AST Antimicrobial Susceptibility - Testing

② methods.

I) Phenotypic methods (3).

a) Disk Diffusion method.

b) Broth Dilution method.

c) Epsilometer Test (E-Test) (a + b).

II) Molecular methods

i) phenotypic :

a) Disk Diffusion Method :

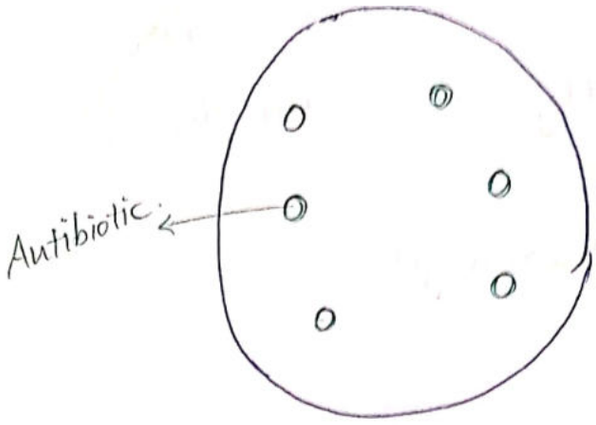
* m/c performed.

Take a solid Agar in a plate
MHA - Muller Hinton Agar

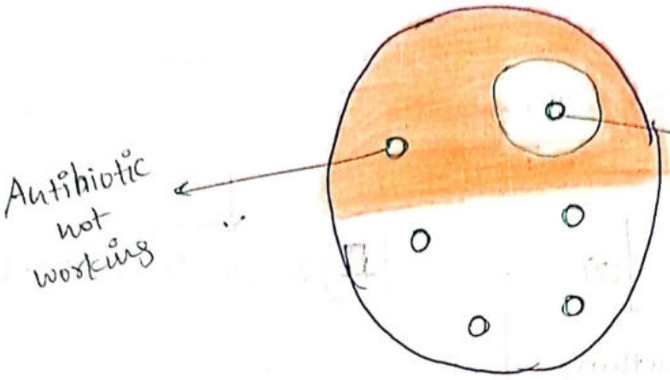
The isolated org. should be lawned.
(spread on the Agar).

We should keep 5-6 Antibiotic discs.

Now Antibiotic will diffuse into the disc.



MHA - Muller Hinton Agar.



aka org. is susceptible to the antibiotic

Antibiotic is working

Zone of inhibition formed

After Zone of inhibⁿ is formed, we have to measure the zone of inhibⁿ (DIAMETER)

For eg: Gentamycin → 15mm.

if zone < 15mm ⇒ Then that Antibiotic Cant be used

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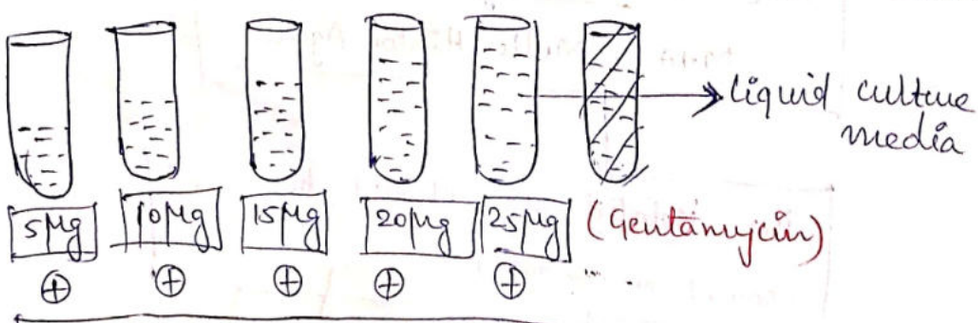
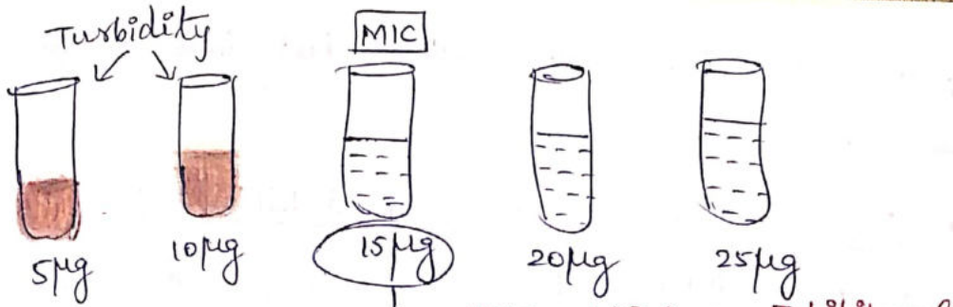
b) Dilution Method / Broth Dilutⁿ method (Gold standard but time consuming)



Fixed concⁿ of org. Suspension (1mL).

After some time



MIC – Minimum Inhibitory Concn.
min. amt. of Gent. reqd. for Antimicrobial susceptibility

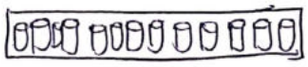
Significance of Dilutn method:

↳ In addn to knowing whether the antibiotic will work/not, we can also find MIC - ie) the min amt. reqd.

- - -

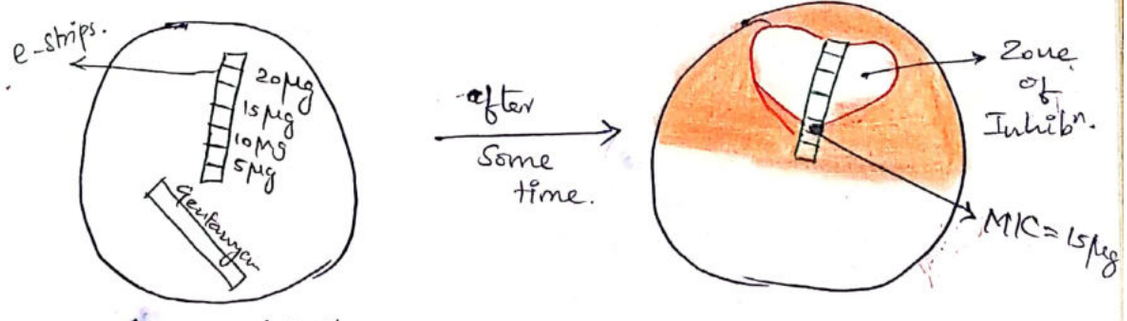
Dilution method.

- macro broth dilutn
  - ⇓
  - if performed in test tube
- micro broth dilutn
  - ⇓
  - if performed in microtitre plate / ELISA plate
  - ↓
  - 96 small wells are present



c) Epsilometer Test (E-Test) -(a+b) (costly).

* E-strips are used which are Antibiotic strips with varying Concn of Antibiotic along the length



MHA - Muller Hinton Agar

* Similar to Disk Diff. method but here E-strips are used.

** We get both Zone of Inhibition as well as MIC here, Thats an Advantage

⇓

Thats why it can be considered as (a+b)

II) Molecular method:

PCR:

* In ID, we used PCR to detect the presence of a particular disease causing org. in the pt's sample.

* But Here we detect whether the Antibiotic Resistant gene is present/not

Eg: Mycobacteria → rpoB gene → present ⇒ resistant to rifampicin

→ absent ⇒ sensitive/susceptible to Rifampicin

GENETICS : How bacteria acquire AMR.? (Antimicrobial Resistance)

I) ① Gene Transfer.

if one bacteria is resistant, then it will transfer that gene to other bacteria

II) ② Mutation

* change in Nucleic a Seq.
* AMR by Mutation is seen only in MYCOBACTERIA - alone

① Gene Transfer: (4).

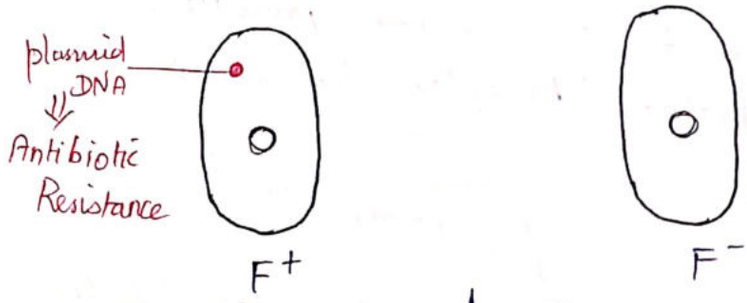
(i) Conjugation. — Conj. Tube
(ii) Transformation — Competence factor
(iii) Transduction — lytic & lysogenic
(iv) Trans-position — Jumping genes (Transposons)

i) Conjugation: (m/c method for all bacteria except staphylococcus to acq. AMR).

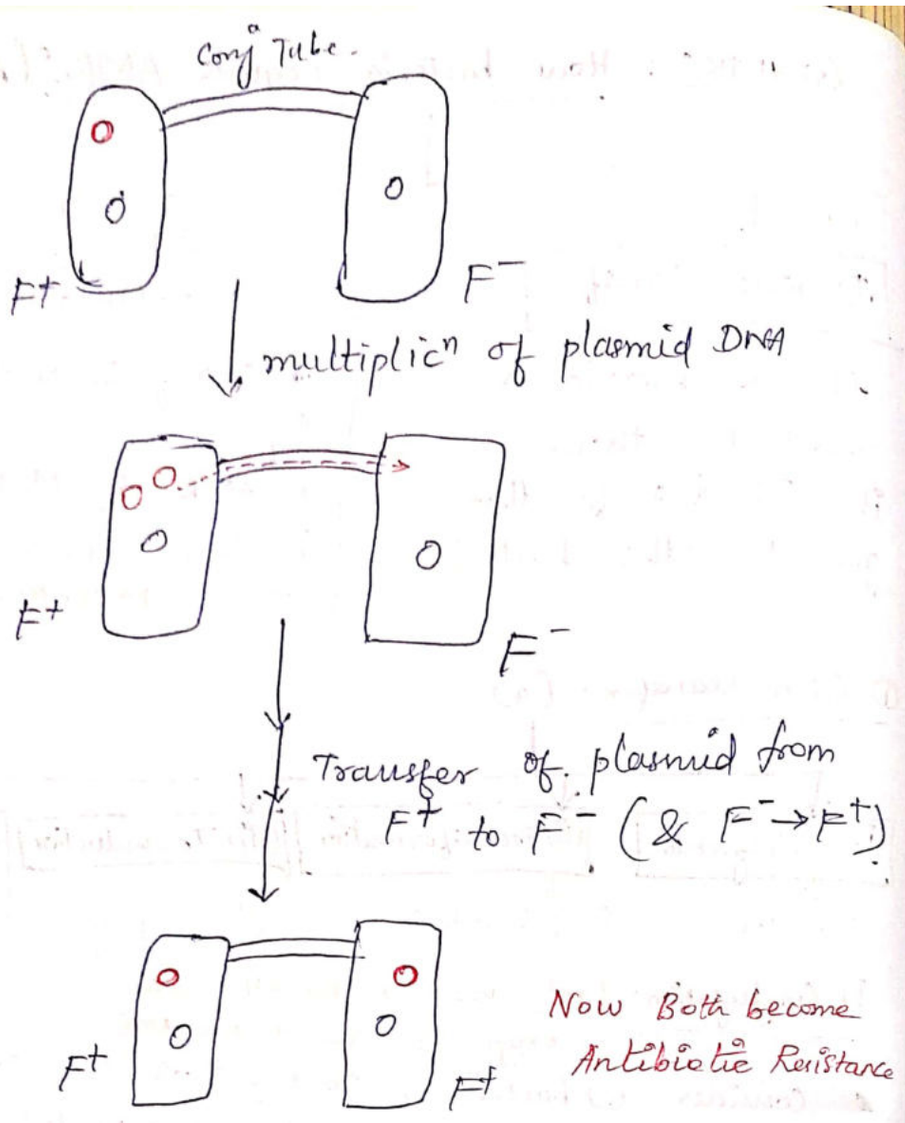
* Consider ② bacteria, among those ②, one contain plasmid DNA in addn to Nucleoid (Chrom. DNA).

↓
aka Fertility Factor (F-Factor)
↓
That bacteria is called $F^{\oplus}$ bacteria

* Resistant gene may be present in both Chrom. DNA (or) plasmid DNA. but m/c in plasmid DNA.

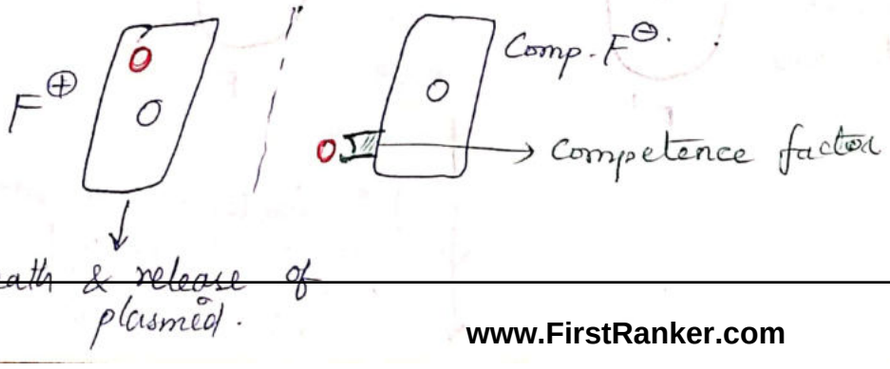


F^+ bacteria can make a communicn with F^- by means of conjugatn



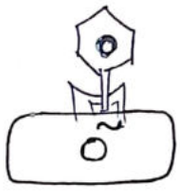
(ii) Transformation:

* Transfer of plasmid without any conj tube
* But this can't happen without death of $F^{\oplus}$ (due to some envir. changes)
* Free plasmid available in environment can be taken up by competent bacteria
* competent bacteria → can produce competence factor (Receptor for DNA).



(iii) Transduction: *** (m/c for Staphylococcus to acquire AMR).

* Transfer of gene/plasmid with the help of BACTERIOPHAGE (a virus that infects bacteria).



infec^{n} of bacteria by bacteria

① LYTIC cycle. (used for Multiplicat^{n}).

② Lysogenic / Temperate cycle.

* Step ①: Nucleic ā of bacteria direct

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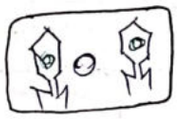
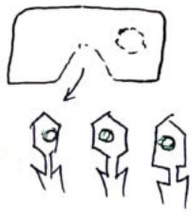
① Lytic cycle:

Step(i): Nucleic ā of bacterio phage directs the bacterial nucleic ā to produce more Nucleic ā, proteins of bacterio-phage.

Step(ii) Assembling of comp. of phage
The parts of phage gets assembled



Step(iii) Lysis of bacteria & Release of Bacteriophages (Daughter bacteriophages)

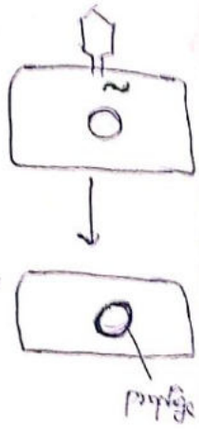


SIGNIFIGANCE OF LYTIC CYCLE

* Bacteriophage use Lytic cycle for their MULTIPLICATION

* Daughter phages contain Nucleic ā same as that of parent.

② Lysogenic / Temperate Cycle:



Step (i): Integ^n of Bacterial DNA & phage DNA
⇓
prophage (DNA) is formed.

Bacteria with prophage is called "Lysogenic bacteria"

Normal bacteria —lysogenic conversion→ Lysogenic bacteria

* Why lysogenic conversion is ⊗?
⇓
Bcoz many bacteria acquire virulence via this lysogenic conversion to produce "PHAGE MEDIATED TOXINS"

Eg:

- G +Ve
  - PVL Toxin (also Leucocidin; WBC lysis)
    - Panton Valentine toxin produced by lysogenic bacteria of Staph aureus. (PVL + γ Hemolysin) (Synergohymenotrophic Toxin).
  - Diphtheria Toxin
    - ⇓ Corynebact. Diphtheria
- G -ve
  - Cholera Toxin
    - ⇓ V. cholerae.
  - Verocyto-Toxin
    - ⇓ produced by EHEC. Entero Hemorrhagic E. coli

⇓
When these lysog. bact exposed to phy/chem. agents (UV)
⇓
Separat^n of Nucleic ā of bacteria & phage occurs
⇓
This is called INDUCTION — O —induction→ SO
⇓
Sometimes ~~bacteriophage~~ ~~takes~~ during induction, some of bacterial DNA gets separated along with phage DNA
⇓

* DNA of phage directs the bacteria to undergo LYTIC cycle.

↓

more phage DNA, proteins formed & Assembled

↓

Daughter Bacteriophages formed.
(But these phages contain some part of Nucleic a of bacteria also).

↓

lysis of bacteria & phages are released.

↓

These phages can infect other bacteria & if lysogenic/Temperate cycle takes place.

↓

Then previous bacterial DNA acquired by the phage During Induction is transferred to the New bacteria

⇓

Gene Transfer from one bacteria to other with the help of bacteriophage

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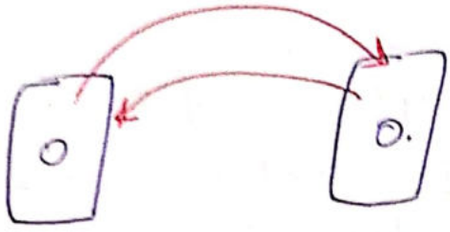
By this way Antibiotic Resistance "gene" can be Transferred from one bacteria to another with the help of phage.

(iv) TRANSPOSITION:

* Transposon aka Jumping genes are small part of DNA which can jump from one bacteria to another freely.



* If they Transposons contain Resistance gene then other bacteria will also become Antibiotic Resistant
* They dont have the ability to replicate by themselves bcoz they small part of DNA.

(v) Mutation:

* change in Nu.ā sequence.
* This change can randomly produce AMR. Sometimes
* Seen only in Mycobacteria. (No other bacteria eg. AMR by this method).