.

★ Types of motility (Imp)

- ✓ Corkscrew (Treponema pallidum) MCQ
- ✓ Darting (Vibrio & Campylobacter)
- Gliding (Mycoplasma - no flagella) or PPLO
- ✓ Tumbling (Listeria) also k/a end to end motility
- ✓ Stately (Clostridium & Salmonella)
- Serpentine (~~Eikinella corrodens~~ Bacillus)
- Twitching (Eikinella corodens)

* Twitching / Jerky / Rotatory
Trichomonas vaginalis
(protozoa not bacteria)
* Falling leaf — Giardia lamblia

HACEK

* Special group of bacteria
* Eikenella belongs to it
* H → Hemophilus parainfluenzae
Hemophilus aphrophilus
A → Actinobacillus species
↓
now k/a Aggregati bacter
C → Cardiobacterium
E → Eikinella
K → Kingella
* They have common properties
1) G-ve (GN) pleomorphic (various size) bacilli
2) Capnophilic (grow better in 5% CO_2)
3) Slow growing organisms (colonies form in 2-3 days)
4) Do not grow on MacConkey medium (extra nutrition)
5) Only grow on blood agar.
6) All are normal oral flora
* But during dental manupulations if bacteremia occur due to HASEK group → they have affinity for cardiac valves → so infect cardiac valves → subacute bacterial endocarditis (3-5% by HASEK bacteria)

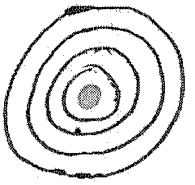
* MCC of SABE (Subacute bacterial endocarditis)
Strep. viridans

Pili

- Two types based on function
  1) Common pili / fimbriae
  2) Sex pili
- All pili are protein in nature → repeating subunits of Pilin.
- Common pili / fimbriae
  - Helps in adhesion
  - 1-1.5 μ long
  - Present only on surfaces of G-ve (GN) bacteria.
- Sex pili
  - Mediates conjugation
  - 5-20 μ long
  - Formed by both G+ve & G-ve bacteria (if they have the 'F' plasmid)

Spore

* Formed by certain bacteria in extremes of conditions
  eg: Nutrient depletion
  Extreme heat / dryness.
* Amongst pathogenic bacteria → only 2 genera are spore forming
  1) Clostridium
  2) Bacillus
* Clostridium → Bulging spores
* Bacillus → Non bulging spore, only grow in soil & culture
* But clostridium → grows in soil, culture, human body
* Each spore contains → @ centre : Core, surrounded by 4 layers



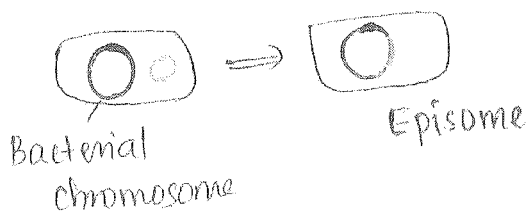
* Core → contains genome,
  MCQ → Dipicolinic acid present which is responsible for heat resistance of spores
* 1st layer - Spore wall / Germ cell wall
  - Made of typical peptido glycans
  - MCQ → It forms future wall of vegetative bacteria
* 2nd layer - Spore cortex
  - Atypical peptidoglycan in nature
  - MCQ → Thickest layer of spore

* 3rd layer - Spore coat
  - Made up of keratin like proteins
  - Responsible for resistance to chemical disinfection (MCQ)

* 4th layer - Exosporium
  - Lipo protein in nature

Plasmids

* These are extra chromosomal ds circular DNA molecules present in bacterial cytoplasm.
* 1-40 plasmids can be present in one 1 single bacterium.
* They are not essential for the life of the bacteria
* They are capable of replication independent of chromosome.
* Sometimes plasmids may integrate with the chromosome
  ↓
  kla Episomes



* If bacteria undergo binary fission → one copy each of plasmids are passed on to both daughter bacteria →
* That is k/a Vertical Transfer
* But sometimes, occassionally some plasmids can also be transferred horizontal by conjugation ↓

  kla horizontal transfer
  ↓
  Only those plasmids which have special "tra" genes
  ↓
  genes for sex pilus or conjugation tubes

Types of plasmids

A) Based on ability for transfer
  1) conjugative
  2) Non conjugative

* Conjugative → capable of both Vertical & horizontal transfer
* But non conj → only capable of vertical transfer

B) Based on function of plasmids
  1) Fertility plasmids / F-plasmids
     ↓
     encodes sex pilus
     ie, responsible for conjugation

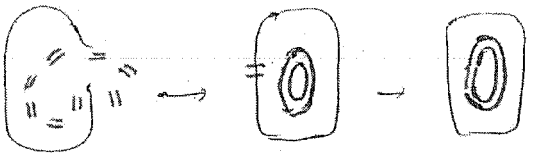
2) Resistance plasmids
Contains antibiotic resistance genes

3) Virulence plasmids.
Have genes for special virulence factors.
eg: Toxin, capsule

4) Col plasmids
Encode bacteriocins - which are antibiotic like proteins which kills other bacteria.
eg: E-coli producing Colicin
Kleibsiella → Klebocins
Corynybacterium diphtheria → Diphthericins
Pseudomonas aeroginosa → Pyocins
(gives survival adva advantage over other bacterias)

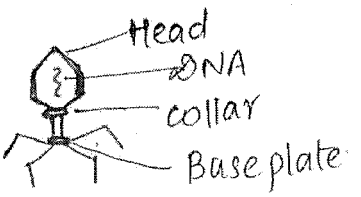
** Imp Mechanisms of gene transfer in bacteria.

(A) Transformation:
* It is uptake of soluble DNA fragments from the environment directly through the cell wall
* This phenomenon was first demonstrated on streptococcus pneumoniae / Pneumococcus by GRIFFITH.



* Ability to take up DNA fragments through cell wall
k/a Competence

B) Via bacteriophages
* They are viruses infecting bacteria → Bacteriophages
* These viruses, bind to receptors on cell wall of bacteria & they inject the DNA in to the cytoplasm.



* After injecting DNA in to cytoplasm → follow either lysogenic or lytic cycle

Lytic cycle
* Phage DNA stops bacterial metabolism
* Uses bacterial enzymes to synthesize phage components
* Assembly of daughter phages →

induces lysis of bacterium → now inject new bacterium

Lysogenic cycle

* Phage DNA integrates with bacterial chromosome → now k/a Prophage
* And bacteria is k/a lysogenic bacterium
* Prophage passed on to future generations
* On exposure to UV radiations/ nitrogen mustard → induction of prophage → phage DNA separates out → enters lytic cycle.

Types of phages

* Phages that only follow lytic cycle k/a Virulent phages
* Phages that follows both lytic & lysogenic cycle k/a Temperate phage
* eg: Virulent phage T_1, T_2 of E. coli
* eg: of Temperate phage Lambda phage of E. coli

(i) Transduction

Imp
* MC mechanism of gene transfer
* DNA is transferred from one bacterium to other by a defective or mispackaged phage
* During assembly, bacterial genes are incorporated in the phage head → defective/ mispackaged phage

Imp
* This virus apart from its viral genes have bacterial genes in it. (Viral mediated bacterial recombination)

(ii) Lysogenic conversion

* In some temperate phages, the prophage itself supplies genetic information such that the lysogenic bacterium exhibits a new property/ a new characteristic which earlier it did not have

eg: Ability to produce toxin
↓
k/a phage mediated toxin

eg: ✓Diphtheria toxin
✓Cholera toxin
Enterogenic E. coli → Verotyto toxin (Shiga like toxin)
By Streptococcus pyogenes → Pyrogenic toxin A, B, C
Botulinum toxin C & D

c) Via conjugation

* Transfer of genetic material from one bacterium to another through a bridge like connection ⊆ is k/a sex pilus b/w two cells (sex pilus / conjugation tube)

↓

Conjugation.

* Ability to form sex pilus is due to presence of fertility / F / sex plasmid.
* Bacteria ∈ F plasmid k/a F^+ bacterium
* Those without F plasmid k/a F^- bacterium

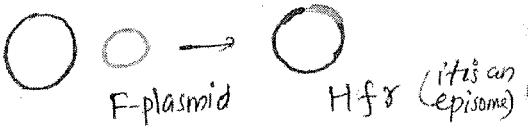
① $F^+ + F^- \longrightarrow 2F^+$

(ds plasmid → one strand passed to F^- → acts as template → forms ds plasmid → both becomes F^+) (F^- to F^+)

* This is first demonstrated by Lederberg & Tatum on E.coli K12 strain.

High frequency ~~conjugation~~ recombinant (Hfr)

* It is a bacterium with a F-plasmid is integrated in to its genomic DNA.



* Hfr bacterium during conjugation, it transfers just some chromosomal DNA to the F^-.
* Usually the Hfr chromosome or sex pilus breaks before it is completely transfered

② $Hfr + F^- \longrightarrow Hfr + F^-$ with chromosomal gene

* So here F^- remains F^-

F' plasmid / F' factor

* Hfr state is reversible
* When F factor is seperating from the chromosome, it may carry some chromosomal genes next to its site of attachment → it is k/a F prime factor (F')

③ (F plasmid (free) ∈ chromosomal gene)

* Transfer of F' via conjugation is k/a Sexduction

R-plasmid

* It is F plasmid with antibiotic resistance genes
↓
now k/a resistance transfer factor

* Antibiotic resistance genes are k/a r-determinants.

④

* Spread of R plasmid via conjugation is the MC mechanism of spread of antibiotic resistance.

MCQ

Col plasmid / Col factor

* F plasmids in which genes for bacteriocin production are also present.

⑤

(5 types of conjugation)

1) $F^+ + F^- \rightarrow 2F^+$ ($F^- \rightarrow F^+$)
2) $Hfr + F^- \rightarrow Hfr + F^-$ c CG
3) F' formation
4) F plasmid + Antibiotic resistance
↳ R plasmid
5) F plasmid + Bacteriocin pdtn gene

MCQs

- Bacteriophages carry portion of bacterial DNA to other k/a Transduction.
- If F plasmid is attached to bacterial genome → k/a Hfr.
- Transduction is viral mediated bacterial recombination
- A bacterial cell transfer chromosomal genes to F-cells but rarely cause them to become F^+, the bacterial cell is Hfr.
- A bacterium containing phage DNA integrated in its chromosome Lysogenic.
- Term used for a bacterial cell that is able to take up naked DNA Competent.

x——————x

GRAM POSITIVE COCCI

GRAM POSITIVE COCCI

Micrococci

- Non pathogenic
- G+ve cocci in tetrads
- Strict aerobe
- Catalase test +ve
- Oxidase +ve

Staphylococcus

- G+ve C seen in clusters
- Aerobes & facultative anaerobes
- Catalase +ve
- Oxidase -ve

Differentiates

Streptococcus

- G+ve C seen in chains / pairs
- Aerobes & facultative anaerobes
- Catalase -ve
- Oxidase -ve
- Enterococcus included

Staphylococcus

* G+ve cocci in grape like clusters
* Non motile
* Non sporing
* Microcapsule +/-
* All are facultative anaerobes (even in absence of O_2 can grow)
* All species → resistant to 10% salt
* Biochemical tests:
  - Catalase test +ve
  - Oxidase test -ve
  - Ferments sugars (Cability to utilize sugar under anaerobic condition)
* Cultivation → Grow on simple media (C & N)

Classification of Staphylococcus

* Slide coagulase test:
  Detects bound coagulase enzyme
  → also k/a clumping factor.
* Tube coagulase test:
  Detects the free coagulase enzyme (tube → slide it → free coagulum @ bottom → +ve test)
* If both test +ve then Staph. aureus
* If one / both are -ve then Any other staph. species k/a CONS (Coagulase -ve staphylococci)

Staphylococcus aureus

* β-hemolytic on blood agar
* Golden pigment
* Mannitol fermentation +ve } Only Staph. species

CoNS

- S. saprophyticus } only species ⊆ are resistant to Novobiocin
- S. xylosus
- S. hemolyticus → only CoNS ⊆ is ~~hemolytic~~ β-hemolytic (complete hemolysis)
- S. lugdunensis → has bound coagulase in its cell wall.
- S. epidermidis

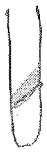
MCQ S. epi & S. saprophyticus are differentiated by Novobiocin resistance.

* S. epid Vs S. aureus
  1) Coagulase test
  2) Hemolysis
  3) Pigment
  4) Mannitol fermentation
* S. aureus Vs. S. saprophy
  All above 4 + Novobiocin

Staphylococcus aureus

* Normal flora in humans
* MC → Nose & Oropharynx (30% human)
* Other sites → Skin, Hair, Perineum, Vagina
* Biochemical test:
  - Slide coagulase +ve
  - Tube coagulase +ve
  - Mannitol fermentation +ve
  - DNAse test +ve
  - Phosphatase test +ve
  - Urease test +ve
  - Novobiocin sensitive
  - Gelatin liquefaction +ve
* Cultural characteristics:

In nutrient agar
- Forms golden yellow non diffusible pigment
- Oil paint appearance on semi slant tube



- Bordetella pertussis → Aluminium paint appearance.
- On blood agar → Large pin head, β-hemolytic colonies

- On Mac Conkey medium:
  forms tiny, lactose fermenting (bright pink) colonies [also by enterococcus]

- Selective medium
  - Salt agar (10% salt)
  - Salt milk agar
  - Ludlam's medium
  - Polymyxin agar

- Selective + Differential medium
  ↳ Mannitol salt agar
  (bright yellow colonies)
  (only by Staph. aureus)
  (all CoNC → produce pale color colonies)

Diseases.

* MC infection → Skin & soft tissue infections

* Soft tissue infections are:
  - MCC of Stye (cornea) / Boil / Carbuncle, Furuncle, Ecthyma, Bullous impetigo, subcutaneous abscess (breast abscess), ~~any post-operative wound infection~~
  - (MCC) Any post. operative wound infection
  - (MCC) Botryomycosis (≈ Mycetoma)

* Bone, muscle & Joint
  - MCC of osteomyelitis
  - " septic arthritis
  - " septic bursytis
  - " Tropical pyomyositis

* Respiratory tract
  - Pharyngitis
  - Sinusitis
  - Otitis media
  - Bronchitis
  - Pneumonia
  - Lung abscess
  - MCC of pneumatoceles

Other causes of pneumatocele
- Strep. pneumoniae
- H. influenzae
- Kleibsiella pneumoniae
- Pneumocystis (fungus)

* Cardiac
  - MCC of infective endocarditis
  - MCC of early & late prosthetic valve endocarditis
  - MCC of infective endocarditis in i.v drug users.

* Others:
  - Meningitis
  - UTI
  - Bacteremia

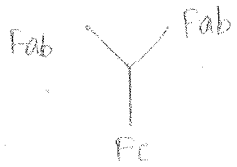
* Toxin mediated syndromes:

1) Toxic shock syndrome
2) Scalded skin syndrome (Ritters disease)
3) Food poisoning

Virulence factors of Staph. aureus

(I) Cell wall associated

1) Microcapsule +/-
2) Slime +/-
3) Peptidoglycan cellwall & Teichoic acids
4) Adhesion proteins
   eg: Fibronectin binding proteins
5) Bound coagulase
   It activates prothrombin which leads to conversion of fibrinogen to fibrin → forms an antiphagocytic barrier
   ↓
   It is detected by slide coagulase test.
6) Protein-A
   It is an antiphagocytic + anti complementary protein
   • It can bind to Fc part of IgG Ab.

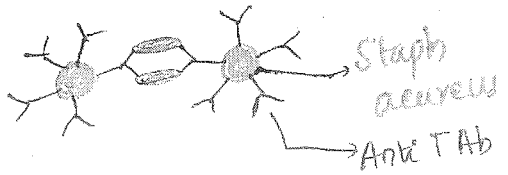


• This ability is used in the coagulation test for detection of soluble antigens.

Sol. Ag + Ab → Immune complex
(occasionally)
(not seen macroscopically)

eg: S. typhi Ag in serum + Ab → invisible immune comp

So Anti S. typi Ab + S. aureus + specimen → visible agglutination



(II) Secreted virulence factor by staph. aureus

1) Free coagulase
   - MOA → same as bound coagulase
   - Detected by tube coagulase test.
2) Hemolysins
   • Has 4 antigenic type α, β, γ, δ
   • α → most important hemolysin
   • shows paradoxical reactivation 80-100°C

- β-hemolysin → sphingomyelinase in nature
  - shows hot cold phenomenon/ hemolysis
  - 37°C → 4°C
- γ-hemolysin → membrane damaging by pore formation
  +
  Bicomponent toxin
  ↓
  Both these properties → k/a Synergohymeno tropic toxins

3) Panton Valentine Leucocidin

4) Exfoliatins / Epidermolytic toxins
   - EF-A or EF-B (2 antigenic types)
   - Serine proteases in nature
   - Cleave protein desmoglein-1 of desmosomes
   - Site of action is stratum granulosum of neonates & young infants.
   ↓
   Colonization or infection by S. aureus → EFA or EF-B secreted
   - Systemic dissemination → stratum granulosum → proteolyse d&g-1 → mid epidermal split → generalized exfoliation → k/a SSSS (Staphylococcus ~~Staphylococcus~~ Scalded Skin Syndrome)

5) Enterotoxins
   * 8 antigenic types
     A, B, C_1, C_2, C_3, D, E, G
   * Heat stable toxins (resist boiling for up to 40 min)
   * MOA: Stimulation of vagus and vomiting centre
   * S. aureus food poisoning
   ↓
   - H/O cooked milk/meat products contaminated by S. aureus carrier → preformed toxins
   - Incubation period: 1-6 hr (Also bacillus cereus has emetic type food poisoning)
   - C/F: Nausea, Vomiting, Abdominal cramps, Diarrhea, No fever
   - Self limited in 10-12 hrs
   - Rx: Fluid & electrolyte supplements, no antibiotics required

6) TSST-1 (Toxic shock syndrome toxin 1)

- Also k/a enterotoxin F.
- Belongs to pyrogenic toxin super antigens → all cause fever
  1) Fever
  2) ↑ lethality of endotoxin in rabbits by 1 lakh fold

* 3) Super antigen

Foreign Ag → phagocytosed by dendritic cells → processing → peptides + MHC II Ag → migrates to regional LN → cell mediated / humoral immunity.

Super antigen

① Do not need processing by APC
② They directly binds to MHC-II Ag of APC at a site lateral to usual Ag-presenting groove.
③ Need to be recognized by just variable-Vβ part of TCR (5-20% of Th may be sharing same Vβ TCR) ← (huge number)
④ Th → activated (all)
⑤ Massive cytokine release → responsible for toxic shock syndrome.

(Generally a foreign Ag is recognized by only < 0.0001% Th)

Types of TSS:

1) Menstrual TSS (Toxic shock syndrome)

* ↑ vaginal colonization by S. aureus + h/o use of high absorbancy tampoons during menstruation.

* 100% cases of menstrual TSS is due to TSST-1

2) Non menstrual TSS

* Either due to infection or colonization by S. aureus or Strep. pyogens.

* Due to TSST-I (or) Staph. enterotoxins - SEB & SEC. (or) pyogenic toxins A, B or C (S. pyogenes)

* DOC for Staph. aureus β-lactam.

β-lactam

* MOA → Binds to penicillin binding protein → inhibition → no cross linking → lysis of bacteria
(Trans peptidase - protein)

* Adverse

Mechanism of resistance to β-lactam in S. aureus

① * MC → betalactamase production
- Mediated by R-plasmids
- In S. aureus → R-plasmids → most often spread by transduction.
(MC in others → conjugation)

② MRSA / ORSA
- Methicillin resistant staph. aureus
- Oxacillin »
- Less common
- Chromosomaly mediated resistance
- Chromosome contains mobile genetic element ⊛ i/a SCC mec → contains "mec A" gene → which encodes an altered transpeptidase (PBP) (PBP 2a / 2')
↓
Not bound ∴ not inhibited by any β-lactam

MCQ - Diagnosis of MRSA

i) Agar / Broth dilution method
MIC for oxacillin ≥ 4 µg/ml then strain is MRSA
(MIC - min inhi concentr)

ii) Disc diffusion test:
- Cefoxitin disk (superior) or Oxacillin disc
- If zone of inhibition is smaller than recommended zone then MRSA.

iii) Rapid test - Latex agglutination for altered PBP 2a

⇒ Gold standard for MRSA → PCR for mec A gene.

MCQ ⇒ Best method for diag of MRSA
a) methycillin disc
b) Oxacillin disc
c) cefoxitin disc
✓d) Oxacillin agar

- mec A gene is expressed best not at 37°C but at 30-33°C. Hence incubation is done at this temperature.

Rx. of MRSA

* Isolation of patient for 2 days after start of Rx.
* mc mode of spread of MRSA / S. aureus in hospitals via hands of hosp health care workers.

* DOC → Vancomycin, Teicoplanin } Glycopeptide antibiotics

Alternative: Linezolid, Daptomycin, Streptogrammins, Tigecyclines

MCQ
* All are used for MRSA Rx except
  a) Vancomycin
  b) Imipenem (β-lactam) ✓
  c) Ciprofloxacin
  d) Linezolid

Only β-lactam effective in Rx. of MRSA → 5th generation Cephalosporins "CEFTOBIPROLE"

CoNS

(Coagulase negative str.

Staphylococcus Epiderdimidis

* Slide coagulase -ve
* Tube coag" -ve
* Novobiocin sensitive
* Mannitol -ve
* Non hemolytic on blood agar
* Non pigmented
* MC staph species present on skin as (N) flora.
* Diseases
  - MCC of bacteremia in iv catheterized individuals
  - MCC of infections in orthopedic implants
  - MCC of dialysis associated peritonitis
  - 2nd MCC of early prosthetic valve endocarditis (1st S. aureus)
  - 2nd MCC of post-operative wound infections after S. aureus
  - S. epi is multi drug resistant
* DOC → Vancomycin

Staphylococcus saprophyticus

* Slide coagulase -ve
* Tube coagulase -ve
* Novobiocin resistant
* Mannitol -ve
* Non hemolytic on blood agar
* Non pigmented
* (N) flora on skin, genito-urinary tract
* Diseases
  - After E. coli, MCC of UTI in young ~~pated~~ ♀ (recently sexualy active)

* Scarlet fever → S. pyogenes
* UTI in young ♀
  E. coli > S. saprophyticus

Streptococci

* Gram +ve coccus → chains & pairs
* Non motile
* Non sporing
* Capsule +/-
* Facultative anaerobes
* Biochemical test
  - Catalase -ve
  - Oxidase -ve
  - Ferments sugar
* Cultivation → Only grow on enriched medium (meat, egg/blood or serum)
* Classification → done by Brown
  ↓
  On the basis of hemolysis on horse blood agar-pour plate cultures.
* Classified in to
  1) α-hemolytic → partial (on blood agar) hemolysis, green color
     - Viridans group
     - S. pneumoniae

	Viridans	S. pneumonia
	• Convex colonies	• Carrom coin colonies
	• G+ve cocci in chains	• G+ve diplococci
Best / Specific	• Bile insoluble	• Bile soluble
	• Optochin resistant	• Optochin sensitive

2) β-hemolytic Streptococci
   - Yellowing
   - Lancefield serogrouping on basis of 'C' carbohydrate antigen in cell wall → in to serogroups A to W (-I/J) (no I & J)
   - Most pathogenic is grp A
     eg: S. pyogenes
   - Grp B → S. agalactiae
   - S. pyogenes is
     Only species { • Sensitive to bacitracin
     • Ribose fermentation -ve
   - S. agalactiae gives
     • CAMP test +ve
     • Hippurate hydrolysis +ve.
   • Neonatal meningitis MCC S. agalactiae

3) Gamma (non) hemolytic

- Group ∅ strep (Grp ∅ carbohydrate Ag)

Enterococcus	Non enterococcus group
• Bile resistance +ve	• Bile resistance +ve
• Esculin hydrolysis +ve	• EH +ve
• PYR test +ve	• PYR test -ve
• Growth in 6.5% NaCl ⊕	• Growth in 6.5% NaCl ⊖

Viridans group

+ Viridis - Green

* α-hemolytic on Blood agar

* Contains
  - S. mutans
  - S. mitis
  - S. salivarius
  - S. anginosus
  - S. sanguis

* S. salivarius → Longest chains
  → Non pathogenic

* Viridans are (N) oral flora and in the upper resp. tract

* Diseases:
  1) MCC of dental carries
     MC species → S. mutans
  2) MCC of subacute bacterial endocarditis
  3) 2nd MCC of late prosthetic valve endocarditis
     (1st → S. aureus)
     < 60 days : Early
     > 60 d (after 2 months) : Late
  4) MCC of human bite infections
     S. anginosus
     - Other causes of human bite infection
       - Anaerobic streptococci
         k/a Peptostreptococci
       - Eikinella

β-hemolytic on Blood agar

+ Lancefield serogrouping:
  A to W (except no I & J)

* It is on basis of 'C' carbohydrate antigen in cellwall.

* 'C' carbohydrate Ag → has to be extracted out from the cell wall (difficult process)

* Lancefield method to extract 'C' carbohydrate Ag → by HCl acid

"C" Ag + Abs prepared in rabbit.

⇓

Ring precipitation test (ring of precipitate is formed)

Group A / S. pyogenes (Imp) (capsulated)

* Griffith typing (typing → is intra species strain characterisation)

↓

on the basis of "M" protein in the cell wall.

↓

in to > 80 "M" types (M_1, M_2, ----)

* Earlier M types, eg: M_1, M_3, M_6 mainly causes respiratory tract infections
* Latter M types eg: M_{49}, M_{51}, M_6, mainly causes skin and soft tissue infections
* Biochemical tests:
  1) Sensitive to bacitracin
  2) Ribose fermⁿ -ve
  3) PYR test +ve
* Culture:

Blood agar → Pinpoint (very small) β-hemolytic colonies

* If colonies are mucoid / matt appearance → Virulent strain
* If colonies - Glossy → Avirulent strain.
* Transport medium: PIKE'S
* selective medium → Crystal violet blood agar.

Virulence factors (S. pyogenes)

Cell wall associated

1) Polysaccharide capsule made up of hyaluronic acid
2) Peptidoglycan & Teichoic acid
3) Adhesin proteins
4) "M" protein

* M protein → Antiphagocytic & anti complementary.

↓

- embedded inside the cell wall
- Ab's to M protein provides type specific life long immunity

Secreted virulence factor

1) Streptolysins / Hemolysins
   - 2 antigenic types
   - Streptolysin O (SLO) & SLS.
   - SLO is
     - O_2 labile (active only in reduced state)

- Hemolysis due to SLO is seen only in pour plate culture (inside medium, not on surface)
- SLO is antigenic → "ASO" is Ab produced against it (Anti SO → ASO)
- ASO Ab titer is used in retrospective diagnosis of a recent S. pyogenes infection in rheumatic fever.
  Significant ≥ 200 todd units titer

- SLS is
  - O_2 stable
  - ie, active in both reduced as well as oxidised state
  - Hence the β-hemolysis seen around the surface colonies due to "SLS"
  - Non antigenic → So no Ab formed against it.

2) Streptokinase (Fibrinolysin)
- It activates plasminogen.
  ↳ lysis of fibrin (MOA)
- Responsible for spread of streptococcus infections along with hyaluronidase.
- It is antigenic
- Same also produced by group C & gp A streptococci

Use: Thrombolysis (MI, Thromboembolism)
- Since antigenic, it should be given once in life for thrombolysis (2nd time → Abs already formed attacks it)
- Streptokinase used for thrombolysis is produced by Group C strep S. equisimilis.

3) Pyrogenic / Erythrogenic / Scarlatini form / Dick toxins.
- It is of 3 antigenic types → A, B, C
- They all belong to pyrogenic toxin super antigens.
- So responsible for streptococcal toxic shock syndrome & symptoms of Scarlet fever.

4) Streptodornase / DNase
* They depolymerize the DNA
* So responsible for (watery exudate) serous character of strep. exudate.
* 4 antigenic types → A, B, C, D
* Most antigenic: DNase B
* Anti DNase B Ab titer is used in retrospective

diagnosis of recent S. pyogenes infection in post. strep. glomerulo nephritis.

5) Hyalurodinase / Spreading factor: (antigenic)

6) NADase

* Diseases of S. pyogenes:

① * MCC of bacterial sore throat (pharyngitis)

* sore throat is further classified in to
- Tonsillitis
- Quinsy
- Otitis media
- Sinusitis
- Ludwig's angina

② * Causes skin & soft tissue infections
• MCC of N - Necrotising fasciitis (Flesh eaters disea
N - Necrotising fasciitis
I - Impetigo
C - Cellulitis
E - Erysipelas
L - Lymphangitis

③ * Other diseases → Endocarditis, Pneumonia, septic arthritis, Bacteremia

④ * Also Toxin mediated syndrome
Toxic shock syndrome & scarlet fever.

⑤ * Immune complex mediated
i) Rheumatic fever
follows only sore throat
ii) Glomerulonephritis
follows sore throat or skin & soft tissue infection

Treatment

* DOC is Penicillin
- No resistance is reported to Penicillin

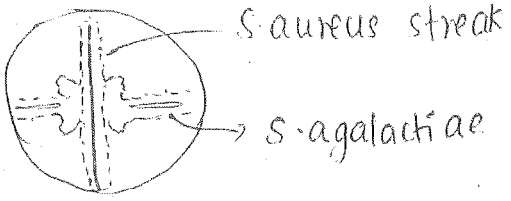
Cross reactions:

* Hyaluronic capsule of S. pyogens × Synovial fluid
* cell wall protein × Myocardium
* cell wall carbohydrate × Cardiac valves

(× : Cross reacts with)

Group B / Strep. agalactiae

* Capsulated
* Biochemical test:
1) CAMP test +ve
(Christie Atkinson Munch Peterson)



- Hemolysin of gp B strep synergises with β-hemolysin of S. aureus
  ↓
  Enhanced zone of hemolysis

2) Hippurate hydrolysis +ve

* Culture
  on
  • Blood agar → Pinpoint ~~colonies~~ β-hemolytic colonies

* Normal flora in GIT & genito urinary tract.

* Diseases

i) Neonatal disease (MCQ)

2 types
→ Early onset
→ Late onset

- Early onset neonatal disease present in 1st 6 days after delivery
- They are infection acquired from maternal tract during delivery

MCQ - Neonatal meningitis, Neonatal pneumonia & N. septicemia

- Late onset → 7th to 3 months after delivery.
- It is acquired from community
- Meningitis, Pneumonia, Septicemia (manifestations)

⇔ Causes of Neonatal meningitis

- MCC : E.coli

MCQ - Grp B streptococci
= Listeria - Klebsiella pneumoniae
- Some caused by
  - Staph aureus
  - Streptococcus pneumonia

ii) Adults

→ Resp. tract infections
→ Puerperal sepsis
→ Endocarditis.
→ Septic arthritis

Rx

- DOC : β lactams / Penicillin

α-hemolytic on Blood agar

1) Enterococcus
2) Non enterococcal gp D strep
   ↓
   S. bovis
   S. equinus

* S. bovis now k/a S. gallolyticus

- S. bovis causes septicemia & endocarditis in colorectal cancer patients.

Enterococcus

* Gram +ve cocci in pairs/short chains.
* Biochemicals
  - Bile resistance (Grow in 40% bile)
  - Esculin hydrolysis
  - PYR test +ve
  - Grow in 6.5% salt
  - Grow @ pH 9.6
  - Grow @ wide temp range 10°C - 45°C
  - Resistant to sodium azide
    ↳ so used as a selective agent.
* Culture characteristics of enterococcus.
  - On blood agar → Non-hemolytic colonies.
  - Mac Conkey medium → forms tiny LF colonies (Lactose fermenting)
  - Selective medium → Sodium azide blood agar.

* It is a normal GIT flora

Diseases

- UTI
- Wound infections
- Intra abd. abscesses
- Peritonitis
- Endocarditis

* MC clinical isolate is Enterococcus faecalis (80-90%)
* Next common → E. faecium (5-15% infection)

Rx

* Inherent resistance to penicillins, cephalosporins & aminoglycosides
* But both resistance to penicillin & cephalosporins are overcome by synergism
* So usual Rx of enterococcal infection → Ampicillin + Gentamicin
* Occasionally some strains are highly resistant to aminoglycosides → synergism does not work → DOC is Vancomycin

Streptococcus pneumoniae
(Pneumococcus)

* G +ve cocci → pairs/short chains
* Flame shape / Lanceolate shaped

* Capsulated
* On the basis of capsular ~~og~~ Ag classified in to >90 capsular serotypes (done by Quellung reactn)
* Out of 90 capsular serotypes → Types 1 to 8 → >75% adult infections.
* Types 6, 14, 19, 23 → >75% children infectn
* Facultative anaerobes
* Capnophilic (grows better in 5% (O_2))

Biochemical tests

1) Bile solubility test +ve
   - ↑ activity of autolytic enzymes by bile salts is k/a bile solubility test.
     (turbid → to clear)
2) Optochin sensitivity test
   (no growth around optochin disk)

Culture characteristics

- On blood agar → α hemolytic colonies formed → carrom coin or draughtsman appearance

Virulence factors

1) Polysaccharide capsule
2) Peptidoglycan & Teichoic acids
3) Adhesin proteins
4) O_2 labile hemolysin k/a Pneumolysin.
5) IgA 1 protease → breaks/ proteolyses IgA 1
   ↓
   Also secreted by 2 bacteria
   - Neisseria mengitidis
   - H. influenzae.

* Can present as normal flora of upper resp. tract

Diseases

* Noninvasive diseases (No bacteremia)
  - MCC of otitis media
  - sinusitis (mcc)
  - Acute exacerbations of COPD (MCC → H. influenzae)
* Invasive diseases (with bacteremia)
  - MCC of ~~lobar or broncho~~ community acquired lobar or broncho pneumonia
  - MCC of meningitis in any age & in immunodeficient except HIV (Cryptococcus neoformans)

(except neonates)

MCQ
* MC infection caused by Pneumococcus
Otitis media

MCQ
* Dangerous organisms in spleenectomized individuals (meningitis & septicemia)
1) Pneumococcus
2) Meningococcus
3) H. influenzae

MCQ
(Vaccines for these ds are recommended to splenectomized individuals)

Rx
- DOC is β-lactams
(few reports of resistance)

Vaccine against Pneumococcus
(Subunit vaccines)

1) Polysaccharide vaccine
- Capsular Ag of 23 capsular serotypes.
- Given as single dose i.m
- Provides type specific immunity for 5 years
- Recommended to be given in
  * Splenectomized
  * HIV
  * Long term steroids & cytotoxic drugs
  * Age > 65
  * Chronic hemolytic anemia
  * Chronic lung, liver, brain or kidney ds
- Not immunogenic in < 2 yrs age children

2) Conjugate vaccine
- For < 2 yr age children
- 13 capsular Ag. conjugated to a carrier protein (non toxic toxin of a mutant Corynebacterium diphtheriae)
- Schedule:
  6 wks
  10 wk
  14 wk
  } 1° immunization
  Booster after 12-15 months

FAMILY - ENTEROBACTERIACEA

* Has 35 genera.

Properties
1) GN bacilli
2) Facultative anaerobes, non sporing
3) Motile by peritrichous flagella except
   - Klebesiella
   - Shigella
   - Yersinia pestis
   - Salmonella gallinarum-pullorum

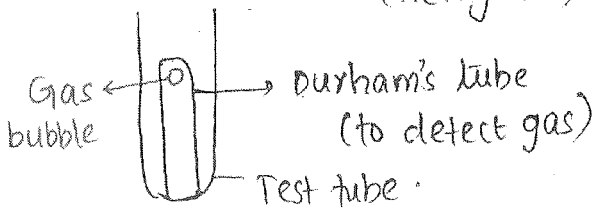
Atypical E. coli (not imp)

* 4) Catalase test +ve
(except S. dysenteriae)

5) Oxidase -ve (also staph., strep.)

6) Reduce nitrates to nitrites
(Nitrate reducth +ve)

7) Ferment sugars → produce acid
(anaerogenic)
→ Acid + Gas (CO_2)
(Aerogenic)



8) Easily grow on simple media

9) Grow on Mac Conkey medium
→ LF colonies (pink)
→ NLF colonies (pale)

- Ingredients of Mac Conkey medium
  - P - Peptones
  - L - Lactose
  - A - Agar
  - N - Neutral red (pH indication)
  - T - Sodium taurocholate } selective agents
  - +/- C - Crystal violet

- Mac Conkey medium here
  - selective
  - differential
  - indicator

Que: Mac Conkey medium is
a) selective (mildly only)
✓ b) differential
c) Enriched
d) Enrichment

10) Contains R plasmids
(resistant to antibiotics)

Members

- Escherichia
- Shigella
- Edwardsiella (less imp)
- Citrobacter
- Klebsiella
- Enterobacter
- Serratia
- Salmonella
- Proteus
- Morganella (less imp)
- Yersinia

⇒ Not psuedomonas, not vibrio
(not member of enterobacteriaceae)

Biochemical test

* IMViC test
  - I → Indole test
  - M → Methyl red test
  - V → Voges Proskauer (VP)
  - C → citrate utilization

- Indole test → converts tryptophan to indole
- M → On sugar fermentation → ↑↑ acid pdtn → pH remain <4 for very long.
- VP → On sugar fermⁿ → Acetoin produced.
- C → Utilize citrate as sole source of carbon

* Shigella ⇒ Causes bacillary dysentery / Shigellosis

* Most antigenic heterogenous S. boydii
* " homogenous S. somnei
* MF → Mannitol fermentation
  NMF → Non mannitol fermentation
* NLF → Non Lactose fermentation
* MC in India: S. flexneri.

Shigella

* Non motile, non capsulated.
* On basis of somatic 'O' Ag → classified in to 4 species.

Species	Serotypes	Mannitol	Lactose	Special comment ★★
Shigella dysenteriae	10 serotypes, 1 → Kla shigas bacillus	No mannitol fermentation	Non lactose fermenting	Most severe disease
S. flexneri	6 serotypes	MF	NLF	MC species in India
S. boydii	>16 serotypes	MF	NLF	Least common species
S. somnei	1 serotype	MF	Late LF	• Mildest ds • MC species in West • Most resistant species

cal reactions

gar fermentation → Anaerogenic (except some S. flexneri)

2S -ve on sugar fermentation

IMViC - + - - (only S. flexneri +ve)

Urease -ve

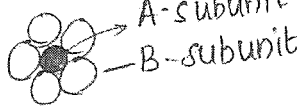
Cultivation

* On Mac Conkey medium → pale colonies (- somnei → LLF)
* Transport medium : Sachs buffered glycerol saline or Cary Blair
* Enrichment → Selenite F broth or G-ve broth.
* Selective medium → • DCA (Deoxycholate citrate agar)
  • XLD (xylose lysine deoxycolate citrate agar)
  • HEA (Hoektoen enteric agar)
  • SSA (Salmonella Shigella agar)

* Diseases : Shigellosis
* Pathogenesis of shigellosis :
  - Invasive diarrhea
  - Site of invasion - Colon & Rectum
  - Causes
    • Entero invasive E. coli
    • Shigella
    • Campylobacter
    • Vibrio parahemolyticus
    • Yersinia enterocolitica
    • Non typhoidal salmonella
  - Invasive due to "plasmid" mediated invasiness.
  - Shiga toxin → plays a supplementary role in the disease. (Shigellosis)

↓

• Only by S. dysenteriae type 1
• TOxin → AB5 subunit toxin



• Site of action → Endothelial cells of small blood vessels of GIT
• MOA : Receptor mediated endocytosis → transported to ER → A splits from B → breaks to $A_1 + A_2$, A_1 is active part
  → A_1 enters cytoplasm & cleaves 60s ribosomal subunit
  → inhibition of protein synthesis
  → necrosis of endothelial cell → activation of coagulation
  → local ischemia
• In 5% of S. dys type 1 infection → shiga toxin disseminates via blood → glomerular endothelial cells → inhibition of

protein synthesis → necrosis of cells
→ coagulation activation
⇓
K/a HUS (Hemolytic Uremic Syndrome)

• Complication of shigatoxin by S. dys type 1 → HUS

Bacillary dysentery

* Strict human disease
* Incubation period → 12 hr – 5 days (average: 2 days)
* Infective dose; ID_{50} → 10–100 bacteria
↓
easily transmitted by
1) Fomites
2) Person to person
3) Human feces
4) Contaminated food/H_2O

* C/F :-
  - Fever
  - Abdominal cramps
  - Diarrhea
  - Blood & mucus in stools
* Self resolution in 5–7 days
* But some develops complications
  - Fluid & electrolyte imbalance
  - Intussesupeption
  - Toxic megacolon
  - Reactive arthritis (only S. flexneri)
  - HUS (only by S. dys 1)

* Diagnosis:
  * Best specimen : Fresh stools
  * Rectal swabs acceptable
  * Gram stained smear :
    Pus cells ++
    RBC ++
  * Culture on selective medium → isolation of colonies
  * Prove invasiveness → by
    ↓ Sereny Test
    Kerato conjunctivitis in rabbit or guinea pig

Rx
* Self limited → fluid & electrolyte supplementation only required
* Antimotility agents should be avoided (worsens disease)
* Very severe / young / old / imm. def → Antibiotics according to sensitivity testing

E. coli

* All are motile except Atypical E. coli
* 80% capsulated
* Typing is done on basis of 3 Ag

"O" Ag → > 170 'O' serotypes (x)

"K" Ag → 100 K "

(capsular)

"H" (flagellar) Ag → >75 H "

Biochemical test

* Sugar fermentation → Aerogenic
* H_2S -ve
* IMViC ++ - -
* Urease -ve

Culture

* MaC Conkey → LF colonies (bright pink)
* Blood agar → β-hemolytic colonies

⇒ Present as normal flora in GIT

⇒ GIT flora :

Strict Anaerobes		Facultative Anaerobes
↓	1000 : 1	↓
• Clostridium		• MC is E. coli
• Bacteroides (MC in human colon)		• Klebesiella
• Actinomyces		• Enterobacter
• Bifidobacterium		• Proteus
• Eubacterium		• Citrobacter
• Peptostreptococci		• Enterococcus
• Lactobacillus		

Diseases of E. coli

1) Diarrhea

- By diarrheagenic E. coli

(i) Entero invasive E. coli (EIEC)

EIEC

- cause disease similar to shigella.
- 2 differences

1) Infected dose, ID50 is 1000 ~~not~~ times more

$10^4 - 10^5$

2) No strain produce Shiga toxin

MCQ

- Sereny test +ve

(ii) Enterotoxigenic E. coli (ETEC)

- MCC of bacterial diarrhea in all ages except infants

(MCC → EPEC)

- MCC of travellers diarrhea
- Infective dose, ID50 is

$> 10^9$ bacteria

- No role of fomite & no person to person transmission
- 1° transmission by food or water.

- Virulence factor:

1) Colonization factor Ag → help ETEC in adhesion to small intestine mucosa

They are either fimbrial protein or outer membrane protein

eg: CFA-I, CFA-III

(2) Plasmid mediated
↳ Heat labile toxin (LT)
↳ Heat stable toxin (ST)

LT	ST
• AB5 subunit toxin (≈ cholera toxin)	• Small, 19 aa long polypeptide
• MOA: ↑ cAMP in SI mucosal cells by ADP ribosylation of Gs protein. (α-stimulatory: G_s)	• MOA: ↑ cGMP ★ by directly ↑ activity of guanylate cyclase ★

⇒ Both ↑ cAMP & ↑ cGMP → ↑ secretion of ions in to small intestine ⇒ lumen

* C/F
- Water diarrhea ± fever & ± vomiting
- Abdominal cramps
- Generally self limited in 2-3 days.

* Diagnosis
- Collect stools
- Gram smear → Pus cell ⊖ RBC ⊖
- Culture on blood agar & Mac Conkey medium
↓
Isolation of colonies
- Prove ETEC → by toxigenicity tests.

Toxigenicity tests

LT	ST
• Rabbit Ileal loop assay (read @ 18 hrs)	→ " (read @ 6 hrs)
• Y1 adrenal tumor cell assay	• Suckling (infant) mouse intragastric assay
• Chinese hamster ovary assay	

(Cytotoxic effect on cell lines)

Rx
* Oral rehydration
* Antimotility drugs → Bismuth subsalicylate or loperamide.

* Fluoroquinolones shorten duration.
* Prophylaxis of travellers diarrhea.
  → Fluoroquinolones (Ciprofloxacin)
  → Now FDA approved Rifaximin.
  (Rifaximin > ciprofloxacin)

iv) EPEC

* Enteropathogenic E. coli
* Mcc of infantile bacterial diarrhea

v) EAEC

* Entero aggregative E. coli
* Mcc of chronic / persistent diarhea in the World.
* Can cause travellers diarrhea.

vi) EHEC / VTEC

* Enterohemorrhagic E. coli or Verotoxigenic E. coli
* Mcc of HUS in World
* Reservoir is GIT of cows (zoonoses)
* MC H/O food → contaminated poorly cooked beef or hamburgers
* Also via contaminated unwashed salads
* Via contaminated water
* Via person to person or fomites
* ID50 < 1000
* Produces verocytotoxin or Shiga like toxin

Verocytotoxin

* Phage mediated toxin
* All features ≈ Shiga toxin
* C/F:
  - Fever
  - Abdominal cramps
  - Bloody diarrhea
  - Self limited in 5 days
* Some develops complication
  - HUS (SLT dissemination)
* Most dangerous serotype (severe ds & common HUS)
  ↓
  E. coli → 0157 : H7

Diagnosis of EHEC

* Fresh stool
* Gram smear → Pus cells+, RBC+
* Isolation of culture on Mac Conkey & Blood agar
* Prove EHEC → 2 steps

a) O157 : H7 → Detected by Sorbitol Mac Conkey test

All E. coli are	O157 : H7
Sorbitol fermⁿ +	No sorbitol fermⁿ
PINK colonies	Pale colonies.

b) Non O157 : H7 EHEC → detected by tissue culture assay or cytotoxicity assay on vero-cell lines.

Rx of EHEC

* Fluid & electrolyte supplements
* Absolute C/I – Antibiotics (worsen the disease)

Other diseases due to E. coli

* Mainly caused by normal GI flora strains having special virulence factors & have entered a normally sterile extra-intestinal site.

a) MCC of UTI → Any age, any sex, community acquired, nosocomial/post catheterization UTI.

* UTI caused by uropathogenic E. coli → have P. pili/fimbriae → have binding ability to urogenital mucosa.

Diagnosis of UTI (any)

* Sample → ~~Mid stream urine~~
  Best specimen : Suprapubic aspirate
  Next best : Catheter specimen
  MC used : Mid stream urine.

* Kass criteria for significant bacteriuria.
  ↓
  says if $\geq 10^5$ fine colony forming unit /ml of a single bacterial type in a midstream urine sample (MSU) is significant
  ↓
  This only for asymptomatic cases

* Symptomatic ♀ → $\geq 10^2$ cfu/ml of MSU
  ♂ → $\geq 10^3$ cfu/ml of MSU

* For catheter specimen $\geq 10^2$ cfu/ml

* For suprapubic aspirate → even 1 colony is significant

b) MCC of intra abdominal abscess
c) MCC of peritonitis
d) MCC of neonatal meningitis & septicemia
e) Wound infection, bed sore infection.

Proteus

* Motile
* Non capsulated
* Biochemical test
  - PPA test +ve → ability to convert phenylalanine to phenyl pyruvic acid.
  - Aerogenic / Anaerogenic (x)
  - IMVic → Variable (x)
    P. vulgaris : + + − +
    P. mirabilis : − + − +
  - H_2S +ve (on sugar fermentation) (ie, H_2S is produced »)
  - Urease test +ve.

---

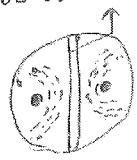
* Urease +ve organisms:
  - Helicobacter pylori (max)
  - Proteus
  - Kleibsiella
  - Morganella
  - S. aureus
  - Ureaplasma
  - Brucella
  - C. neoformans (Yeast)

Cultivation of Proteus

* Mac Conkey → NLF
* Blood agar → swarming + +
  Fishy smell.

* Normal flora in GIT
* Infections → UTI, wound infection, Intra abd. abscesses, nosocomial pneumonia.

* Diene's phenomenon ⇒ Used in epidemiological typing of proteus strains.
  - Urine → P. vulgaris
  - Pus swab → P. vulgaris
  - To prove both as same strain
    ↓
    Take in a culture media
    ↙ ↘
    if same strains → swarmings of both strain merges
    if different strains → ↓ seperation zone b/w swarming



- Diene's stain is used for demonstrating colonies of mycoplasma.

Klebsiella pneumoniae

* K/a Friedlander's bacillus
* Non motile
* Capsulated

Biochemical test

* Aerogenic on sugar ferments
* H_2S -ve
* IMViC → − − + +
* Urease + ve

Culture

* Mac Conkey → LF
* Blood agar → Mucoid colonies
* (N) flora of GIT

Infections

- Community
  - UTI
  - Friedlander's pneumonia
    - Risk factor: Alcoholism, DM, COPD, CRF
    - Mainly affects upper lobe
    - Blood stained: Currant jelly sputum
- Nosocomial (hospital)
  - Post catheterization
  - Neonatal meningitis & septicemia
  - Intra-abdominal abscess
    - Peritonitis
    - Pneumonia
  - Wound & bed sore infection

* ↓ DOC
* Multidrug resistant bacteria.

Enterobacter

* Closely related to Klebsiella
* But motile

Biochemical test

* Aerogenic
* H_2S -ve
* IMViC − − + +
* Urease -ve

Cultivation

* Mac Conkey → LF
* (N) flora in GIT

Diseases

- Intra abd. abscesses
- Nosocomial pneumonia

MCQ

* Klebsiella Vs Enterobacter

Klebsiella	Enterobacter
• Non motile	• Motile
• Urease +ve	• Urease -ve

* UTI ⟹ Recurrent UTI is due to proteus → persistant alkalinization of urine → Struvite stones (Mg ammonium PO_4 stones) are deposited.

Serratia marcesans

* Red pigment k/a Prodigiosin
* Pigment producing bacteria
  - S. aureus (golden yellow)
  - P. aeroginosa (blue green)
  - Serratia marcesans
  - Flavobacterium
  - Atypical mycobacteria
  - Chromo bacterium

Salmonella

* All Salmonella are motile, except S. gallinarum pullorum.
* Non capsulated except S. typhi, S. paratyphi C, S. dublin.
* 2 species of Salmonella
  - S. enterica
    - ↓
    - 6 subspecies
    - eg: enterica, indica, anzonae
    - ↓
    - > 3000 serotypes
  - S. bongori

done by Kaufan & White → used 3 Ag i.e are "O", "H", "Vi" Ag

O → Somatic Ag
H → Flagellar Ag
Vi → Capsular Ag

* S. enterica subspecies enterica serotype typhi

O : H (Vi) capsulated
9, (12) (Vi) : d

* S. enterica subspecies enterica serotype paratyphi A

O : H (No capsule)
1, 2, (12) : a

* " " paratyphi B

O : H (No capsule)
1, 4, 5 (12) : b ; 1, 2

Phase / antigenic variation

⇒ In India → all serotypes causing typhoid shows O Ag 12.

⇒ Widal test → Typhoid
↓
detecting significant titre of O, H Ag → add O Ag of S. typhi to O Abs → detects Abs of paratyphi A & B too.

Biochemical test (Salmonella)

* Aerogenic (except S. typhi) *
  (Anaerogenic)
* H_2S +ve (on sugar fermentation)
  except paratyphi A.
* IMViC (except typhi −+−−)
  −+−+ (×)
* Urease −ve.

Culture

* Mac Conkey → NLF
* Transport medium → Cary Blair
* Enrichment → Selenile F broth
  GN broth
  Tetrathionate broth
* Selective medium → DCA, XLD, SSA, HEA
  ↓
  Best → Wilson & Blair medium
  (Green coloured medium)
  ↓
  Contains Bismuth sulfite

Bismuth sulfite $\xrightarrow[\downarrow H_2S]{\text{Salmonella}}$ Bismuth sulfide (Black)

★ Imp

H - Antigen

- Flagellar Ag
- Protein in nature
- Highly antigenic
- Phase / Antigenic variations ++

H-Ab's appear earliest after infection & persists for several months

O - Antigen

- Somatic Ag
- Polysaccharide in nature
- Lower antigenicity
- O Ab's follow appearance of H Ab's & disappear in 2 wks time.

Vi Antigen

- Capsular Ag
- Polysaccharide in nature
- Least antigenic
- Only present in 3 serotypes of Salmonella
  - S. typhi
  - S. paratyphi C
  - S. dublin.
- It covers the 'O' Ag & prevents agglutination with O ~~antiserum~~ Ab
- Appear for a short time during convalescence
- Absence of Vi Ab's indicat poor prognosis
- Persistence of Vi Ab indicates it becomes a carrier state

Diseases caused by Salmonella

- **Enteric fever / Typhoid**
  - Only by 4 serotypes
    - S. typhi (> 98% in India)
    - S. paratyphi A (2nd MC in India)
    - " B
    - " C (Not in India)
  - All these 4 are strictly human adapted.
- **Invasive diarrhea**
  - Rest of all serotypes of Salmonella
  - They are GI commensals or pathogens of reptiles, birds or mammals other than man (Zoonoses)

Enteric fever / Typhoid

* Strictly human disease acquired by human feces contaminated food or H_2O.
* It might be a case / carrier
* ID50 → 10^2-10^5 bacilli (mcq)
* Incubation period → 1-2 wks (mcq) (3-21 days)
* C/F : Step ladder pyrexia, vomiting, malaise, anorexia
* Signs → Bradycardia, Rose spots on skin, Hepatomegaly, Spleenomegaly
* Illness lasts for 3-4 wks

* Complications :
  - MC : Intestinal hemorrhage (mcq)
    ↓
    Intestinal perforation
    MC in 3rd wk of disease
  - Meningitis (mcq)
  - Deafness
  - Psychosis
  - Arthritis
  - Nephritis
  - Osteomyelitis
  - Periostitis
  - Visceral abscesses
  - Cholecystitis

* Diagnosis
  - Isolation by culture
    (1) Blood culture → 5-10ml of sterile blood of patient + 50-100 ml of bile broth (1:10 dilution)
    ↓
    Daily subculture on blood agar & Mac Conkey × 1 wk

    OR

    Can use biphasic medium / Castaneda medium
    ↓
    Brain heart infusion agar & broth

* Rates of positivity of a blood culture:

* 1st wk → > 90%
  2nd wk → ~ 75%
  3rd wk → ~60%
  4th wk → ~ 25%

(2) Stool culture:

Becomes +ve at the end of 2nd wk of disease in 40-50% of cases.

Stool → Inoculate in selective medium
Stool → Also in enrichment medium → Subculture on to selective medium.
@ 6 hrs & 24 hrs

(3) Urine culture:

Becomes +ve at the end of 3rd wk of disease in 30-50% cases

- It is same as stool culture

(4) Bone marrow culture:

- 2 advantages
  1) Most sensitive @ all stages of disease
  2) Remains +ve for 5 days after start of Rx.
- Done similar to blood culture

* Enteric fever is the only bacterial disease for ≤ a bone marrow aspirate is WHO recommended.

II) Serology (Poor sensitivity & specificity)

* Abs in serum

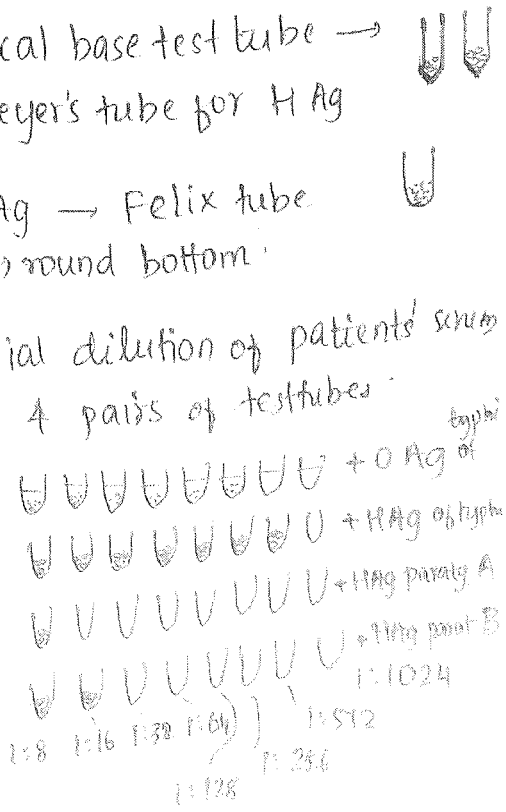
(A) Widal test

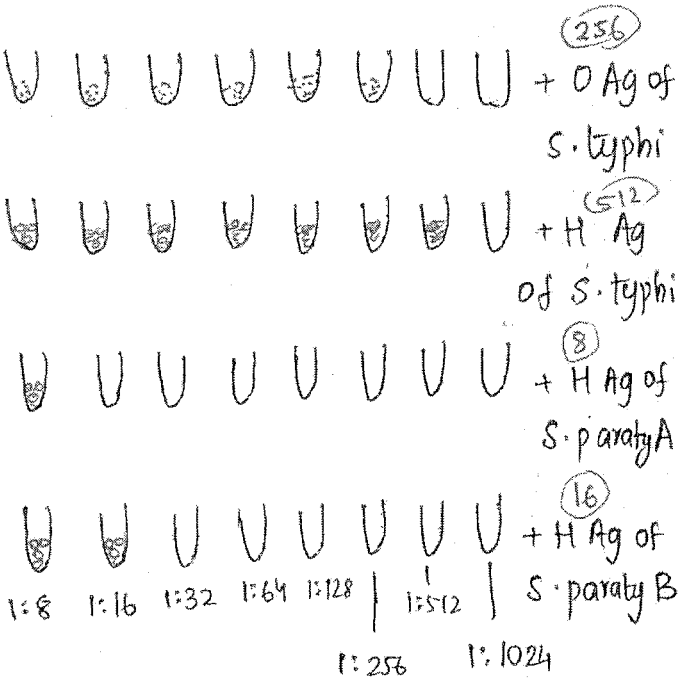
- Type of tube agglutination test
- Significant titre of 'H' Ab in the serum detected
- Significant titre of 'O' Abs in serum detected.

H Abs + H Ag → Fluffy or woolly agglut n

O Ab + O Ag → Granular agglut n

- Conical base test tube → Dreyer's tube for H Ag
- O Ag → Felix tube → round bottom.
- Serial dilution of patients' serum in 4 pairs of testtubes.





* O-Ab ⇒ Recent infection
* H-Ab ⇒ Serotype of infection
* Serology becomes +ve at the end of first wk of disease
* For every population, a baseline titer of both 'O' & 'H' Abs has to be determined.

[In India, O Abs ⇒ ≥100
H Ab ⇒ ≥200 ↓ considered as significant]

* Significant titre of O Ab indicates recent infection
* Significant titre of H Ab indicates serotype of infection
* Rise in Ab titer should be demonstrated.
* A single high titre may may an anamnestic reaction (ie, false +ve)

(B) Typhidot

* It is an immuno chromato graphic test.
* Both IgM & IgG Abs detected seperately by ELISA.
* Disadvantage → does not quantify the titre of Ab.
* Rapid & easy to do unlike Widal

III Diazo test

* Detects a phenolic compound in urine of patient
* Has >90% sensitivity & specificity
* Disadv: +ve only in 1st wk.

IV Antigen detection in serum

* By Latex agglutination or co-agglutination.
* Only disadv: Only +ve in 1st wk.

(V) Molecular methods

* PCR or Nucleic acid probes detect DNA in blood.
* Has poor availability in India

(*) Best method for diagnosis at 10 days of disease

Imp
a) Widal
b) Typhidot (Fast than widal) (x)
✓ c) Blood culture (75% chance)
d) Stool culture (@ end of 2 wk)

(*) If que: during culture
Ans: Widal

(*) If que: @ 15 day -
~~Ans~~: a) Widal (x)
✓ b) Blood cul (60-75%)
c) Stool cul (40-50%)
d) Urine cult (20th d x)

Treatment

* DOC: Fluoroquinolones
Ciprofloxacin or Ciflox
orally x 5-7 day

+ Alternatives: Oral Azithromycin x 7d
or
I.V Ceftriazone x 10-14 day

Carriers

* According to ~~type~~ site of persistence → 2 types

1) Fecal carrier → site of persistence is gall bladder
↓
Seen in pts of biliary tract abnormalities

2) Urinary carrier → Site of persistence in kidney
↓
seen in pts of urinary tract abnormalities

* According to duration of persistence ⇒

1) Convalescent carriers
• Up to 3 months after clinical cure.

2) Temporary carriers
• 3 m – 1 yr after clinical cure

3) Chronic carrier
• > 1 yr after clinical cure

Diagnosis of carrier (2 steps)

1) Screening → Vi agglutination test for persisting Vi Abs
2) Confirmatory → By culture:
• Stool or duodenal aspirate
• Urine

2nd disease – Invasive Diarrhea

* Caused by rest of all serotypes
+ i/a Non typhoidal salmonellas
+ Zoonosis (animals not humans)

* H/O unpasteurized milk or milk products
  eg: Icecreams directly prepared using unpasteurized milk
* H/O contaminated poultry
* H/O raw eggs (Salmonella can penetrate egg shells)
* Incubation period: 6-24 hrs
* C/O: Fever, abd cramps & diarrhea, occasionally blood & mucus in stools.
* Generally self limited.
* Diagnosis → done by pt stool culture. (Rx: fluids & electrolyte supple)
* MC serotypes is

Salmonella typhimurium

* In young, old immunodeficient, chronic hemolytic anemia pts (eg: Sickle cell anemia)
  ↓
  Enteritis becomes septicemia
* MC serotype causes septicemia is S. enteritidis or S. choleraesuis.
* Septicemia can lead to
  - Meningitis or
  - Osteomyelitis or
  - Nephritis
* Here Δ by blood culture (Δ: Diagnosis)
* MCC of osteomyelitis in sickle cell anemia.
  Non typhoidal salmonella (S. enteritidis or S. choleraesuis)
* Rx: 3rd generation Cephalosporins

Yersinia Pestis

* Category A bioterrorism agent
  1) Bacillus anthracis
  2) Variola virus (Small pox)
  3) Yersinia Pestis
  4) Botulinum toxin
  5) Francisella tularensis
  6) Viral hemorrhagic fever
     ↳ High mortality rate like Ebola virus & Marburg virus
     ↓ ★
     Belongs to filoviridae family.
     ↳ Lassa fever virus
     ↳ Crimean Congo hemorrhagic fever.

Yersinia Pestis

* Non motile
* Capsulated
* Highly pleomorphic G-ve rods
* Grow at wide pH range of 5-9.6

* Grow at wide ~~xx~~ temperature range of ~~2-45~~ 2 - 45°C

* Optimum temp. of growth 27°C

Temperature requirements

- Optimum temp of growth for all pathogenic bacteria is 37°C
- Mesophiles → Grow b/w 25 - 40°C (all bacteria)
- Psychrophiles → Grow < 20°C
  - Includes Yersinia (up to 2°C)
  - Listeria (up to 4°C)
  - Enterococcus (up to 10°C)

MCQ

k/a cold enrichment (incubating in the fridge)

- Thermophiles → Grow > 40°C (can grow up to 45°C)
  - Yersinia
  - Listeria
  - Enterococcus
  - C. perfringens

⇒ Campylobacter jejuni grows up to 42°C

MCQ

* Optimum temp → for all → 37°C
* except:
  Y. pestis : 27°C
  C. jejuni : 42°C

(Yersinia pestis) (continues)

Biochemical test

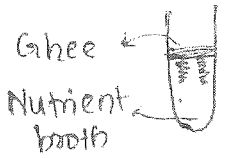
* ≈ Shigella
* Anaerogenic on sugar ferm?
* H_2S - ve
* IMViC - + - -
* Urease - ve

Cultivation

* Mac Conkey → NLF (pale)
* Blood agar → Dark brown colonies (due to hemin deposition) (×)

MCQ

* On ghee broth → Stalactite growth.



Virulence factor

1) Polysaccharide capsule
   k/a Fraction 1 Ag (F1 Ag)
2) Lipopolysaccharide / Endotoxin

Disease : Plague

* Plague → Zoonoses (Wild rats)
* Vector - Rat flea (Xenopsylla cheopis)
* Also endemic typhus has vector rat flea

* 3 types of Plague:

① • MC → Bubonic → due to bite of flea

↓

- Painful enlarged regional LN K/a Buboes. (MCQ)
- Incubation period : 3-7 days
- 50% untreated individuals shows spontaneous resolution
- Rest → Septicemia

② Pneumonic Plague

- Due to aerosol inhalation
- Incub. period : 1-3 days
- Symptoms of hemorrhagic pneumonia c/c out pleuritis or mediastinitis or meningitis
- 80-95% individuals will go in to septicemia

③ Septicemic Plague:

- Generally secondary to bubonic or pneumonic plague.
- Symptoms of severe endotoximia eg: High grade fever, ↓ BP, gangrene of toes & fingers due to DIC, shock due to multi organ failure.

Diagnosis of plague

* Bubonic → Bubo aspirate
* Pneumonic → Sputum
* Waysons stain instead of Grams

① → stain with Methylene blue
→ Bipolar staining / safety pin appearance

② Culture :
• Done only in a specified bio safety level 3 laboratory

③ Ag detection (F1)
- by direct fluoresant Ab testing (DFAT)
- Specific to capsular Ag

④ Ab detection (specific Ab in serum)
- by indirect fluorescent Ab test (IFAT)

⑤ PCR or probes

Treatment

* WHO → DOC : Streptomycin or Gentamycin
× 7-10 days.
* Alternatives to aminoglycosides Doxycyclin or Chloramphenicol
* Post exposure prophylaxis DOC : Doxycyclin
* Pre-exposure prophylaxis Killed vaccine (lab technician)

Enterobacteriacea → 2 que confirm
→ either from E. coli, Shigella, Salmonella or Y. pestis

× ——— ×

VIBRIONACEAE

* G -ve, curved rods
* Vibrio (Genus)
* Monotrichous flagellum in all
* Special covering → k/a sheathed flagellum.
* Darting / shooting star motility
* Facultative anaerobes.
* All are alkaliphites (optimum pH ⇒ 8.2 - 8.4)
* Require optimum salt conc. of 0.5 - 1 % → hence k/a Halophiles
* If we ↑ salt conc → Beyond 6 % → grow gets inhibited.
* Up to 8 % salt conc → survived by V. parahemolyticus
* Up to 10 % salt conc → V. alginolyticus
* Non halophilic → do not require salt to grow
  V. cholerae
  V. mimicus

Biochemical test

* Catalase +ve
* Oxidase +ve
* Nitrate reduction +ve
* Ferment sugars
* String test +ve

* V. cholerae → shows cholera red reaction +ve.

$NO_3^- \longrightarrow NO_2^-$
(nitrate) (nitrite)

Tryptophan → Indole

NO_2 + Indole ⇒ Nitroso-indole
↓
H_2SO_4
↓
Red colour

Cultivation

* Easily grow on simple / basal media.
* Mac Conkey → NLF colonies
* Transport medium
  V R medium, Cary Blair, Autoclaved sea water.
* Enrichment medium:
  Alkaline peptone H_2O,
* Monsur's taurocholate tellurite peptone water
* Selective M : Bile salt agar, Monsur GTTA

* Selective + Differential → TCBS
Thio sulphate citrate
Bile salt sucrose media

- Sucrose fermenting Vibrio → Yellow → V. cholera
- Non SF Vibrio → Pale → V. parahemolyticus

Classification

* Gardner & Venkat raman classification of Vibrio
* Based on 'H' Ag (flagellar) of V. cholerae

- H Ag +nt → Group A → V. cholera → On basis of "O" Ag ↳ Serogrouping → $O_1 \longrightarrow O_{138}$ serogp → Only O_1 produces cholera toxin
  ie, disease cholera is caused only by O_1 V. cholera
- H Ag -nt → Group B → Rest all species

* Rest all serogroups k/a nonchólera vibrio (NCV)
* Also k/a non-agglutinable vibrio (no agglutination ~ O_1 antiserum) (Ab's)
* Latest serogroup of V. cholera
O 139

O139 V. cholera

* 1st isolated in Madras in 1992
* Also k/a Bengal strain (cause epidemic there)
* Like O_1 V.C it produces cholera toxin & produces disease similar but unlike Vi cholerae O_1, it is capsulated & agglutinates ~ O_{139} Ab's.
* Presently reported only from India & Bangladesh.

---

* O_1 V. cholera on basis of biochemical test → in to biotypes :
  - EITor
  - classical

Biotypes

ElTor (serotypes a,b,c Ag)
- Ogawa (a,b)
- Inaba (a,c)
- Hikojima (a,b,c)

Classical
- O (a,b)
- I (a,c)
- H (a,b,c)

Biotyping Criteria (of V. cholerae O1)

	ElTor	Classical
1) Sheep RBC hemolysis	+	−
2) Chick RBC agglutination	+	−
3) VP test	+	−
4) Polymyxin susceptible.	(R)	S
5) Susceptible to phage	(S)	IV

(R: Resistant, S- sensitive)
(VP - Vokes Proskauer test)

Diseases due to biotype

ElTor	Classical
- Milder	>
- < Mortality	>
- < SAR	>
- > Carrier	<
- > Subclinical cases	<

SAR: Secondary Attack Rate.

Virulence factors

1) Sheathed flagellum
2) Toxin co-regulated pilli
   (helps in adhesion to the small intestine mucosa)
3) Cholera toxin
   - due to CTX ϕ phage
   - Phage mediated toxin
   - AB5 subunit structure

   - Receptor is GM1 ganglioside
   - Site of action: Small intestinal mucosa cells.
   - MOA: Receptor mediated endocytosis
     ↓
     Toxin is transported to the endoplasmic reticulum
     ↓
     A splits from B
     ↓
     further broken down to $A_1 + A_2$
     ↓
     A_1 → Actual toxin
     - A_1 enters cytoplasm and causes ADP ribosylation of Gs protein → ↑ adenyl cyclase activity causes ↑↑ cAMP

→ leads to secretion of ions in to the lumen.

4) LPS / Endotoxin has no role in ~~the~~ cholera.

Cholera

* It is a strict human disease
* Reservoir is sea water (MCQ)
* Incubation period is 2-5 days
* ID_{50} c H_2O → $>10^6$

  ID_{50} c food → $>10^3$

Vibrio are highly susceptible to acidic pH of stomach, food raises gastric pH.

* 2-5% infected are symptomatic
* Risk factor to symptomatic disease:

  Antacids, PPI, H_2 blocker, chronic gastritis, Gastrectomy, Achlorhydria

* C/F
  - Effortless vomiting
  - Severe watery diarrhea. (Rice water stools)
* Complication:
  - Hypovolemia c severe metabolic acidosis

* Diagnosis:
  - Fresh stools
  - Wet mount of mucus plate → Fish in stream appearance
  - Gram smear → Pus cell ⊖, RBC ⊖, G1-ve curved bacilli
  - Culture on selective medium → colonies
  - Toxigenicity test ≈ Labile toxin of ETEC

Rx

* Oral or parenteral rehydration
* Single dose of Doxy: 300 mg / Ciprofloxacin: 1g / Azithromycin: 1g in adults
* Children → 3 days → Ciprofloxacin / Erythromycin / Septran
* ♀ → Furazolidone (MCQ) / Erythromycin
* Chemoprophylaxis to be given for all household contacts
  - Doxy 1g single dose
  - Tetracyclin x 3 days

GROUP B VIBRIO

① Vibrio parahemolyticus

* Grows up to 8 % salt
* Swarming on blood agar
* Disease → Contaminated seafood (shell fish or Oysters)
  ↓
  Invasive enteritis
* Kanagawa phenomenon: *
  (on Wagatsuma blood agar)
  ↓
  Human blood agar with 2 % salt.
  ↓
  - Pathogenic strains: Hemolytic
  - Non-pathogenic strains: Non-hemolytic

2) Vibrio vulnificus

* Also k/a L + Vibrio (only lactose fermenting species)
* Disease → By ingestion of seafood which causes
  - In healthy → Diarrhea
  - In iron overload / cirrhosis pt → septicemia
* Causes infections of wound exposed to sea water

3) Vibrio alginolyticus

* Grows up to 10% salt conc.
* Shows swarming (++)
* Disease → by infections of wound exposed to sea water
  - Eye & Ear infection in sea water swimmers.

PSEUDOMONADACEAE

Pseudomonas aeruginosa

* G-ve baillus
* Motile, monotrichous.
* strict aerobe
  (strict aerobes include
  - Mycoba. tuberculosis
  - Nocardia
  - Brucella
  - Bordetella
  - Micrococcus
  - Legionella pneumophila)
* Non capsulated
* slime producing

Biochemical test

* Catalase +
* Oxidase +
* NO_3 reduction
* Non fermenters

Cultivation

* Easily grows on simple media
* Mac Conkey → NLF colonies
* Blood agar → Mucoid colonies (Slime)
* Selective medium: Cetrimide agar.
* Fruity smell / Grape like smell
* Pigments:
  - 100% produce pyocyanin
  - Also pyoverdin +/-
  - Pyomelanin +/-
  - Pyorubin +/-

(Never normal flora in healthy)

⇒ Normal saprophyte in moist enviroment.

⇒ In hospital → isolated from resp. therapy equipments, antiseptics, soap, sink, mop

⇒ Community reservoirs → Swimming pool, contact lens solution

Diseases

A) Community
- MCC of corneal ulcers in the contact lens users
- Causes swimmers ears
- Malignant otitis in diabetis
- Generalized folliculitis in Kla Jacuzzi syndrome.

B) Hospital (Nosocomial)
- MCC of burn infections
- MCC of ventilator associated pneumonia.

⇒ MCC of nosocomial pneumonia (MCQ) G-ve bacilli

⇒ MCC of ventilator ass. pneumonia (MCQ) P. aeroginosa

- In immunodeficient in ICU causes septicemia manifesting as Ecthyma gangrenosum. (MCQ)
- Cause post. catheterizn UTI
- Post lumbar puncture meningitis

Rx

* Multidrug resistant bacteria because of
  1) Lots of R-plasmids encoding hydrolytic enzymes
  2) Lack of porin proteins in cell wall. (↓ entry of antibiotics)

* Uridopenincillin.

Bacillus cereus

* Spores widely distributed in nature (soil, vegetables, cereals, spices, poultry)
* Swarming
* Causes — 2 types of food poisoning
  1) Diarrheal type
  2) Vomiting type.

Diarrheal type

* Incubation period : 8-16 hr
* Poorly cooked meat contaminated with spores
* Toxin : 'Nhe' toxin is formed in GIT
* Symptoms: Abd. cramps + watery diarrhea
* Self limited

Emetic type

* Incubation : < 6 hrs
* Chinese rice / fried rice ~ preformed toxin
* Toxin → Emetic toxin or cerulide.
* Abd cramps + Vomiting
* Self limited.

* Selective medium for Bacillus cereus is MYPA
  (Mannitol Egg Yolk phenol red polymyxin agar)

GRAM NEGATIVE SPIRALS

Spirochaetes

Treponema Pallidum

* Only pathogenic species of genus Treponema.
* Many non-pathogenic species → oral & genito-urinary tract flora.
  eg: Reiter's strain
  ↳ Treponema phagedenis
* Divided in to 4 subspecies

① TP. pallidum → Causes venereal syphilis

② TP. endemicum → Endemic venereal GR Bejel

③ T. P. pertenue → Yaws

④ TP carateum → PINTA

① → STD
2, 3, 4 → Not STD but skin to skin contact

2, 3, 4 → k/a Nonvenereal Treponamatosis (NVT)

* 4 subspecies are morphologically as well as antigenically similar

* Test → RPR, VDRL, FTA-Abs all may be +ve in NVT.

Treponema pallidum subspecies pallidum

* Discovered by Schauddin & Hoffman as causative agent of syphilis.

* G-ve spiral.

* 10-14 μ long

* ~~broad~~ MCQ → 0.1 μ

Not seen by light microscope (0.2-0.3 μ)

* 10 regularly spaced spirals

* Corkscrew motility because of endo flagella - present in the periplasmic space of cell wall.

* Non cultivable on cell free media → eg: M. Leprae (1st - foot pad of mice, Armadilloes - better) & MC used

- T. pallidum → Rabbit testis (1st strain grown is k/a NICHOL'S strain)
- Chlamydia } Yolk sac of chick egg or cell lines
- Rickettsia }
- Spirullum minus → Non cultivable anywhere

(Cell lines - generally used for cultivating virus)

* Disease → Syphilis

* Spirullum minus causes ~~xxx~~ rat bite fever / Sudoku fever.

Syphilis (Imp)

* Incubation period - 9-90 days

* STD

* 1° stage → Painless, indurated genital ulcers + Regional lymphadenopathy

- Ulcers generally resolves spontaneously in 2-8 wks

* In 2-6 wks of 1° chancre appearance → 2° stage

* 2° stage is also k/a great imitator

* 2° stage :
  - Mucous patches
  - Maculopapular rash
  - Condyloma lata (most infectious skin lesion)
  - Generalised lymphadenopathy
  - Hepatitis
  - Uveitis, chorioretinitis
  - Nephrotic syndrome
  - Meningitis.
  - Periostitis

⇒ Symptoms resolve spontaneously
⇒ But relapse for 2-5 yrs after infection
⇒ 5-30 yrs after infection → 30-33% develops tertiary sp syphilis.
Rest become latent for life (only serological test +ve, no manifestations)

* 3° stage :
  - Granulomas k/a Gumma in skin, liver, spleen, kidney etc.
  - CNS → Meningo vascular ① syphilis (due to endarteritis obliterans) ↓ Paresis, aphasia, ataxia
(CNS) → due to actual ② destruction of neurons ↓ Tabes dorsalis.
  - CVS → Cardiovascular syphilis Aortitis & Aortic regurgitation

* Diagnosis → By microscopy
(i) Negative staining ē India ink or Nigrosine.
(ii) Dark field microscope. (the reflected light reaches observers eye)
(iii) Silver stains
  a → Fontana silver stain
  b → Levaditi stain.
(iv) Direct fluroscent Ab testing. (DFAT)
For detection of treponemal antigens.
  - Most sensitive microscopic method among above 4.

* Diagnosis → By serological test (detection of Ab in serum)
(i) Non treponemal tests / Standard tests of syphilis (STS)
  - Ag used → Cardiolipin Ag (alcoholic extract of beef/ox heart)

• Ab → Non specific Ab k/a. Reagin Ab.

eg: of Nontreponemal tests

1) Wasserman test

- First serological test used for diagnosis of syphilis
- Complement fixation test (Type of)

2) Kahn test

- Tube flocculation test
- Flocculation → precipitation
- So its a type of precipitation test (precipitate floats on to the top instead of settling down)

3) VDRL test

- Venereal disease research lab slide
- ~~Type of~~ flocculation test
  Type of → (precipitation)
- ~~Type of~~ microscopic test. (needs microscope)

4) RPR test

- Rapid plasma reagent test
- Cardiolipin Ag coated on to carbon particles + serum
  ↓
  Macroscopic agglutination.

* Biological false positive →
  No syphilis for patient but gives false +ve non trep. test.
  ↓
  Causes for this

Acute bio. FP
↓
- Last for < 6 months
- Infectious mononucleosus/ glandular fever
- Pregnancy
- Measles, mumps, Malaria, Chickenpox, Viral hepatitis, recent immunizations

Chronic
↓
- > 6 months
- SLE
- Lepromatous leprosy
- Collagen disorders
- Several malignancy.

(serological test)

ii) Treponemal Tests

- Ag used → T. pallidum (live or Ag)
- Ab detected → Specific T. pallidum Ab.

egs :-

a) TPI (T. pallidum immobiliz^{n} test)

- Serum of patient +

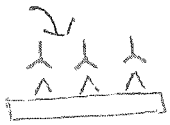
Live T. pallidum + Complement
↓
This live T. pallidum gets immobilized under Darkfield microscope.

- Most specific

b) FTA-Abs (Fluroscent T. pallidum Ab absorbed test)

- Indirect fluroscent Ab test (IFAT) (Type)

Serum (? Ab)



Slides precoated ε T. palli Ag

→ Wash the slide

↓

Add Rabbit antihuman Ab Ig + Flurosunt dye

← Wash

↓

Under fluorescent miscroscope

- Most sensitive test.

⇒ Fluroscent dye :

c) TP-HA or MHA-TP

- T. pallidum haemagglutination (test tubes)
- Micro haem agglutination T. pallidum (in microtitre plates)
- T. palli Ags are coated on to sheep RBC

 + Serum (Ab) →

Agglutination of sheep RBCs

d) TPPA :

- T. palli particle agglutination
- T.p Ag coated on to gelatin particles + Serum (? Abs)

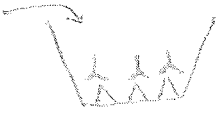
↓

Agglutination of gelatin pasticles.

e) Indirect ELISA / ELISA

- Done in microtiter plates (96 wells) MCQ.

Serum (? Ab) →



Well coated ε TP Ag

→ Wash → Add rabbit anti human Ab Ig

↓

Conjugated ε enzymes

Wash ←

Wash → Add substrate for the enzyme & look for color change

↓

if color changes then +ve test.

Serology (Imp) *

1) Serology becomes +ve at 10-14 days after infection

2) 1st test to become +ve is FTA Abs.

3) For diagnosis of Syphilis → Always use a two step approach

↓

(i) Screening done c Non Treponemal test (VDRL or Kahn)

(ii) Confirmation done c Treponemal test (MC used : TPPA)

4) Follow up (look of 4 fold fall in Ab titre)

Done c a Non-treponemal test (VDRL or Kahn)

- TPHA, TPDA, FTA-Abs remain +ve for yrs after Rx.

5) Most specific : TPI followed by TPPA

6) Most sensitive → FTA-Abs

7) Congenital syphilis → diagnosed by IgM Ab detected by FTA-Abs on ELISA.

Non Venereal Treponematosis

Yaws, Pinta, Bejel

* Transmission via skin to skin contact. c lesions of disease

* Only NVT reported in India is Yaws → Earlier declared eradicated in India in 2006.

* For all of them DOC ⇒ Penincillin.

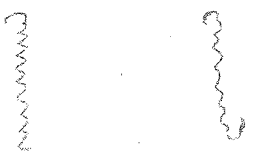
* If allergic to Penicillin → pregnant female → can given Penincillin G ~~desensitization~~ desensitization followed by Penicillin.

Stages:	1°	2°	3°
• Bejel / Endemic Syphilis	- No 1° chancre	• Mucous patches, maculo papular rashes	• Gummas • No CVS / CNS involvement
• Yaws	- Ulcerating extra genital chancre (kla Mother Yaw)	• Mucous patches, • MP rashes, • Mother Yaws like lesions at several sites	»
• Pinta (in South America)	- Psoriaform extragenital 1° chancre	• Hypo / hyper pigmented skin lesions.	• Rare

LEPTOSPIRA

Leptospira Interrogans

* 1st pathogenic species
* G-ve spiral, closely wound spirals ~ one end / both ends hooked



Shepherd crook / Umbrella handle appearance

* 1 µ broad, @ 20 µ long
* Motile ~ endoflagella.
* Easily grown on serum containing medium

  eg: EMJH, Korthoff's medium
* Leptospirosis → MC zoonoses in the world

  Reservoir: Rodents

  ↓

  Most imp - Rats

  Other - Pigs, Dogs, Cow, Sheep etc
* Asymptomatic colonization of collecting tubules of kidneys → urine of reservoir - has leptospires

* Mode of infection

Mucous membrane / skin breaks exposure to urine contaminated water.

* Occupations at risk:

Veterinarians, Rice field farmers, Sewer maintenance workers.

* Also k/a disease associated c 3 R's

- Rats
- Rice fields
- Rainfall

* 5-10% infected → develops Ictero hemorrhagic fever or Weil's disease.

- Jaundice
- Renal failure (oliguria, Hematuria, ↑ BUN)
- Hemorrhages.

* Mortality rate: 15-20%

* Diagnosis → serology (Abs in serum)

- Macroscopic agglutination test
- Microscopic agglutination test

MCQ

* DOC: Penicillin

* Severe → i.v penicillin x 7-10 days.

* Anicteric leptospirosis (only fever)

DOC: Doxycycline / Ampicillin / Amoxycillin.

MCQ

* Prophylaxis in people @ risk

* Doxycycline (weekly - 200 mg)

BORRELIA

* G-ve spiral

* 0.3-0.5 μ broad (seen by light microscope)

* Disease caused:

1) Relapsing fever

- Shows antigenic variations
- Two types
  - Louse borne / Epidemic relapsing fever.
  - Tick borne / Endemic relapsing fever.

Louse-borne

* Caused by Bordelia recurrentis
* Vector : Body Louse (Pediculus humanus)
* Transmission → Crushing of louse against the skin.
* No extra human reservoir (Strict human pathogen)
* Severe disease c/c just 1-2 relapses.
* Case fatality → 4-40%.

Tick-borne

* 15 species of ~~Beard~~ Borrelia (eg: Borrelia parkeri)
* Vector : Soft tick (Ornithodorus species)
* Transmission → Bite
* Reservoir : Rodents (Zoonoses)
* Milder disease c/c 5-7 relapses
* Case fatality : < 1%.

Diagnosis

* Collect blood during fever
* Giemsa stain → Irregular spiral shaped bacteria.

Rx

* Tetracycline × 7-10 days or Erythromycin × "

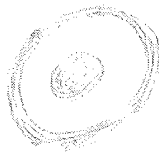
↓

Tick-borne RF

* Louse borne RF → Single dose Tetracycline (0.5g) or Erythromycin (0.5g)

2) Lyme's disease:

* Borrelia burgdorferi MCQ
* Other → B. afzelli, B. garinni
* Vector : Hard tick MCQ (IXODID)
* Reservoir : Rodents, Deer
* 1° stage → Erythema (IP: 3-30 d) chronicum migrans or Bull's eye rash



Chlamydia

* G-ve cocco-bacilli
* Obligate intracellular *
* Filtrable through standard bacterial filters.

(Thought of as viruses)

* But Chlamydia contains both DNA & RNA
* Respond to antibacterials

(Thus classified as bacteria)

* Lacks enzymes for ATP synthesis → Hence kla Energy parasites
* Forms inclusion bodies like viruses → which are basophilic → hence kla Basophilic viruses
* Also kla PLT agents (diseases)
  - Psittacosis
  - LGV
  - Trachoma
* 3 pathogenic species
  - Chlamydia psittaci
  - Chlamydia pneumoniae
  - C. trachomatis

* C. psittaci ⇒
  - Reservoir – Psittacine birds eg: Parrot
  - Mode of infection → Inhalation of dried feces/ body fluids of infected birds
  - Causes atypical pneumonia kla Psittacosis

* C. pneumoniae
  - Strict human pathogen
  - Causes RTI → Pharyngitis, bronchitis, Atypical pneumonia

* C. trachomatis

Stains
- No Grams stain
- Use Giemsa, Castaneda stain

Cultivation
- Non cultivable on cell free media.

a) Yolk sac of chick embryo
b) Irradiated Hela / McCoy cell lines.

⇒ On the basis of Ag → 15 serotypes of C. trachomatis
⇒ A, B, Ba, C → Trachoma
⇒ L_1, L_2, L_3 → LGV (Painless genital ulcer)

LGV : Lympho granuloma Venerum.

⇒ D-K Serotype → Genital Chlamydiasis

- MC bacterial STD Worldwide
- MCC urethritis / Non gonococcal urethritis
- MCC of reactive arthritis
- MCC of Ophthalmia neonatorum.

Genital Chlamydiasis

Male	Female
• 50% asymptomatic	• 80% asympto.
• Cause watery urethral discharge	• Watery urethral/ vaginal discharge
• Urethritis	• Urethritis
• Epididymitis	• Cervicitis
• Proctitis	• Endometritis
• Reactive arthritis	• Salpingitis
	• PID
	• Fitz-Hugh Curtis syndrome (Perihepatitis)
	• Pregnant infected ♀ → Perinatal transmission during delivery

50% → Inclusion conjunctivitis

10% → Infant pneumonia

* ♂/♀ ∈ Genital Chlamydiasis while swimming transmit disease to other → Kla Swimming pool conjunctivitis

* Diagnosis → Specimens :
  - Not discharge
  - Endo urethral swabs
  - Cervical scrapings
  - Conjunctival scrapings

* Most sensitive method
  Molecular method
  (Nucleic acid probes / NAATs - Nucleic acid Amplification Tests)
  ↓
  eg: PCR

* Rx → WHO recommends → Single dose of Azithromycin (1 gm)

- Earlier → Doxycycline → But for 7 days

* 2 stages in life cycle of Chlamydia
  - Extracellular : Elementary body
  - Intracellular : Reticulate body

(MCQ)

Rickettsiaceae

Vectors, Reservoirs, Cause* - MCQ

* GN cocco bacilli
* Obligate intracellular (except Bartonella)
* All transmitted by arthropods except Coxiella & Bastonella henselae)
* All parasitize in endothelial cells of small blood vessels.
* Fever, rash, vasculitis.
* Rash is never seen in Coxiella infection
* Forms basophilic inclusion bodies (Giemsa & Castaneda)
* Cultivation
  1) Lab animals (Guinea pigs)
  2) Yolk sac of chick egg
  3) Cell lines

1) Rickets

CNS symptoms are dominant → Typhus group

Rash is dominant → Spotted fever

Typhus group

A) Epidemic typhus
- By R. prowazekii
- Vector - Body louse
- Reservoir : Only man (no zoonosis)
- Can become latent → Followed by reactivation k/a BRILL ZINSSER disease
- Epidemic typhus also k/a GAOL fever.

B) Endemic typhus :
* Rickettsia typhi
* Vector - Rat flea (Xenopsylla cheopis)
* Reservoir - Rodents
* Milder disease

Spotted fever Ricketsial ds (SFR)

* 1) Rocky mountain spotted fever
- Most severe
- R. ricketsi
- Vector : Hard ticks
- Reservoir : Rodents
- NO ESCHAR at the site of bite of tick (Black lesion) MCQ

(Rest all SFR & Scrub typhus have eschar)

TICK transmitted diseases:

BEL ORST

* Babesiosis (Protozoa)
* Ehrlichiosis
* Lyme's disease
* Q fever (only amongst animals)
* Relapsing fever → Only this by soft tick
* Spotted fever — other - Hard tick
* Tularemia

2) Indian tick typhus

* In Africa ⇒ Boutteneuse fever
* Kenyan tick typhus
* For all → by R. conori
* Vector: Hard tick
* Reservoir: Rodents
* Eschar +

3) Rickettsial pox

* Only SFR not tick transmitted
* R. ~~ankari~~ akari
* Vector: Mites (MCQ)
* Reservoir: Rodents
* Mildest SFR
* Eschar +

② Orientia

Orientia tsutsugamushi

* Scrub typhus (Chiggerosis)
* Vector: Chigger (larvae) of mites
* Reservoir: Rodents
* Eschar +
* Zoonotic tetrad MCQ *
  - Orientia
  - Chiggers
  - Rodents
  - Scrub vegetations

MCQ
Que: Zoonotic tetrad is seen in scrub typhus

Weil Felix Reaction

* Heterophiles agglutination test
* Heterophile Ags → Ag's shared over classes & kingdom.

* OX-19 of proteus vulgaris
  OX-2 of P. vulgaris
  OX-K of P. mirabilis

⇓

Ag's shared by Proteus and Rickettsia

	OX-19 of P. vulg.	OX-2 P.V	OX-K of P. mirabilis
Epidemic & Endemic typhus	++++	++	⊖
RMSF, ITT, Boutteneuse fever, KTT (Not Rick. pox)	++	++	⊖
Scrub typhus	–	–	⊕

* Doc for all:
Tetracycline x 2-4 wks

* Disease caused → Q fever
(Q: Query)
↓
(Zoonoses)

③ COXIELLA

Coxiella burnetti

⊛ Not Arthopod transmitted
⊛ No rash
* Resewoir → Domestic animals- cows, goats, sheep
* Coxiella in large numbers in milk & placenta.
* Mode of infection – Ingestion of raw milk, Aerosols, handling products – percutaneous transmission.

* Q-fever: 2 stages

Acute
↓
- Fever
- Myalgia

Chronic
↓
- Nodular vegetation on heart valves (endocarditis)

* Diagnosis – Serology (mcq)
* Doc: Doxycycline

④ Bartonella

* Cultivable on blood agar & chocolate agar.

B. henselae

* Cat associated (Cat lick, bite / scratch)

(a) Healthy individuals → Cat scratch disease
- Incubation P : 3-10 days
- Papule/vesicle + regional lymphadenopathy.
- Resolves spontaneously

(b) HIV → Vascular proliferative lesions.
- MC on skin k/a Bacillary angiomatosis
- Resembling Kaposi sarcoma
- Also in liver - k/a Baillary hepatis
- In spleen - k/a Bacillary splenitis
- In multiple organ - k/a Bacillary peliosis

* DOC for all: Erythromycin or Doxycycline

B. quintana

* Causes Trench fever / 5 day fever

MCQ
* Body louse (by crush against skin)

B. bacilliformis

* S. America
* Vector: Sandfly.
* Carrion's disease or ORoYA fever.

G -ve Coccobacilli

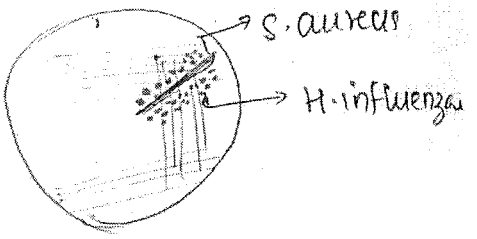
1) Hemophilus influenzae

* Pfeifer's bacillus
* Capsulated
* Capnophile. (needs 5% CO_2)
* Faultative anaerobes
* Requires X & V factor to grow

Present in blood { X-factor → Hemin; V-factor → NAD / NADP

Hemophilus : Blood Loving

* Culture :
  - Poor growth on blood agar (because V-factor is trapped inside the RBC)
  - Good growth on chocolate agar.
  - Can grow on blood agar ⊂ staph. aureus streak inoculation of specimen
    S. aureus → β-hemolytic



  - Kla Satellitism (H. inf grow around S. aureus streak)

* Diseases :
  - Otitis media
  - Sinusitis
  - Pneumonia

  } MCC is Pneumococcus

  - Exacerbation of COPD
  - MCC of Epiglottis (MCQ)
  - Meningitis
  - Conjunctivitis

MCQ
* DOC for H. influenzae meningitis
  3° cephalosporin - Ceftriaxone
  (3rd generation)

2) Bordetella pertussis

* strict human pathogen
* G-ve coccobacilli
* Non motile
* Capsule is Non-antigenic
* strict aerobe.
* Cultivation : Bordet Gengou medium.
* Disease : Pertussis / 100 day cough

MCQ
* IP of pertussis
  1-2 weeks
* 3 stages :
  → Catarrhal - Stage of max. infectivity
  → Paroxysmal
  → Convalescent.
* pertussis / whooping cough
* All stages for 2-3 wks each (hence long - 100 days)
* MC complication
  Pneumonia
* Others ⇒ Emphysema,
  # ribs due to severe coughing,
  hernia
* ~~Diagnosis~~ ⇒

* Cultivation colonies → Mercury drop / Bisected pearl appearance

* If in slanted medium → Aluminium paint app.

* Diagnosis:
  - Best specimen is
    Nasopharyngeal aspirate
  - Next best
    Nasopharyngeal swab
    (by ~~na~~ pernasal or peroral route)
    (PN > PO)
  - Least desirable
    Cough plate method
    (cough on plate)

* DOC: Macrolides (Erythromycin or Azithromycin)

DPT
⇓
D – Diphtheria toxoid
T – Tetanus toxoid
P – whole cell killed B. pertussis
↓
assoc. c some neuro paralytic complications

So now → DTaP

aP: ~~Acellular~~ Acellular component of B. pertussis (Virulence factors)
eg: Pertussis toxoid.

IMMUNITY

* MHC ~~I Ag~~ (Major histo compatibility cells)

* MHC I Ag → In all nucleated cells + Platelets

* MHC II Ag → Expressed on (APC) Ag presenting cells

* T cells are MHC restricted

* cytotoxic T cells → Tc → MHC I restricted

* helper T cells → Th → MHC II restricted

* Restricted means they can only recognize pathogenic peptides on that particular group

Tc → can recognize only peptides on MHC I

Innate Immunity

* 1st line of defense
* Always present
* Immediate response
* Receptors recognize broad molecular patterns ~~recognized~~ shared by several pathogens → k/a PAMP's - Pathogen assoc. molecular patterns (eg: LPS, Flagellin, Teichoic acid)
* Receptors are k/a pattern recognition receptor
  eg: Toll like receptors
* No memory response.

Adaptive / Specific / Acquired Immunity

* 2nd line of defense
* Provoked only on exposure to the pathogen.
* Lag time of response (4-7 days required to provide immunity)
* Receptors recognize-organism specific Ag.
* Receptors are T-cell receptors and B-cell receptors.
* Memory response is present

Components of Innate Immunity

1) Anatomical & Physiological barriers:
   a) skin c its acidic pH (lactic acid, other fatty acids)
   b) Acidic pH of stomach
   c) Mucous membranes c are lined by cilia & covered by thick mucus
   d) Commensal flora - provides colonization resistance.
   e) Lysozyme & other hydrolytic enzymes in tears, saliva & mucous secretions

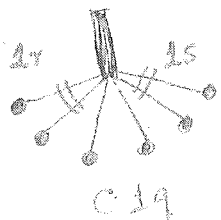
2) Cells
   - Monocytes } CD 14 on surface
   - Macrophages }
   - Neutrophils } CD 66 b
   - Eosinophils }
   - Basophils }

- Mast cells
- NK cells → CD16, CD56
- Dendritic cells

CD: Cluster differentiation.

3) Complement system (Alternate & Lectin pathway)

* It is a trigerred enzyme cascade in which there is a sequencial activation of proenzymes (complement proteins)
* Proenzymes are also k/a zymogens.
* Cobains 11 complement protein + 9 regulatory proteins.
* Regulatory proteins →
  eg: Factor H & I (inhibitory)
* Complement proteins
  eg: C_{1q} C_{1r} C_{1s} $C_2 \rightarrow C_9$
  C_1 complex: $C_1q r_2 s_2$



C1 complex

* Most complement proteins are synthesised in liver.
* Most abundant complement protein → C_3

4) Antimicrobial peptides in blood & mucous ~~pa~~ secretions
  ↳ α & β defensins, hepcidins

Pattern ~~regn~~ recognition repeptors of innate immunity

eg: TLR's (in human - 11 TLR)
Toll like receptors
↓
* named after toll proteins - present in drosophila or fruit fly.

MCQ
* TLR-4 recognise LPS (lipo polysaccharides)

* TLR-1,2,6 recognizes cell wall components
  eg: Teichoic acid

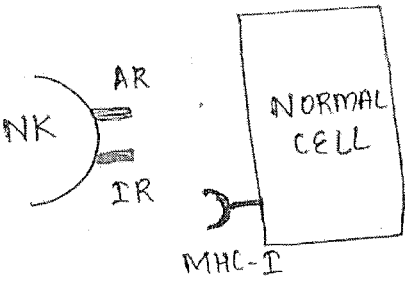
* TLR-5 recognize Flagellin

NK cells

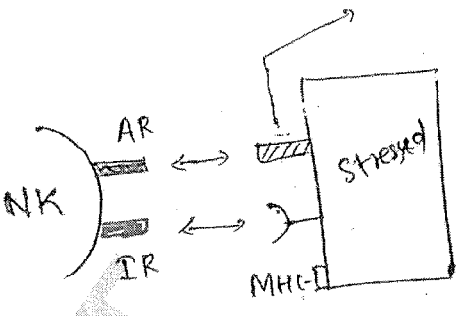
* Also k/a Null cells because it lacks Ag specific receptor like T & B cells
* Also k/a LGL (large granular lymphocyte)
  - 12 to 15 μ
  - Granules → Perforin etc
  - Originates from lymphoid progenitor cell.
* NK cells are recognized by CD16 & CD56
* CD16 → ~~key~~ receptor for Fc part of IgG

* Function: (Part of innate I)
  - Killing virus infected & cancerous cells
* Receptors → 2 types
  1) Activating receptors
     Ligand is not known
  2) Inhibitory receptor
     Ligand is MHC I Ag

(substance to bind - ligand)

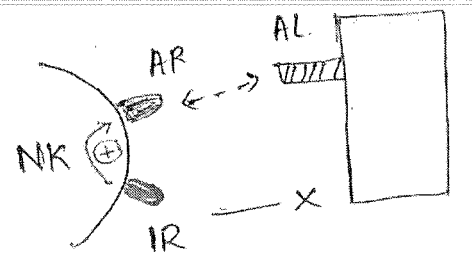


Stressed cell → May express activating ligand



NO KILLING

IR - recognises MHC-I & inhibits it from killing the cell.



↓↓ MHC-Ag I Ag expression

Since ↓↓ MHC I Ag → IR sends +ve stimulation to AR ⇒ KILLING.

* NK cells kills those cells of body which have ↓↓/ Lost expression of MHC I

(Fc attracts CD16)

* Mechanism of killing :

1) Degranulation ⇒ Perforins → forms pores in the cell.

⇒ Granzyme B → Enter through pores & activates caspase → apoptosis

Activation → perforins & granzyme

* IFN-α & IFN-β → are secreted by virus infected cells to attract NK cells

2) Expression of Fas ligand (Fas L) → binds to Fas protein on cell → activation of caspases → apoptosis.

3) Secrete TNF-α → binds to TNF receptors on target cells → activating caspases.

4) Secretes chemokines (cytokine attracting cells)

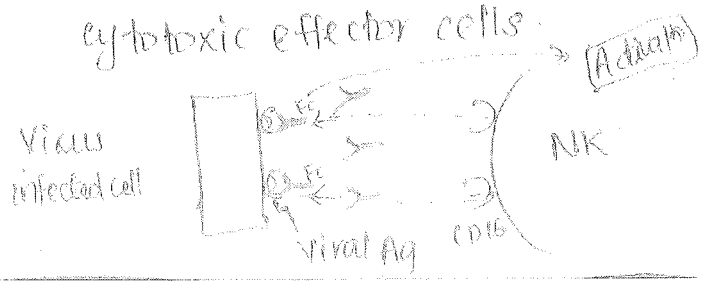
eg: CCL 3, CCL 4 etc

5) Secretes IFN-γ (cytokine ε is activating macrophages)

6) ADCC (Ab dependent cell cytotoxicity)

↓

Non phagocytic killing of an Ab coated cells by cytotoxic effector cells



* Antigen presenting cells (APC)

(A) Professional

* They present peptides of pathogen to helper T cells to stimulate an adaptive immune response

(Th → also k/a CD4)

* 2 requirements for Th cells activation:

1) APC should present the peptides in the groove of MHC II Ag for recognition by TCR (T cell receptor) → gets signal 1

2) Signal 2 ⇒ Costimulation ie, APC expresses B7 which binds CD 28 on Th cells

B-7 < B7.1 → CD80
B7.2 → CD86

* APC → eg:

MIA
- ✓ Dendritic cells
- ✓ Macrophages
- ✓ B-cells

	Dendritic cells	MΦ	B-cells
MHC-II expression	Always	Only on activation	Always
B-7 expression	»	»	Only on activation

* So dendritic cell is the most efficient APC (always alert)

B) Non-professional APC

* Present peptides of pathogens to Th for short periods
* Includes -
  - Fibroblast
  - Vascular endothelial cells
  - Glial cells
  - Pancreatic β-cells
  - Thymic epithelial cells
  - Thyroid epithelial cells.

* Adaptive Immunity

* 1° lymphoid organs are: Bonemarrow, Thymus
* B-lymphocyte mature in the bonemarrow
* T lymphocyte mature in the thymus.
* ~~Each~~ de Peripheral /2° lymphoid organs are: (actual adaptive immune response is developed here)
  - Lymphnodes
  - MALT (mucosal Associated Lymphoid tissue)
  - Spleen.
* Pathogens in tissue dealt by LN
  » blood → spleen
* Humoral immunity eliminates extracellular pathogens
* Cell mediated immunity eliminates intracellular pathogens

* Each T/B cell is genetically programmed in such a way that it is going to recognize one single Ag
↓
k/a antigenic specificity.

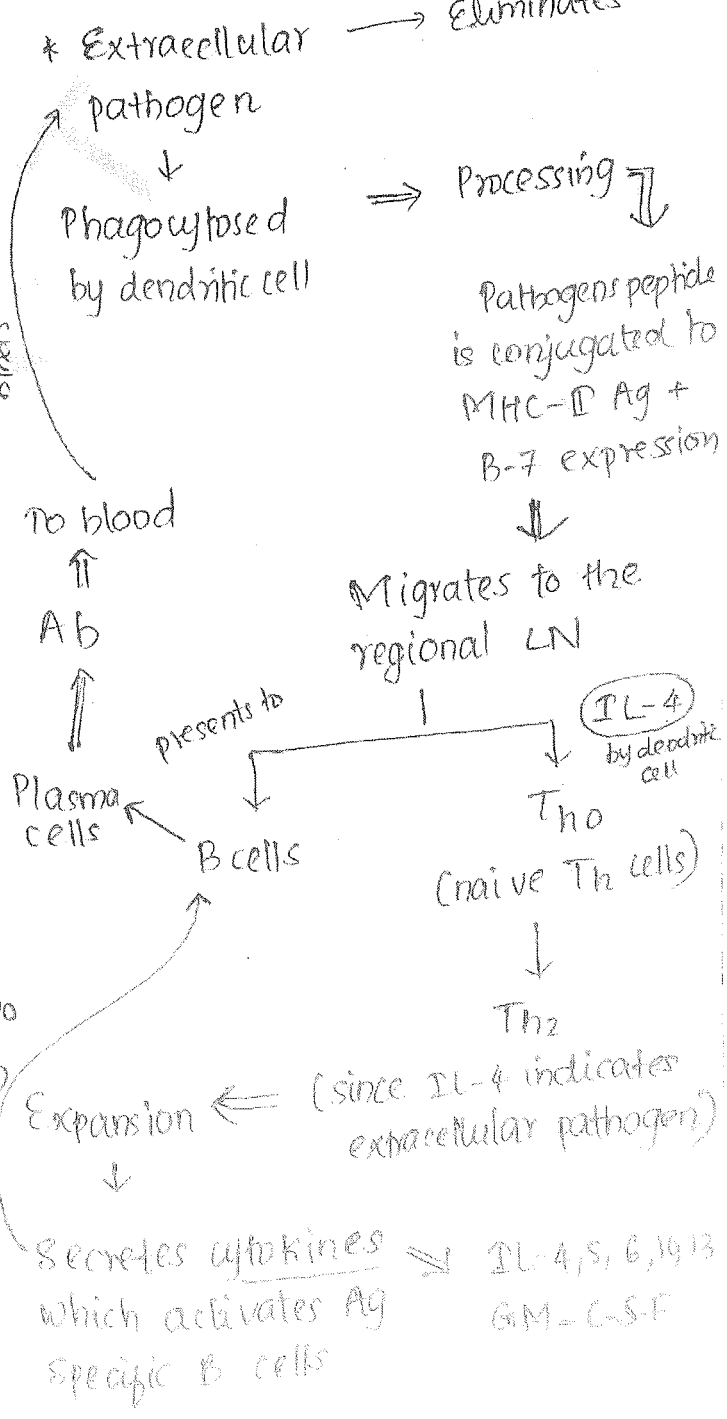
* Each T/B cell has $> 10^5$ T-cell receptor (TCR) or B cell Receptor (BCR) ⟶ each having same Ag binding specificity.

* The huge diversity of receptors is not encoded in our genome but the genes for the receptors are generated by a special complex phenomenon · k/a
Gene rearrangements
or
Somatic recombinations
which occurs in the 1° lymphoid organ during maturation of the T/B cell.
MCQ
ie, B cell ⟶ Bonemarrow
T cell ⟶ Thymus.

* Mature (naive - not yet recognized by an Ag) T/B cells released from 1° lymphoid organs undergo constant peripheral recirculation looking for Ag to recognize.

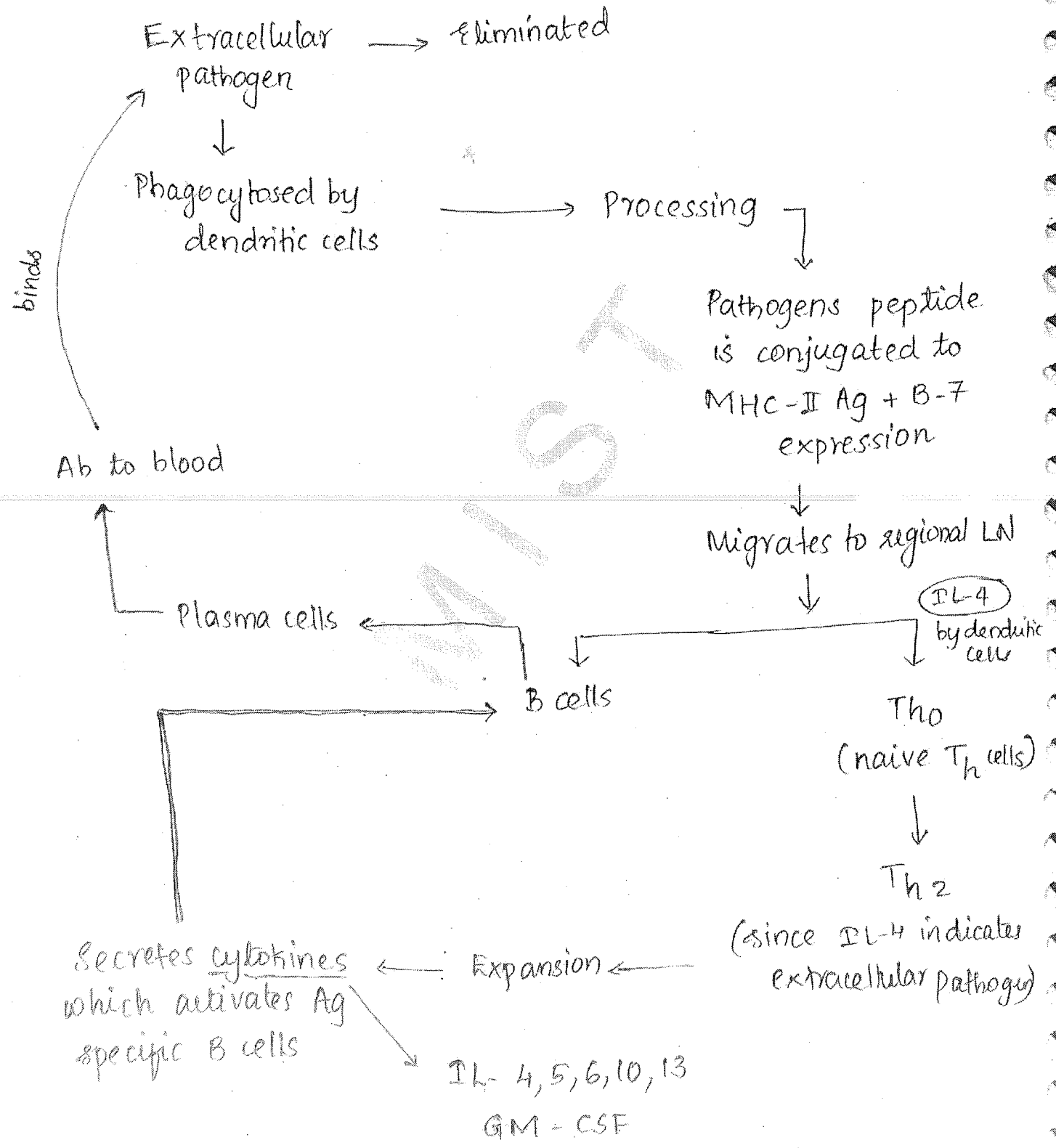
* On recognizing a particular Ag, that particular T/B cell is selected out to undergo expansion ⟶ to give rise to thousands & thousands of T/B cells & memory T/B cells → k/a
Clonal selection & Expansion.

* HUMORAL IMMUNITY



(If IL-12 ⟶ then Th2 produces)
GM CSF - Granulocyte monocyte colony stimulating factor

Humoral Immunity



Adaptive Immunity – T cell mediated

Virus infected cell phagocytosed by dendritic cell ⟹ Processing ⟹ Viral Peptides + MHC I & II Ag + B7 expression

⇓

Migrates to regional LN (presents viral peptides to)

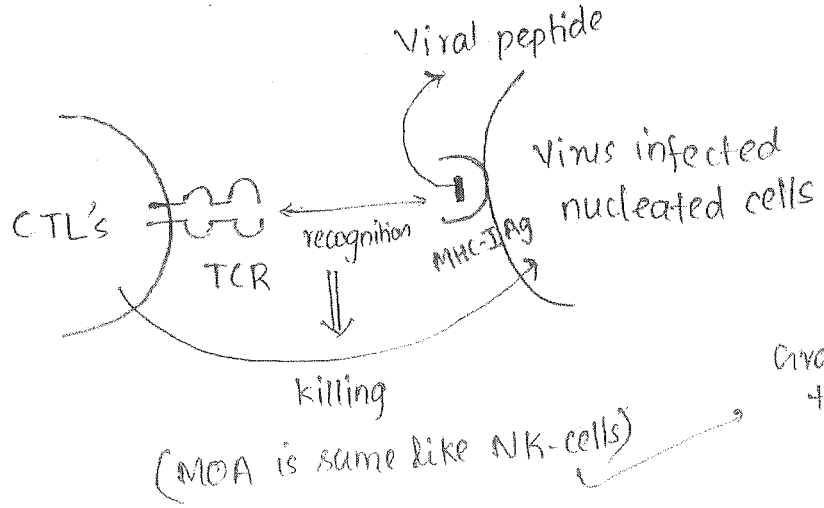
MHC I + B7 – MHC II + B7 – Th0

Th0 → IL-12 → Th1

Th1 → Activates by secreting IFN-γ, TNF-β, IL-12, IL-18 → Tc

Tc → Activation → CTL's (cytotoxic T lymphocytes) → Blood

Tc → Expansion

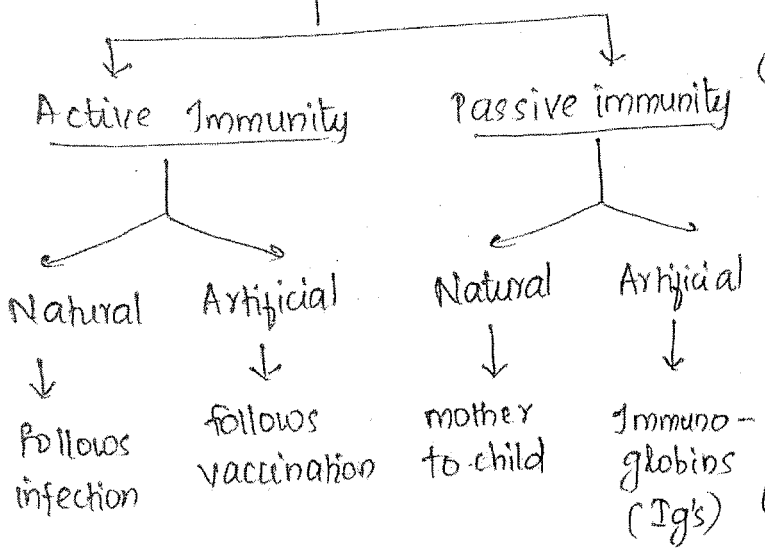


(MOA is same like NK-cells) → Granzyme, Perforins etc.. then apoptosis

⇒ Those cell virus infected cells ⊆ ↓↓ MHC-I will be killed by NK cells

⇒ Those " ⊆ are expressing MHC-I will be killed by CTL's (cytotoxic T-lymphocytes)

✱ Adaptive Immunity



Antigens vs Hapten

Antigen	Hapten
• Substance ⊆ is recognised by the immune system (Immunogenicity) + can bind specifically to Ab (Antigenicity)	• Not immunogenic but it can bind to preformed Ab (ie, have antigenicity) ⊙ Hapten + Carrier protein ⇓ becomes immunogenic

Class switching ⇒ first IgM Ab formed → on continous exposure to Ag → B cells start to produce IgG, IgA & IgE instead of IgM

Determinants of Immunogenicity of an Antigen

(1) Chemical nature ✱

Proteins > Polysaccharide > Nucleic acids > Lipids

- More complex an Ag, more immunogenic (ie, more residues – more immunogenic)

(2) Molecular weight

- Minimum molecular weight of 5000 – 10,000 Daltons required for immunogenicity
- More MW → More immunogenic

(3) Foreignness

- More evolutionary distant origin of Ag → more is its immunogenicity

  eg: Hen to human

(4) Susceptibility to host enzymes

- More the susceptibility → more immunogenic

Classes of Antigens

T-independent	T-dependent
• B cells do not need help of Th to form Ab	• B cells need the help of T_h to form Ab against them.
• eg: Polysaccharide, lipids, nucleic acids, lipopoly saccharide.	• eg: Proteins
	• Only Ag specific B cells are activated.
• Polyclonal activation of B-cells (large number)	• Initially IgM formed then class switches to IgG, IgA, IgE
• IgM Ab's formed mainly [Class switching is absent here]	• Class switching present
• Low affinity formed Ab	• Low → higher affinity Ab
• Memory absent.	• Memory present

Epitope

* It is the small distinct part of an Ag that is recognized by immune system, specifically by Ab, B cells or T cells.

* One single Ag is made of multiple epitope against many of which an immune response is generated.

  ie, 1 Ag → Polyvalent Immune Response

* 2 types of epitopes:

  (1) Linear / Sequential epitopes
  formed by residues right next to each other in the 1° structure of Ag.

  (2) Conformational / Discontinous epitopes:
  formed by residues far apart from the 1° structure but brought close to form an epitope due to 3° or 4° folding of Ag.

MCQ

* T cells recognizes LINEAR epitopes ONLY.
* B cells recognizes → BOTH

Heterophile Ag

* A group of similar Ag present in unrelated species.

eg: Forssman Ag → present in all prokaryotes & eukaryotes (except rabbits)

Superantigens (S. aureus)

* Discussed in staph. aureus.

Isoantigens

* Present only in some members of the same species.

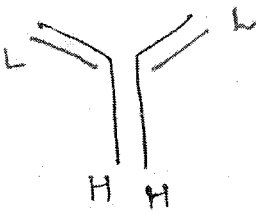
eg: Blood group Ags, Rh factors, MHC Ag

ANTIBODIES

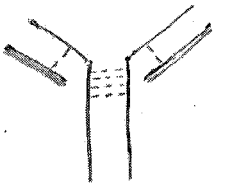
* It is a tetrameric glyco-proteins (Not polypeptide)

* Each Ab → 2 identical light chains (L) : 2 Kappa or 2 lambda
→ 2 identical heavy chains (H) (2 μ or 2 ε etc...)

→ 5 types H-chain
- (IgM) · μ
- (IgE) · ε
- (IgD) · δ
- (IgG) · γ → $\gamma_1, \gamma_2, \gamma_3, \gamma_4$
- (IgA) · α → α_1, α_2



* The class of heavy chain tells us class of Ab.
* Each L chain links to H chain by 1 disulfide bond
* Both H chains linked by 1-5 disulfide bond (Hinge region)



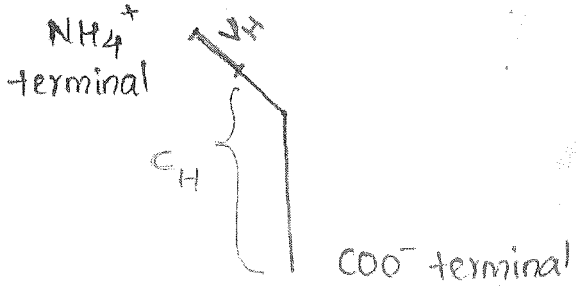
* IgM → 2 μ H chain & 2 Kappa or 2 lambda L chain.

* Heavy chain → molecular weight is 50,000 to 75,000 Da (dalton)

50,000 > 50000-75000

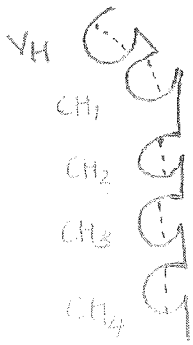
* Each heavy chain contains 446-576 aminoacids with carbohydrate substitution (Glycoprotein)

* First 110 aa from the amino terminal → they determines Ag binding → V_H



* Rest is k/a constant part (C_H) → class of H-chain → class of Ab

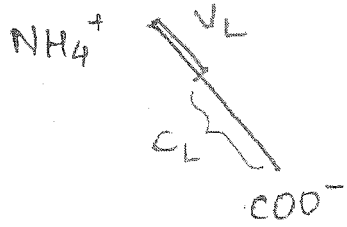
* Within the H-chain has foldings because of disulphide bonds.



* Light chain → 25,000 Da molecular weight

* L chain → 211-217 aa ~~c~~ carbohydrate substitution

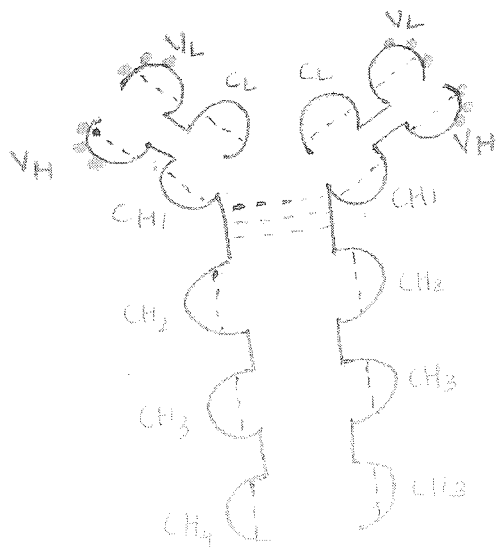
* 1st 110 aa from amino terminal → determine Ag binding → V_L



* Rest all C_L → Class of L-chain

* Has foldings due to disulphide bonds

Antibody



* ~~c~~ hype

* 6 hypervariable loops →

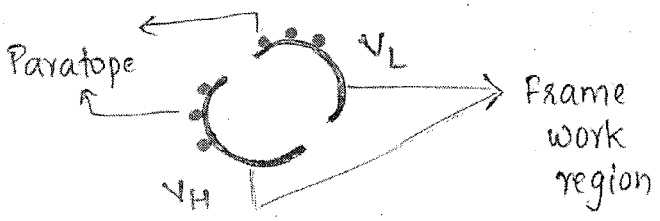
(a) 3 in V_L & 3 in V_H ∈ actually contact the Ag →

k/a Paratope

or complementarily determining region's (CDR's)

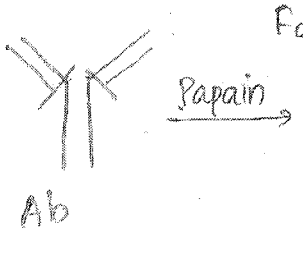
(b) Part of V_L & V_H not forming Ag binding part is k/a

Frame work region



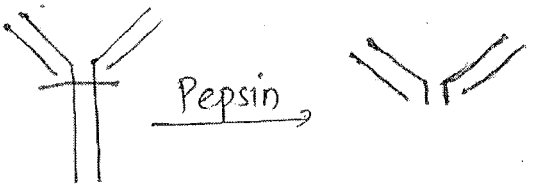
Papain action on Ab

* When papain binds on Ab → it breaks the Ab in to 3 fragments



2 Fab + 1 F_C

* When pepsin acts on Ab →



$F_{(ab)_2}$ + Peptides

No Fc

breaks Fc in to small peptide fragments.

Papain → 2 Fab + 1 Fc

Pepsin → $F_{(ab)_2}$ + Peptides

MCQ ⊛ Max. concentration in blood

Ig G

$IgG_1 > IgG_2 > IgG_3 > IgG_4$

MCQ ⊛ Least concentration in blood

Ig E

MCQ ⊛ Max. $t_{1/2}$ ⇒ Ig G (23 days)

MCQ ⊛ Minimum $t_{1/2}$ ⇒ Ig E (1-2 d)

MCQ ⊛ Pentameric ⇒ Ig M

k/a millionaire molecule

MCQ

* Dimeric in mucous secretion & monomeric in blood.

IgA

MCQ

* J-chain is present in (J-joining)

IgM & IgA

* Primary Ab → IgM
* Secondary Ab → IgG
* 1st Ab formed by fetus

IgM (20 wks)

* Only Ab crosses placenta

$IgG_1 > IgG_3, IgG_4$

(Not IgG_2)

* Ab providing mucosal immunity

IgA

* During intrapartum → infection in fetus → produce IgM inside fetus not IgG.
* Ab mediating immediate HSR

IgE

* Mast cells & Basophils have receptor for

IgE

* Heat labile Ab → IgE

Biological functions of Ab

1) Neutralization:

- Before toxins or viruses can bind to cellular receptors, they are bound & thereby neutralized by specific Ab
- IgG and IgA are the neutralizing Abs
- Valency of

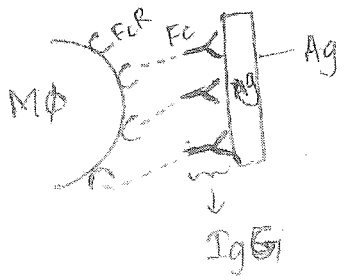
MCQ

IgG → 2

IgM → 5-10

2) Opsonization

- Ab marks an Ag for phagocytosis
- There are receptors for the Fc part of IgG Ab on the surface of Macrophages (MΦ) & neutrophils



- Cross linking of FcR leads to activation of MΦ or Neutrophil → phagocytosis of Ag-Ab complex

∴ Opsonizing Ab is IgG.

3) Ab dependent cell cytotoxicity (ADCC)

↓

- It is nonphagocytic killing of an Ab-coated target cell by a cytotoxic effector cell.
- There are FcR for IgG on NK cells, eosinophils.

(FcR - Fc receptors)

- Usually big pathogens.

4) Activation of classical complement pathway

↓

also k/a Complement fixation

mea * IgM > IgG3 > G1 > G2

(can activate classical C. pathway)

① Classical Pathway

↓

Fc part undergoes a conformational change

↓

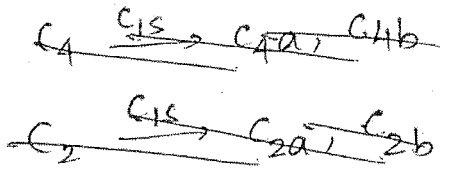
exposes C1q binding → causes spontaneous activation of C1r

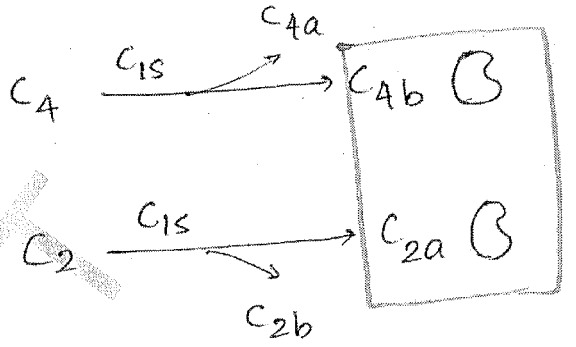
it activates C1s

activates C_{1s} → activated C_1s

↓

Cleaves C_4 & C_2 to





C_{4b} C_{2a} ↓

(C_{4b2a}) = C3 convertase.

* C_3 is most abundant in blood.

* C_3 $\xrightarrow[n=1000]{C_3 \text{ convertase}}$ C_{4b} C_{2a} C_{3b} + C_{3a}

C_{4b} $2a$ $3b$

↓

C_5 convertase

* C_5 $\xrightarrow{C_5 \text{ convertase}}$ C_{5b} + C_{5a}

C_{5b} ⇒ Deposits on Ag

C_{5b} → Deposits on Ag

↓ C_6, C_7, C_8, $C_9{}^n$ (complement system activated)

C_{56789^n}

⇓

Membrane attack complex

↓

Makes pore in Ag

↓

leads to Osmotic lysis of Ag.

MCQ

⇒ Excess of C_{3b} deposits on ~~Ag~~ the surface of Ag.

⇒ There are C_{3b} receptors on MΦ & Neutrophils → binds to C_{3b} on Ag surface → activation of MΦ & Neutrophils → Phagocytosis

---

* MCQ Opsonizing Ab → IgG

Opsonizing complement → C_{3b}

MCQ

* Anaphylatoxic property

$C_{5a} > C_{3a} > C_{4a}$

(↑ local vascular permeability, ↑ more neutrophils to come there)

MCQ

* Chemotactic (attracts more complement proteins)

C_{5a}

* Classical pathway → part of adaptive immunity

② Alternate pathway

(Part of Innate Immunity)

* ~~Classical~~ Activated by lipopolysaccharides, cobra venom, nephritic factors, IgA or IgD aggregates.

* G-ve bacteria → cellwall → lipopolysaccharide (LPS)

* Normally, slow spontaneous hydrolysis of C_3 in to

$C_3 \longrightarrow C_{3a} + C_{3b}$

↓

Immediately hydrolysed by serum factor H & serum factor I

(Not seen in blood)

G-ve bacterium

LPS (binds) → C_{3b} (becomes stable)

C_{3b} ⇓ Serum factor B binds to it

$C_{3b}B$

$C_{3b}B$ ↓ Factor D cleaves → Ba ↑

$C_{3b}Bb$

Kla C_3 convertase

C_3 —($C_{3b}Bb$)→ $C_{3b}Bb3b$ (cleaves C_3 → C_{3a})

Kla C_5 covertase

C_5 —($C_{3b}Bb3b$)→ C_{5b} (cleaves C_5 → C_{5a})

↓

Rest same as classical

⇒ First complement pathway discovered

Classical pathway.

③ Mannose binding lectin Pathway

(Innate Immunity)

* Serum MBL encounters mannose residues on a pathogen → bound MBL

bound MBL

MASP-1, MASP-2 ↓

Bound MBL-$(MASP\text{-}1)_2$-$(MASP\text{-}2)_2$

⇓

C_4 —(C_{4a})→ C_{4b}

C_2 —(C_{2b})→ C_{2a}

$C_{4b}2a$

Kla C_3 convertase

↓

Rest all same as classical pathway.

* Here C_1 is not participating ($-C_1$)

* In alternate pathway → No C_1, no C_2, no C_4

* Alternate pathway is also kla Properdin pathway

Lectin → $-C_1$

Alternate → $-C_1$, $-C_2$, $-C_4$

* All the 3 pathways converge at C_3.

Regulation of complement system

1) Activation of complement proteins occurs on pathogen surface NOT host cells (by)

a) Decay accelerating factor on cell membrane competes with factor B for binding to C_{3b}.

b) CD_{59} / Protectin on the host cells → prevents the assembly of MAC on the host cell (MAC - membrane attack complex)

⇒ Absence of CD59 or decay accelerating factor → causes Paroxysmal nocturnal hemoglobinuria (PNH)

2) Hydrolysis of activated complement proteins after some time of ~~activation~~ action → preventing further activation → by

a) Serum C_1 esterase or C_1 inhibitor → dissociates C_{1q} from C_{1r-1s}

⇒ Deficiency of C_1 inhibitor → Hereditary Angio neurotic edema.

⇒ Deficiency of terminal complement proteins C_5 to C_9 → ~~makes~~ ↑↑ susceptibility to Neisseria infections.

Monoclonal antibodies

* Ab's directed against same Ag.
* First produced by ~~hyd~~ hybridoma technology by KOHLER & MILSTEIN
* Done on mice.
* They did fusion of mouse plasma cells + Myeloma cells

↓

Hybridoma cells

(Cancerous cells & can produce no: of Ab indefinitely)

B-cells

* Pan B cell markers

  CD_{19}, CD_{20}, CD_{21}, CD_{22},

  Surface Ig.

* Enters cell cycle (blast transformn) on exposure to lipopolysaccharide & pokeweed mitogen → thus expands no:

* Forms EAC rosettes

  Sheep RBC + Specific Ab + Complement

  E → Erythrocyte
  A → Ab
  C → Complement.

* B cells forms → 2 types of Ab

  a) Membrane form / Surface Ig.

  ↓

  acts on BCR (B cell ®)

  IgM IgD

  b) Secreted form of Ab

  IgM, IgA, IgG,
  IgE, IgD

  IgA → A_1, A_2

  IgG → G_1, G_2, G_3, G_4

  ∴ 9 in total.

T-cells

* Pan T cell marker

  CD3

* They undergoes blast transformn on exposure to 2 chemicals → Concavalin & Phyto-hemagglutinin.

* Forms E rosettes (c̄ sheep RBC)

* 2 subsets of T cells

  1) T_c (cytotoxic)
  2) T_h (helper)

Tc

* Tc cell marker : CD_8
* They are MHC-I restricted
* Killing cells infected c̄ intracytoplasmic pathogens like viruses.
* MOA : Perforins, Granzymes, Fas L (Fas ligand)

Th

* Th cell marker : CD_4
* MHC-II restricted
* Function → Stimulation of both humoral & cell mediated immunity

* MOA : Secrete cytokines

T_{h1} ⟹ IFN-γ, TNF-β, IL-12, IL-18

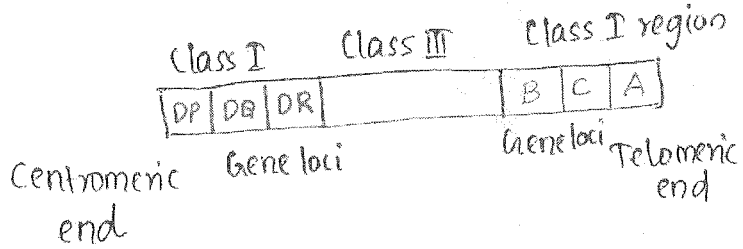
T_{h2} ⟹ IL 4, 5, 6, 10, 13, GM-CSF (Granulocyte monocyte colony stimulating factor)

MCQ

* Class I & II region → contains most polymorphic genes
↓
10's to 100's of alleles exist for each gene locus.
↓
different in all individuals
↓
responsible for rejection in organ transplant.

MHC / HLA complex

* Major histocompatability complex
* Gene cluster on short arm of chromosome 6 (MCQ)
* Has 3 regions:



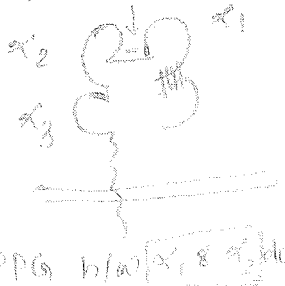
* Class III → has region contains conserved genes (MCQ)
→ it encodes for
  - C_2 & C_4 (complement protein)
  - Factor B
  - TNF α, TNF β
  - Heat shock protein

• same in all individuals

MHC-I Ag

- Encoded by the HLA-A, B, C gene loci
- Expressed on all nucleated cells & platelets.
- Function - presents peptides of intracellular pathogens to T_c

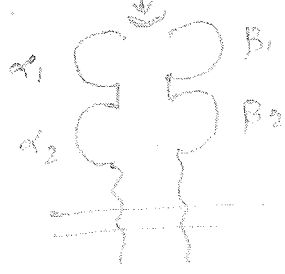
- Has α chain, β_2 microglobulin



Peptide presenting groove →
• PPG b/w α_1 & α_2 domains

MHC-II Ag

- Encoded by DP, DQ, DR gene loci
- Expressed only on APC (DC, MΦ, B cells)
- Presents peptides of both intra & extracellular pathogen to T_h.
- α chain & β chain



• PPG b/w α_1 & β_1 domains

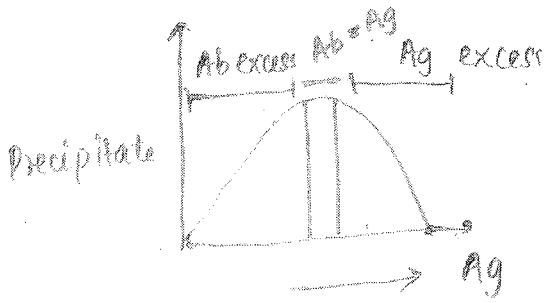
Antigen - Antibody reaction

* Bond b/w Ag & Ab
* NO covalent bonds
* Have electrostatic's, Van der Waals, hydrophobic & H-bonds.

Precipitation reactions

* When soluble Ag reacts c Specific Ab → leads to precipitation & settles down.
* Flocculate ⇒ when the precipitate floats on to the top.
* Precipitation reaction is less sensitive for detection of Ab.
* Take same amount of Ab in
  + Add ↑ amt of Ag

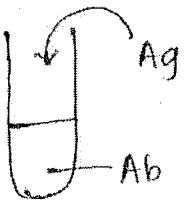
max. precipitate



* Prozone → Ab excess region
* Postzone → Ag excess
* Equivalence zone → Ab = Ag
  → Here maximum precipitate is formed.

Types of precipitation test

1) Ring precipitation test:



- Ring is formed when Ag meets Ab
  a) Lancefield serotyping of β-hemolytic streptococcus
  b) Ascoli's thermo precipitation test (Anthrax)

2) Slide flocculation test:

- Flocculation test
- Syphilis → VDRL (12 wells) slide

3) Tube flocculation test

eg: KAHN test

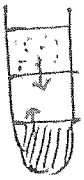
4) Precipitation in gel → k/a Immunodiffusion test.

a)

Plate / Tube (1D)

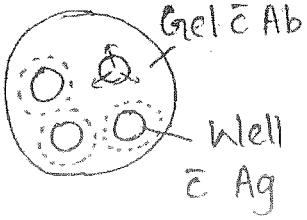
Single diffusion in 1 dimension

b)



Double diffusion in 1 dimension

c)

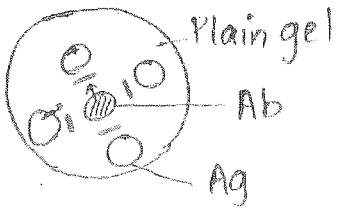


Petriplate (2D) MCQ

Single diffusion (only Ag) in 2 dimension / Radial immuno diffusion.

- Used for quantification of Ab ~~of~~ in blood.

d)



Double diffusion (Ag & Ab moves) in 2 dimension.

Agglutination reaction

* Particulate Ag reacts c̄ specific Ab → Clumping or Agglutinate.
* It is more sensitive for Ab detection than precipⁿ reacⁿ.

Types

1) Slide agglutination
   - Used for identification of bacterial ~~agg~~ isolates
   - Done for blood grouping and cross matching

2) Tube agglutination
   - eg: Widal test, Weil Felix test, Paul Bunnel test

3) Passive agglutination:
   - Coating a soluble Ag in to a neutral carrier like gelatin, latex, RBC, charcoal.
   - More sensitive for Ab detection

   eg: Treponema pallidum heme agglutination (TPHA)

RA Rose Waaler (rheumatoid factor detection)

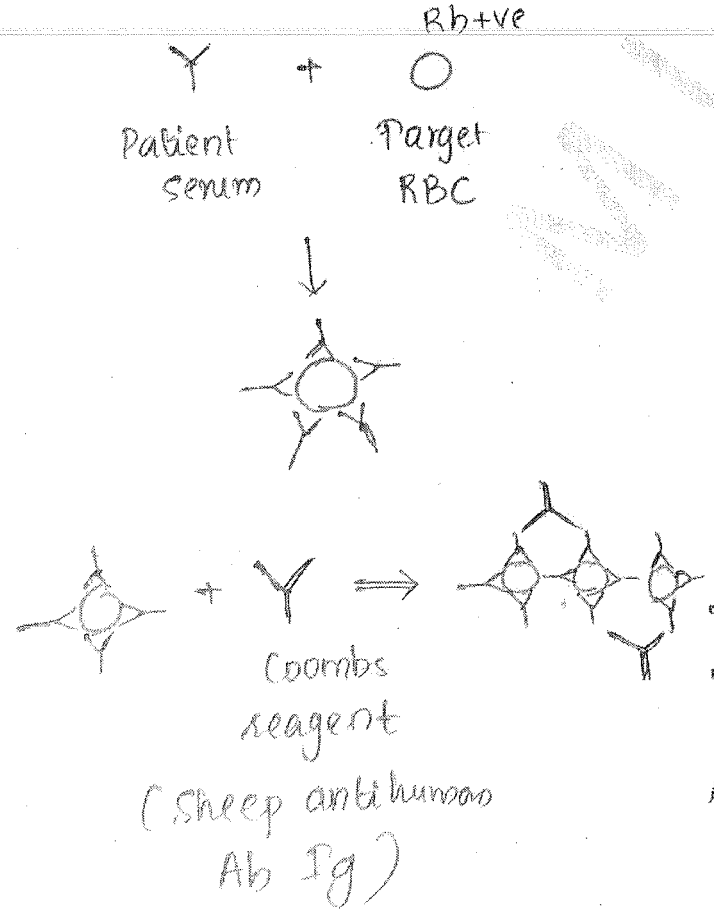
TPPA (T. pallidum particle agglutination)

Coomb's Test

* Antiglobin / Antiglobulin test
* 2 types → Indirect & Direct

Indirect Coombs

* Used in prenatal testing of pregnant women for anti Rh Ab



Direct Coombs

* Done on fetal serum for erythroblastosis fetalis (autoimmune hemolytic anemia)

Fetal serum + Coombs reagent → Agglutination of RBCs

VIRUSES

* Smallest living units
* Obligate intracellular (lack all synthetic enzymes)
* All are ultrascopic (not seen by light microscope)

Imp
- chickenpox
- Mumps
- Measles

- Diseases caused by virus (name)
- Belongs to which group

* All are ultrascopic except Pox viruses (300 nm size) (large)
  ↓
  Largest pathogenic virus

* Smallest pathogenic virus
  Parvo B19

* Pox virus → Small pox,
  Molluscum contagiosum

* Contains either DNA or RNA, never both.

* Nucleic acid is surrounded by a protein coat kla Capsid.

* Capsid → made of subunits kla Capsomers.

* Arrangement of capsomer determines the symmetry of the virus.

  ↓

  - Helical
  - Icosahedral / Cubical
  - Complex (if not H (IH)

* Only virus ∈ complex symmetry
  Pox virus.

* Certain viruses have lipid envelope → derived from the host cell membrane

* Lipid envelope has virus encoded glycoprotein embedded in it.

DNA viruses

* 7 families
  1) Herpes → 8
  2) POX
  3) Hepadna
  4) Parvo
  5) Adeno
  6) Polyoma
  7) Papilloma

* Herpes → HHV 1 to HHV 8
  (Human Herpes Virus : HHV)

* Pox → Variola & Molluscum virus

* Hepadna → Hepatitis B (HBV)
  ↓
  Only hepatitis virus ⊆ is DNA virus.

* Parvo → Parvo B19

* Polyoma → JC virus (JCV) & BKV

* Papilloma → As types 1-150.

* Enveloped DNA viruses
  - Hepadna
  - Herpes
  - Pox

* Rest all naked → P, A, P, P

* All DNA virus has Icosahedral symmetry except POX (complex)

(*) All DNA virus have a dsDNA except Parvo (ss DNA) MCQ

* All DNA viruses replicate in the nucleus except POX (in cytoplasm)

RNA viruses

* 15 families
* All are enveloped except
  - P – Picorna virus
  - A – Astro V
  - R – Reo V
  - C – Calici V
  - H – Hepe V
* Hepatitis E virus, in Hepe virus.
* Earlier it belongs to Calici virus

  Hepe V > Calici V.
* Picorna virus
  - P – Polio
  - E – Echo
  - E – Entero
  - Co – Cox sackie
  - Rn – Rhino
  - A – Hep A virus (HAV)
* ReoV → Rotavirus
* Calici Virus → Norwalk virus
* Hepatitis :
  - A – Physically – Picorna
  - B – Handicapped – Hepadna
  - C – Friend – Flavi
  - D – Danced – Delta
  - E – Happily – Hepe V
* All RNA viruses are ssRNA genome except Reo virus (ds) (dsRNA)
* All RNA viruses replicate in cytoplasm except
  - P – Paramyxo V
  - O – Orthomyxo V
  - R – Retro V

  } Nucleus
* Paramyxo V → Measles, Mumps, Para-influenza, RSV
* Orthomyxo V → Influenza
* Retro V → HIV, HTLV-1, HTLV-2

  (Human T-lymphocyte virus)

* Negative stranded RNA virus:

They have a special RNA dependent RNA polymerase.

Always Bring Polymerase Or Fail Replication

- Arena V
- Bunya V
- Paramyxo V
- Orthomyxo V
- Filo viruses
- Rhabdo V

* Arena V → Lassa fever virus
* Bunya V → Hanta V, CCHF

CCHF: Crimean Congo Hemorrhagic fever

* Filo V → Ebola, Marburg
* Rhabdo V → Rabies.

* Helical viruses:

ABPOFR + Corona virus (above)

Corona V → 'SARS' virus

* Rest all have Icosahedral symmetry

* SARS → Severe Acute Respiratory Syndrome virus.

* Segmented virus: RNA genome

They shows genetic recombination

✓ R - Reo V
✓ O - Orthomyxo V
B - Bunya V
A - Arena V

* Reo V → Rota V → 10-12 segments in genome

* Orthomyxo V → Influenza

Influenza A & B : 8 segments
" C : 7 segments

Shapes of viruses

- Brick → Pox V
- Bullet → Rhabdo V
- Star → Astro V
- Space ship → Adeno V
- Wheel → Rota V
- Filament → Filo V

Inclusion bodies

- Negri → Rabies (max in hippocampus & cerebellum)
- Torres → Yellow fever
- Cowdry A → Herpes V
- Cowdry B → Adeno V
- Henderson Peterson → Molluscum
- Paschen/ Guarnieri → Variola

Diarrhea causing viruses

1) MCC of infantile diarrhea
   Rota (Reo V)
2) Enteric Adeno Viruses
   (Serotypes 40, 41)
   2nd MCC of infantile diarrhea
3) Norwalk V (Calici V)
   MCC of adult diarrhea
4) Other calici viruses
5) Astro virus.

Tick transmitted

1) Flavi → Kyasanur forest disease MCA
   Omsk Hemorrhagic fever
   Russian spring summer Encephalitis virus
2) Bunya V → CCHF
   (Crimean Combo Hemor. fever)

Steps of Viral replication inside host cell

1) Binding to receptor on the cell membrane
2) Adsorption in to host cell
3) Uncoating of nucleic acid
4) Synthesis (DNA/RNA of Virus → transcriped in to early mRNA → early proteins by translation)
   - Early proteins → Synthetic enzymes
   - Several copies of DNA/RNA synthesized → late mRNA → late proteins (capsid protein)
5) Assembly of daughter virions
6) Release occurs by lysis or by budding of host cell.

* Virus having Reverse Transcriptase

1) Retro virus
2) Hepadna virus (HBV)

Cultivation of viruses

* Non cultivable on cell free media
* So use lab animals
  MC used → Mouse
* But in
  Coxsackie V, Arbo virus } Only grow on suckling mice

2) Chick embryo

- In amniotic cavity / allantoic cavity / yolk sac / chorio allantoic membrane.
- Amniotic cavity → All myxoV (para or ortho)

3) Cell lines:

DNA viruses

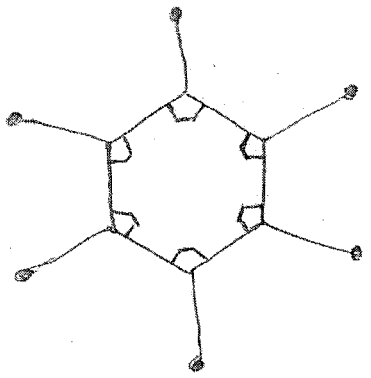
1) Parvo B19 virus

* ss DNA
* Icosahedral
* Non enveloped
* Affinity for erythroid precursor cells → infects normoblast & pronormoblast.
* Then causes lysis of cell.
* MC route of infection: Respiratory
* Disease is only seen in the 1° infection
  - (i) 1° infection in child ⇒ leads to 5th disease / Erythema infectiosum.
    - Fever + Slapped cheek rash
    - No Rx required
  - (ii) 1° infection in adults → Leads to Arthropathies (small joints)
  - (iii) 1° infection in chronic Hemolytic anemia (eg: sickle cell)
    ↓
    leads to Transient aplastic crisis (sudden ↓↓ Hb)
    - Rx: RBC transfusion.
  - (iv) 1° infection in 1st half of pregnancy:
    - 33% risk of transplacental ~~infections~~ transfer
    - 5% fetus → severe hemolytic anemia → hydrops fetalis
      ↓
      k/a Non immune hydrops fetalis

⇒ MCC of non immune H.F = Parvo B19 virus.

2) Adeno virus

* MC viruses used as gene vector in genetic engineering.
* Non enveloped
* Capsid → made of 252 capsomers
  ↓
  12 pentons, 240 hexons



Adeno virus

Imp. topics

1) Giardia
2) Entamoeba
3) Kala-azar
4) Plasmodium
5) Round worms

* 51 serotypes
* Common infections
  = Respiratory tract infection
    - Acute pharyngitis
    - Pneumonia
    - Pharyngo conjunctival fever / swimming pool conjuctivitis

  = Eye infection
    - Epidemic kerato conjunctivitis or shipyard eye.

MCQ

MCQ ⇒ Mcc of acute hemorrhagic conjunctivitis

Coxackie A-24
or Enterovirus 70

= Diarrhea
Enteric adeno virus
(40, 41 serotypes)

= Hemorrhagic cystitis
11, 21

INTRODUCTION

Microbiology:- Study of micro - organism

Virus	Bacteria	Fungi	Protozoa
-Smallest infections agent -Size -20-200nm = 109m	-Prokaryotes -Size :- 1-5µm	-Eukaryotes -Size:- 5-10 µm	-Eukaryotes -Size :- 10-20 µm
-smallest virus → **Parvo Virus** -Biggest virus → **Pox Vi**	-Smallest bacteria :- **Mycoplasma** -Largest Bacteria :- **Bacillus anthracis**	-	-
-All viruses obligate intracellular	-Unicellular	-Multicellular	-Multicellular
-Contain either DNA or RNA -(Never present both) -Artificial media not possible	-Contains DNA + RNA -Surrounded by rigid cell wall	-DNA + RNA -Surrounded by cell wall → Chitinous	-DNA + RNA -Cell wall present
-All are non- motile	-Motile (Flagella) -Non – motile	-Non –motile	-Motile
-Multiply by viral infective cycle	-Multiply by asexually – binary fission	-Sexually – Meiosis -Asexually – Mitosis	-Sexually – Meiosis -Asexually - Mitosis

Prokaryotic Cells	Eukaryotic Cells
always unicellular	often multicellular
no nucleus or any membrane – bound organelles	**Always have nucleus and other membrane – bound Organelles**
Single chromosome **DNA is circular , without proteins**	Multiple chromosome DNA is linear and associated with proteins to from chromatin
ribosomes are small (70S)	**ribosomes are large (80S)**
no cytoskeleton	always has a cytoskeleton
cell division is by binary fission	**cell division is by mitosis or meiosis**
reproduction is always asexual	reproduction is asexual or sexual
Cytoplasmic • **Cytoplasmic Streaming → Absent** • **Pinocytosis → Absent** • **Membrane bound intracellular organelles → Absent**	Cytoplasmic • Cytoplasmic Streaming → Present • Pinocytosis → Present • Membrane bound intracellular organelles → Present
Cell Envelope • **Peptidoglycan cell wall present** - **Except - Mycoplasma** • **Sterols in cell membrane – absent** - **Expect - Mycoplasma** • **Diaminopimelic acid – present in some**	Cell Envelope • Peptidoglycan cell wall absent • Sterols in cell membrane – present • Diaminopimelic acid – absent

Historical Introduction

1. Bacteria were first observed and discovered by **Anthony Van Leuwenhook (1683)**

2. Louis Pasteur:-

- Established that fermentation was result of microbial activity
- Developed differing growth needs or bacteria
- Started studies on anthrax , chicken pox, cholera & hydrophobia
- Coined the term vaccine , when he first attenuated culture of Anthrax bacillus & proved that inoculation of such cultures in animals induced specific protection against anthrax
- Developed Rabies vaccines
- Disproved " ***the theory of spontaneous generation***"

3. Robert Koch:-

- Studies on culture & character of Anthrax bacillus
- Introduction of staining technique & methods of obtaining bacteria in pure culture
- Bacilli of Tuberculosis (1882) & Vibrio cholera (1883)
- **using criteria developed by his teacher, Jacob Henle (1809 -1895) , established the relationship between Bacillus anthracis and anthrax:**
- **His criteria became known as koch's postulates and are still used to establish the link between a particular microorganism and a particular disease:**

Koch's Postulates
• The causative (etiological) agent must be present in all affected organisms but absent in healthy individuals • The agent must be capable of being isolated and cultured in pure form • When the cultured agent is introduced to a healthy organism , the same disease must occur. • The same causative agent must be isolated agent from the affected host. • Exception of Koch's Postulates:- ✓ **M. Leprae:-** Because is can't be cultured on artificial medium however it can be grown in foot pad of mice/armadillo ✓ **TreponemaPallidum** – Grown in rabbit testis.

4. Edward jenner:- used a vaccination procedure to protect individulas from smallpox.

5. Joseph lister (1827 – 1912) – "Father of Antiseptic Surgery"

- Developed a system of surgery designed to prevent microorgamisms from entering wounds – phenol sprayed in air around surgical incision
- Decreased number of post – operative infections in patients
- his published findings (1867) transformed the practice of surgery

6. Emil von Behring (1854 - 1917)

- induced the formation of diphtheria antitoxin

7. Elie Metchnikoff (1845 - 1916)

- demonstrated the existence of phagocytic cells in the blood, thus demonstrating cell – mediated immunity

8. Charles Chamberland (1851 - 1908)

- identified viruses as disease – causing agent – Tobacco Mosaic Virus

Microscopy:-

- Direct Microscopy
  a) Wet preparation (low power microscopy)
  b) Fixed stained smear (oil immersion)
- Objective lenses:-

- Medium Power = 10x
- High Power = 40x

Microscopes

Simple	- Similar to a magnifying glass and has only one lense.
Compound	-Lets light pass through an object and then through two or more lenses
Stereoscopic	Gives three dimensional view of an object. (Examples: insects and leaves)
Light Microscope	-Bright field microecope -Transmitted light, field – bright, object –dark -Both live & killed organism can be seen by light microscope -Resolving power -0.2 µm = 200nm -None of virus can be seen by light microscope Except – Pox virus (200nm)
Dark field / Ground Microscope	-Transmitted light, object – bright, field –dark -Reflected light -Used to demonstrate – spirochaetes
Electron microscope	-uses a magnetic field to bend beams of electrons: instead of using lenses to bend beams of light. -Highest resolving power – 0.2 nm -Only microscope can seen killed organism -Two type a) Transmission EM -2D b) Scanning EM -3D

Staining

- **Increase contrast of microorganism**
- **Classified into types of stains**

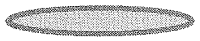
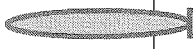
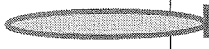
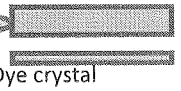
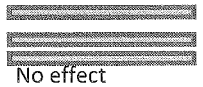
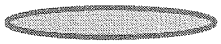
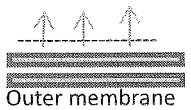
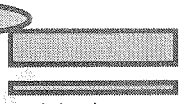
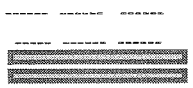
Simple stain	Differential stain	Structural or special stains
-One dye, one step -Direct stain using basic dye -Negative stain using acidic dye -i.e. **Methylene blue**	-More than one dye -**Gram stain, acid fast** -Primary dye Decolorizing step -Counter stain	-Identify structures within or on cells -Different parts of cell are stained different colors -Acid fast stain / ZN stain -Albert stain

- **Acid fast stain & Albert stain**

Acid fast stain	Albert stain
-Acid fastness of bacteria is due to presence of mycolic acid in the cell wall **Organism** / **% of acid tolerance/Fastness** M.TB — 20% M. Leprae — 5% Nocardia — 1% Bacterial Spores — 0.5% Cryptosporidium — .1% -In this stain bacteria is seen as **Red** in color and the surrounding is stained **Blue**.	-It is used only for corynebacterium to demonstrate metachromatic granules -Using this stain bacteria is stained green & the granules are stained purple.

- **Dyes:-**
  - Organic salts with positive and negative charges

Acidic dye	Basic dye
✓ Chromphore repelled by negative cell wall ✓ Background is stained, bacteria are colorless ✓ Negative stain – look at size, shape ✓ Eosin, India ink	✓ Works best in neutral or alkaline pH ✓ Cell wall has slight negative charge at pH7 ✓ Basic dye (Positive) attracted to cell wall (negative) ✓ Crystal violet, methylene blue, safranin

	Microscopic Appearance of cell		Chemical Recation in cell wall (very magnified view)	
Step	Gram (+)	Gram (-)	Gram (+)	Gram (-)
1. Crystal violet (primary dye)			 Both cell walls stain with the dye	
2. Gram's iodine (mordant)			 Dye crystal trapped in cell	 No effect of iodine
3. Alcohol (decolorizer)			 Crystal remain weakened In cell	 Outer membrane cell loses dye
4. Safranin (red dye counterstain)			 Red dye has no effect	 Red dye stains the colorriess cell

Morphology of Bacteria / Structure of Bacterial cell wall

A. Cell Wall

- Major function is to gives **shape & rigidity** to the bacteria
- The basic structural unit of cell wall is called as peptidoglycan which is composed of 2 compounds i.e. N- **acetyl glucosamine (NAG) & N- acetyl muramic acid (NAM)**
- **Difference between cells walls of gram posirive& gram negative bacteria**

Gram Positive	Gram Negative
• Cytoplasm surrounded by lipid bilayer – a/k/a cell memb. • It is essential for life of bacteria • Extension of cell membrane → **Mesosomes** contains multiple layer of peptidoglycan cell wall ✓ More thicker ✓ Site of respiration ✓ Separation of bacterial cell during binary fission	• Cell membrane surrounded by outer membrane • Space between outer & inner plasmic membrane Periplasmic space Contains single layer of peptidoglycan cell wall ✓ More complex

Hair like structure – made up teichoic acid present	Hair like structure present – Pilli / fimbriae, Which is made up of protein pillin / f Ag
Lipo – polysaccharide – Absent	Lipo – polysaccharide – Present
Aromatic and sulphur containing amino acids – Absent	Aromatic and sulphur containing amino acids – Present

- Some of G+ & G- are motile by flagella – made of protein subunits → Flagellin /H Ag

- **Difference between exotoxin and endotoxin**

EXOTOXIN	ENDOTOXIN
Proteinaceous Heat labile **Actively secreted by bacteria** Can be separated from cultures easily **Enzymatic action** Specific Pharmacological effect for each toxin **Specific tissue affinity** Highly antigenic Active in very minute doses Action neutralized by specific antibody **Can be toxoided**	**Lipopolysaccharides** Heat stable **Part of cell wall** Obtained only by cell lysis **Not enzymatic action** Nonspecific effect **No specific tissue affinity** Weakly antigenic Active only in large doses Not neutralized by antibody **Cannot be toxoided**
Produced mostly by Gram positive bacteria	Produced mostly by Gram negative bacteria

- Protoplast – it is a gram +ve bacteria without a cell wall
- Spheroplast – it is a gram –ve bacteria with only some parts of cell wall
- L- form of bacteria
    - ✓ These are the bacteria produced in the lab without a cell wall by growing them in a medium containing penicillin (lister institute)

B. Flagella

- 3 parts
  a) **Filament** – long thin, helical structure composed of protein **flagellin**
  b) **Hook** – Curved
  c) **Basal body** – Stack of rings firmly anchored in cell wall
- Rotates 360°
- It provides motility to the sheath
- Number and arrangement of flagella varies:
    - Monotricchous : flagella
        - ✓ Pseudomonas aeruginosa
        - ✓ Vibrio cholera
    - Lophotrichous : tuft at one end
        - ✓ Helicobactor pylori
    - Amphitrichous :- tuft at both ends
        - ✓ Spirillum minus
    - Peritrichous : all around bacteria

- ✓ E.coli
- ✓ Salmonella
- ✓ Proteus

- **Peculiar MItolity:-**

Motility	Example
Darting	• Vibrio Cholerae
Tumbling	• Listeria Monocytogenes
Gliding	• Mycoplasma
Stately	• Bacillus • Clostridium
Corkscrew Flexion – Extension Translatory	• Spirochetes
Twitching	• TrichomonasVaginalis
Falling Leaf	• Giardia Lamblia

- **Demonstration of flagella**
  - Can be done by direct or indirect methods
  - Motility can be seen by Dark – ground microscope & by hanging drop method
  - Direct method- by increasing the thickness of flagella using silver **impregnation method**
  - Bacteria which are motile without flagella
    - ✓ **Mycoplasma**
    - ✓ **Spirochetes**

C. **Fimbriae**

- Fine, proteinaceous, hairlike bristles emerging from the cell surface
- Function in adhesion to other cell and surface

D. **Pilli**

- Rigid tubular structure made of **pilin** protein
- Found only in gram – negative cells
- Function to join bacterial cells for partial DNA transfer called **conjugation**

E. **Capsule**- Found in some G+ & G- bacteria

- Polysaccharide in nature
  Except – Bacillus anthracis (Polypeptide in nature) & Yersinia sps.
- Demonstration of capsule:-
  - Negative staining as indialnk stain
  - Quellung reaction / Capsular swelling reaction – it is a type of Ag – Ab reaction in which Ab's against capsule are used to make it more prominent

Examples:- H. influenza, **Pneumococci**, N. meningitis, Kleibsiella.

F Mesosomes:-

- These are infoldings of cell membrane into the cytoplasm of bacteria
- They are the principle site of respiratory enzymes in bacteria i.e.functionally they are similar to mitochondria.
- Additionally they also play a role in cell division.

G. Inclusion body

- They are the energy stores present in cytoplasm of bacteria
- They are more developed in cases of starvation

  Examples- Metachromatic granules / polymetaphosphate / Babe earnst granules / polar bodies
- These are present in Corynebacterium diphtheria as energy store
- They cannot be seen by ordinary gram stain but can be seen by a special call as "Albert's stain/Neisser's stain/ ponder's stain.

***. Grouping of bacteria**

- Strepto – chains
- Staphylo – Grapelike clusters

***. Shapes:-**

Spherical	**Coccus /Cocci**	**Gram =ve**	• Staphylococcus • Streptococcus
		Gram -ve	• Neissaria ✓ Gonorrhea ✓ Meningitis
Rod	**Bacilli / Bacillus**	**Gram +ve**	• Mycobacteria • Clostridium • Corynebacterium • Bacillus • Listeria
		Gram -ve	• E. Coli, salmonella, shigella, henmophilus,brucella, bordetella
Coma Shaped		**Gram +ve**	• Vibrio Cholera
Filamentous			• Nocardia • Actinomyces
Spiral / Helical bacteria		**Flexibal**	• Spriochetes
		Rigid	• Helicobacter • Campylobacter

STERILIZATION AND DISINEFECTION

Sterilization	Disinfection	Antisepsis
• A process by which an articles is removed off **all livingorganism** including spores • Follows all or none	• A process by which a **non– living objective** (in – animate org.) is removed off **all pathogenic org**. Leaving behind non – pathogenic org. & spores • applied on Non living obj.	• A process by which a **livingtissue** is removed all **pathogenic organism** leaving behind non-pathogenic org. & spores.

Physical Methods

a) Sunlight & drying – For disinfection, not reliable

b) **Heat**

- Most common method of sterilization & disinfection

Dry Heat :- causes oxidative damage to cell wall of bacteria			**Moist Heat:-** denaturation/ coagulation of proteins		
Flaming	**Incineration**	**Hot air oven**	**<100° C**	**100°C**	**>100 °C**
Sharp metallic - Forceps - Scalpel - Incisors	-	- Instruments used for sterilization of few specific - Articles like:- ✓ Cotton swab ✓ Antibiotic & dusting powder ✓ All sharp material ✓ All glass material ✓ Any media containing lipid/fat. - 160 °C for 60 min - Controls ✓ Biological control – spores of Bacteria	- Inspiration ✓ 80-85 degrees for ½ hour on 3 successive days ✓ Used for sterilizing LJ medium and Loeffler's serum slope - Water bath/vaccine bath ✓ 60 degrees for 1 hr. ✓ For vaccines bacteria - Pasteurization ✓ Method of disinfection ✓ Holder method -63 degrees for 30min ✓ Flash method	- Boiling-method of disinfection steamiest-method of disinfection - Tyndalisation - 100 degrees for 20 minutes on 3 successive days - For sterilization of media containing sugar or gelatin.	- Autoclave/steam under pressure **- 121 degrees under 15 psi for 15 minutes.** - Sterilization control – spores of **Bacillus**

		bacillus subtillis ✓ Browne's indicator	– 72 degrees for 15 seconds followed by rapid cooling to 13 degrees or lower. ✓ Used in pasteurization of milk ✓ To see the effects of pasteurization a) Phosphatase b) Standard plate count c) Coliform count		**stearothermophilus** - Sterilization of culture media, masks and caps, surgical instruments.

c) Radiation

- Non – ionizing radiation :- UV – rays, IR- rays
- Ionizing radiation :- X –rays. Y-rays

UV rays	**y- rays**
- They have less penetrative power as compared to ionizing radiation - Less harmful & at the same time less effective - They are normally used for **sterilization ofdisposable plastic syringes**& are also used in open areas like **hospital wards (blue light)**	- They have less penetrative power as compared to X- rays and hence are more effective - They are mainly used for sterilization of disposable plastic synringes& are more effective than UV – rays - They don't generate much heat hence method is called as " **Cold sterilization"**

d) Filtration:-

- It is used for **Heat sensitive liquid like Ab solution, Serum & Vaccines**
- **Type of filter:-**

Candle filter	**Asbestos/Seitz filter**	**Millipore filter**	**Glass filter**

- It is only removes the physical impurities solution & has on bacteriocidal activity	- MC used type of filter & only disadvantage is that it changes the pH of solution, has carcinogenic property	- Best available filter - A pore size of 0.22 μm	- Not used now a days

Chemical Methods
a) Phenol - Obtained from distillation of coal tar - Unlike other chemical agents its activity is least affected by organic waste matetial hence it is used to clean dirty surfaces - Phenol us used to clean surfaces . - Dettol as it has chloroxylenol: also savlon as it has Chlorhexidine + Cetrimide - Savlon is much better than Dettol **b) Alcohol** - Ethyl alcohol ⟶ used in 60-70% concentration (if 100 % is used it will cause dehydration of skin protein, so used in 60-70% - Isopropyl alcohol - **Methyl alcohol** - it causes dehydration of bacterial cell wall ✓ Used in 60- 70% conc.& used to kill fungal spores hence used to clean old wooden furniture. & old books - Isopropyl alcohol is better than ethyl alcohol as it is more bacteriocidal& less volatile. **c) Aldehyde** - Formaldehyde ✓ Used in 2 forms – gaseous & liquid form ✓ Gaseous form is **used for fumigation of OT**

- ✓ Liquid form is used for a preservative of anatomical specimen
- Glutaraldehyde
  - ✓ Used 2% conc. & is called as Cidex
  - ✓ Used to sterilize Endoscope including Cystoscope& Bronchoscope
  - ✓ Also used to sterilize Anaesthesia equipment like Face mask & ET tube
  - ✓ Sometimes also used to clean Thermometers (Alcohol is better choice)

d) Halogens {Cl_2 & I_2}

- Chlorine
  - ✓ Used for purification of water
- Iodine
  - ✓ Used as an antiseptic
  - ✓ However in pure form it is toxic & irritant, hence it is used in combination with alcohol & called as Iodophore.

e) Ethylene oxide

- Gaseous sterilant
- It is alkylating agent
- Used for sterilization of
  - ✓ Disposable plastic material, syringes, catheter, cannulas
  - ✓ Suture material, Dental equipment & heart, lung machines

f) Quaternary NH_4 compound

- 3 group – Cationic, Anionic, Non ionic
- Used as Antiseptic / Disinfection

BACTERIAL GROWTH

Growth requirement of the bacteria:-

O2	Temperature		CO2	Speical growth factor
Obligatory - Grow only in presence of O2 - Eg.- M. Tb, nocardia, Pseudomon	Thermophilic bacteria	Grow best at high temperatures **55-80 ° C**	- Capnophiles – optimum 3- 10% CO_2 - Many microaerophiles are also	a) X & V factor – H influenza b) Glutathione – Gonococci c) Tryptophan –
	Mesophilic	Grow best at		

as, Hemophillus, bordetella, brucella Obligatory Anaerobes:- - Grow in absence of O2 - Eg,- clostridium, bacteroidis Microaerophilic - Grow in less O2 (7-10%) - Eg.- H.pylori, campylobacter	bacteria: **25-40 ° C** Psychrophilic bacteria: Grow best below **20 ° C**	capnophiles - Cultured in a candle jar	Salmonella typhi d) Cholesterol Mycoplasma

Bacterial Growth Curve

- **Each growth curve has 4 phases**

Lag Phase	Growth Phase	Stationary Phase	Death Phase
- Occurs immediately after inoculation - Cells do not grow: cells per volume do not increase	- Exponential phase - Log Phase - During this phase the microbe is growing at possible. - Cells per volume increases dramatically	- Growth levels off. - Cells per volume does not increase or decrease - Growht Rate = Death Rate - Due to ✓ Depletion of Nutrients ✓ Increase in Waste Products	- Death Rate exceeds growth Rate - Cells per volume decrease - Dye to:- ✓ Very low concentration of Nutrients ✓ Very high concentration of Waste Products.

- End of the Lag phase - Max. Size
- End of Stationary phase - Spore formation
- Toxin production – Stationary phase

- **Generation Time**
  - Length of time required for a cell to divide during log growth
  -

BACTERIAL GENETICS

Transformation	- It was Ist described by Griffth in Pneumococci - Its is the most primitive method of genetic transfer in bacteria - In this method there is no contact between bacteria & the genes of bacteria are released into the surrounding from where they are taken up by another bacteria
Transduction	- In this method bacteriophage enter the bacteria and attaches its own DNA to the bacterial DNA. - However this association is NOT permanent and after sometime bacteriophage DNA gets separated and during its separation it takes the part of bacterial DNA with it & transfer it to another bacteria
Transposons	- Jumping genes consisting of inverted repeat sequence, responsible for transferable drug resistance.
Lysogenic / Phase conversion	- Bacteriophage DNA permanently gets attached to bacterial DNA & provides some additional characters to bacteria. - Example- in Diphtheria genes responsible for toxin production are not present in bacteria but also present in virus called as Betaphage& these are acquired by process of lysogenic / phage conversion.
Conjugation	- Transfer of genetic elements after actual physical contact between a male donor bacterium and female recipient. - Only method in which there is actual contact between 2 bacteria through the formation of conjugation tube / sex pilli - Genes responsible for coding of sex pilli are present in a plasmid called as F- Plasmid / F – factor - It was first described by Laderburg& Tatum.

- **Plasmid:-**
  - It is aextrachromosomal / Extranuclear genetic material.
  - It is not essential for the life of bacteria but it provides some additional characters like drug resistance in bacteria.
- **Episome**
  - Sometimes Plasmid get attached to the chromosomal DNA
- **Transposons**
  - Jumping genes
  - These are the mobile genetic element Which can migrate from one bacteria to another & they usually do not provide any additional character to the bacteria.
- **Drug Resistance in bacteria**

Plasmid mediated resistance	Chromosomal mediated resistance
- It is because of the presence of R – plasmid - It confers resistance to multiple drugs at a time - It is usually transferable & Transferred by conjugation - Both Horizontal & Vertical transfer - Degree of resistance is very high which cannot be overcome by increasing the concentration of antibiotic - Example- Resistance in Staphylococcus with penicillin.	- It is because of mutation in genes - It confers resistance to I drug at a time - It is usually non – transferable - Only vertical transfer - Lower degree of resistance & sometimes high concentration of Ab is effective - Example – Drug resistance in TB