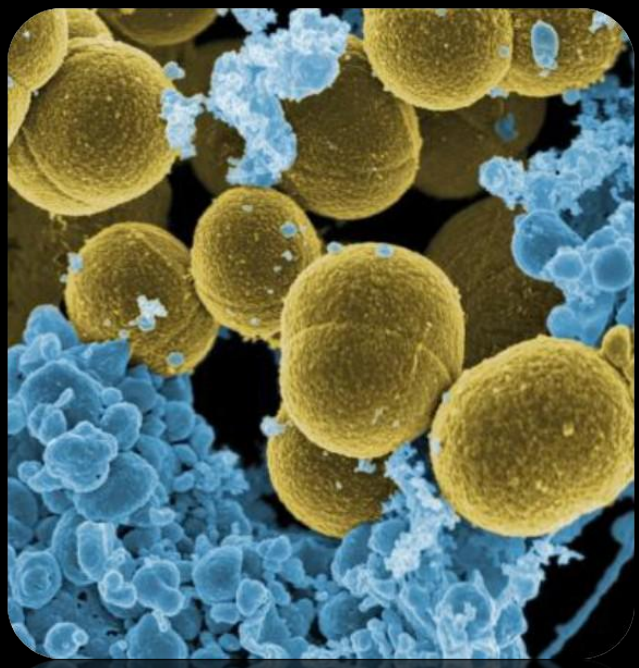
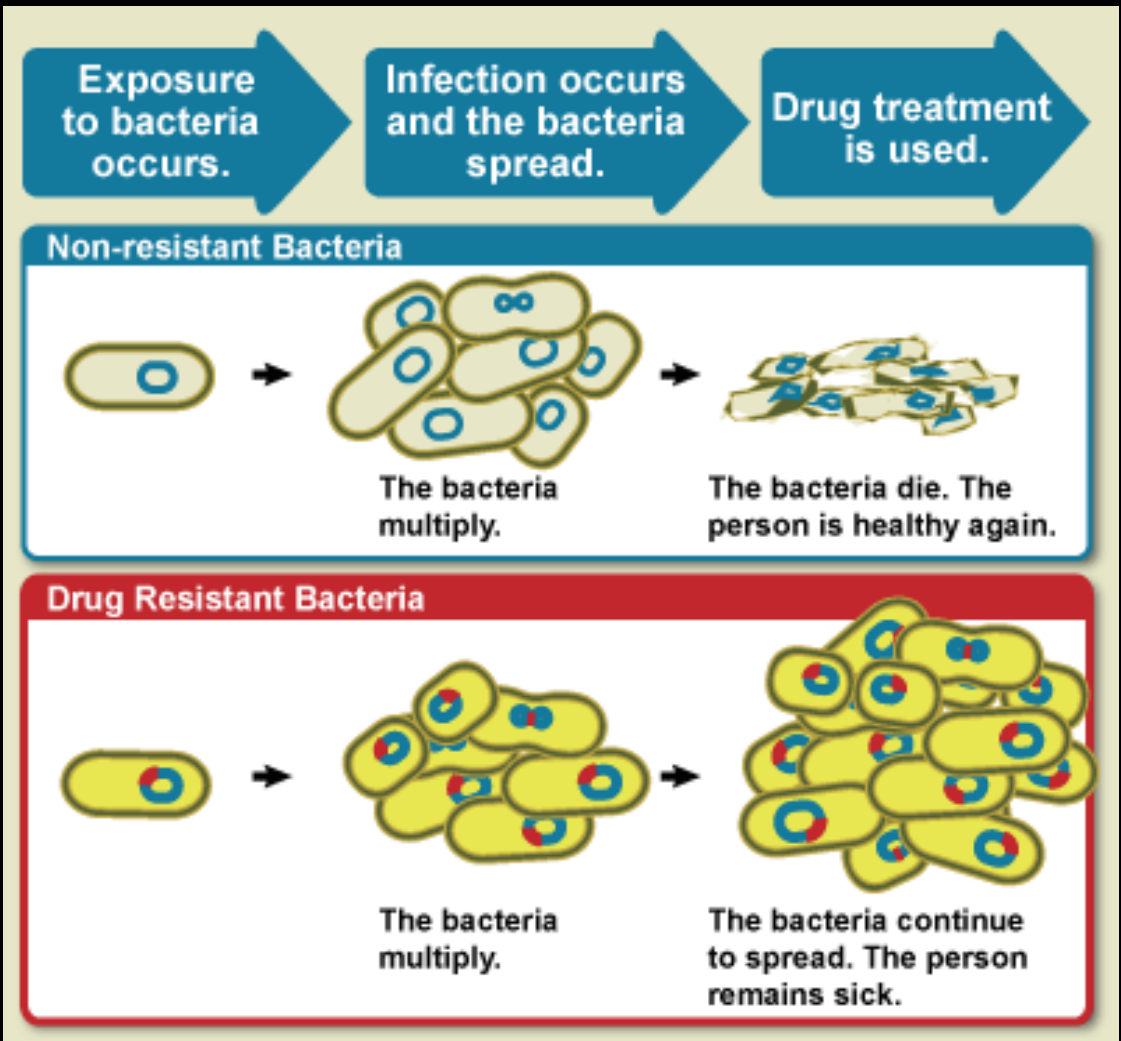
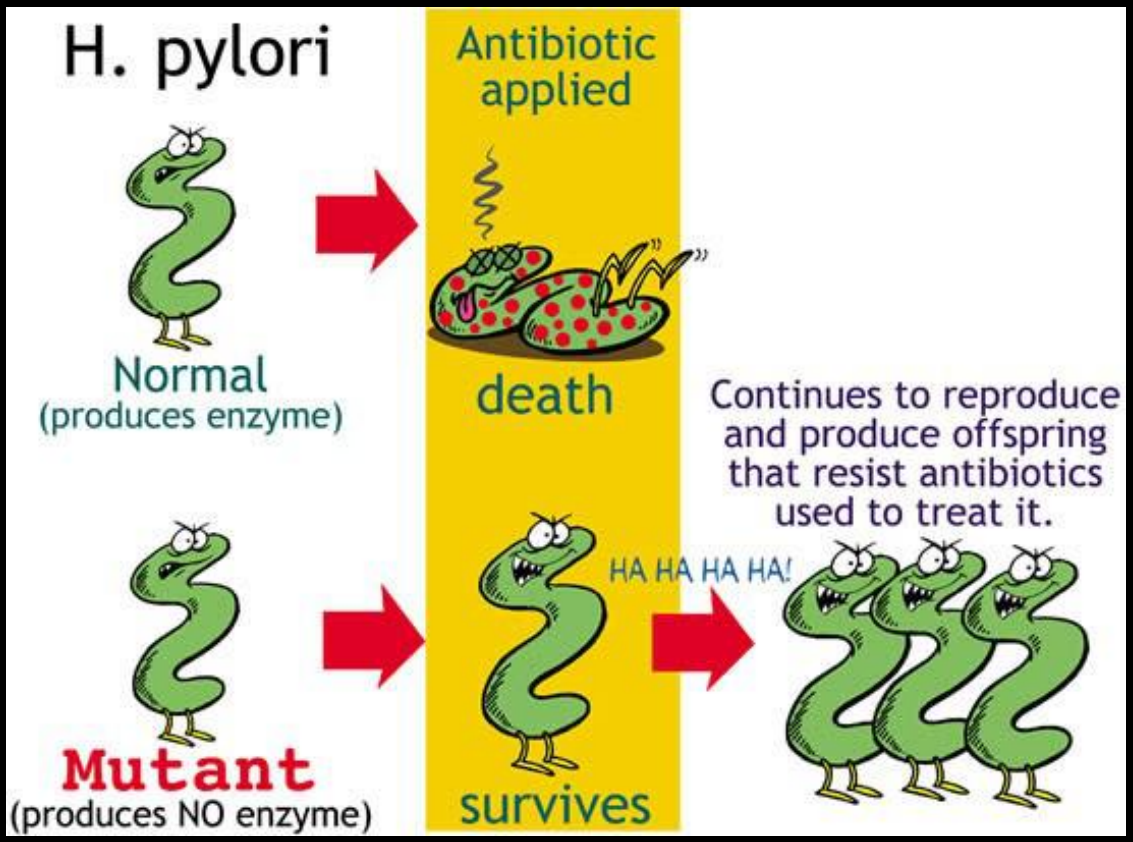
DEFINITION

- Drug resistance is the ability of microbes, such as bacteria, viruses, parasites, or fungi, to grow in the presence of a chemical (drug) that would normally kill it or limit its growth.

- It is the reduction in effectiveness of a drug in curing a disease or condition.




Exposure to bacteria occurs.
Infection occurs and the bacteria spread.
Drug treatment is used.
Non-resistant Bacteria
The bacteria multiply.
The bacteria die. The person is healthy again.
Drug Resistant Bacteria
The bacteria multiply.
The bacteria continue to spread. The person remains sick.

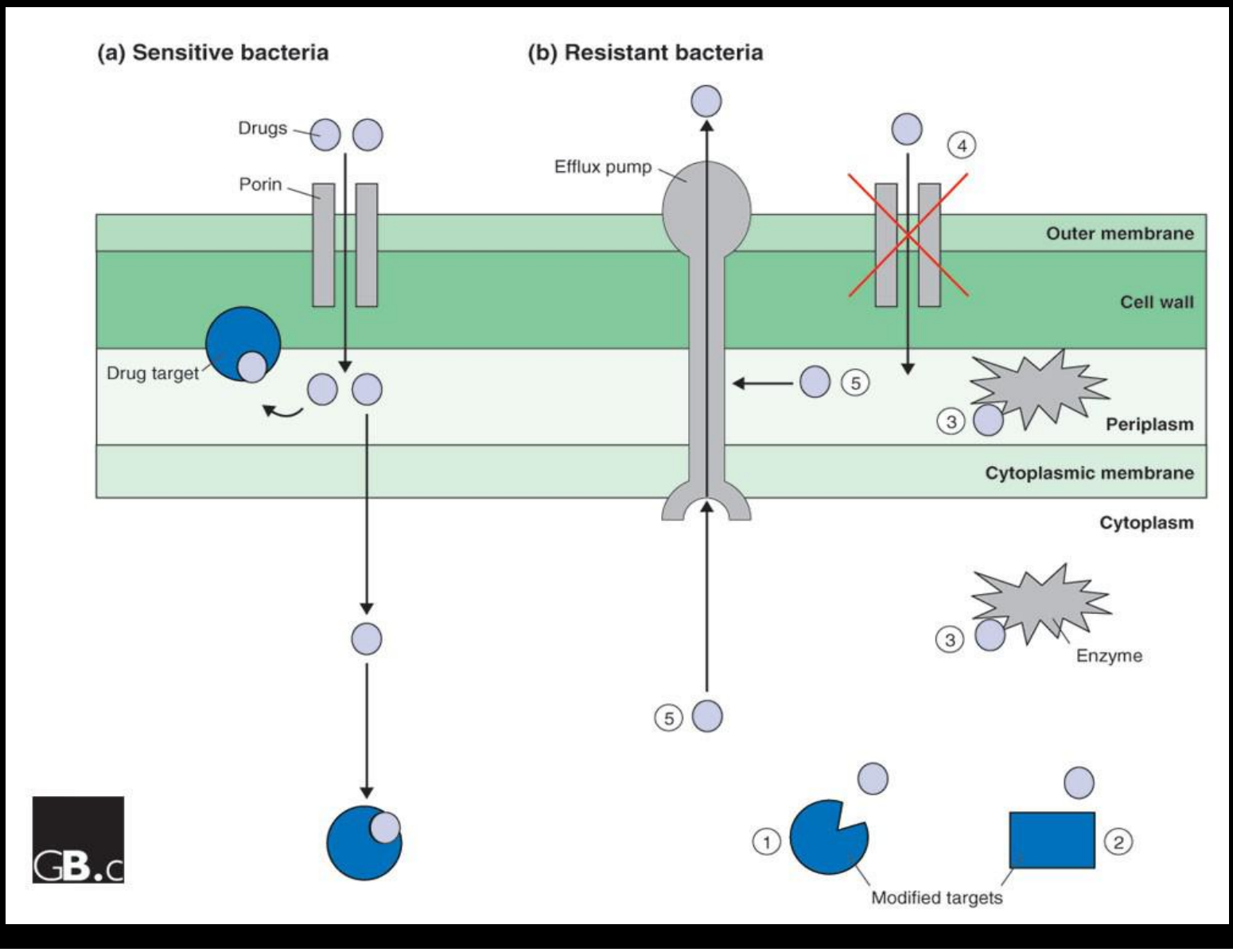

H. pylori
Antibiotic applied
Normal
(produces enzyme)
death
Continues to reproduce and produce offspring that resist antibiotics used to treat it.
HA HA HA HA!
Mutant
(produces NO enzyme)
survives

MECHANISMS OF DRUG RESISTANCE

1. Production of enzymes that destroy/modify the active drug.

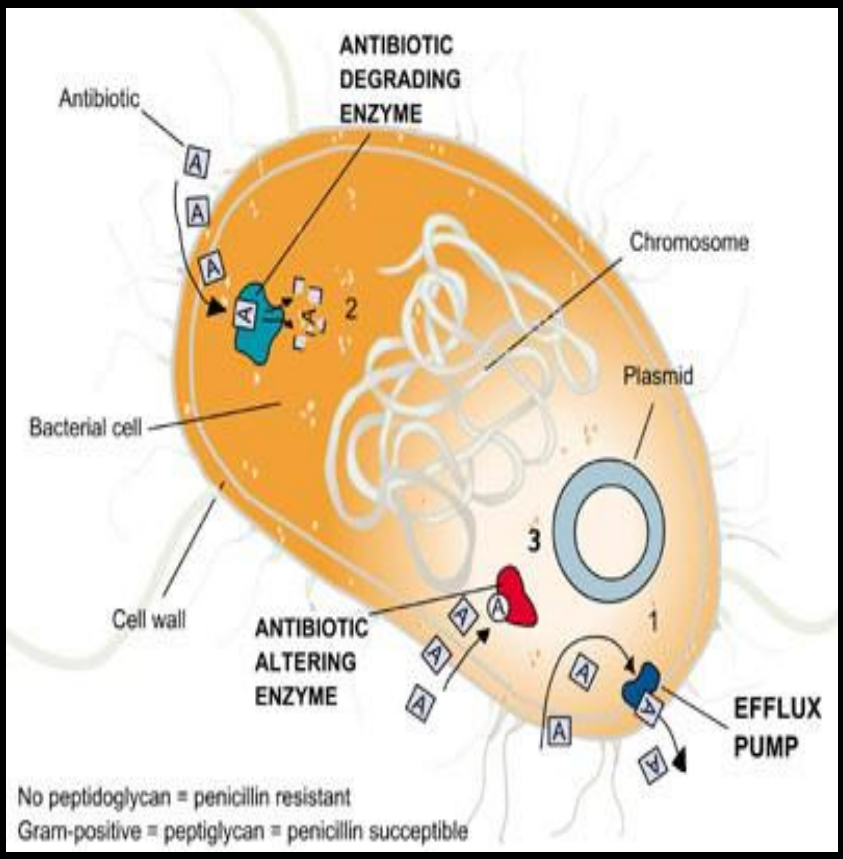
2. Synthesis of an altered target site against which the drug has no effect.

3. Reducing drug accumulation through:
   a) **Decreasing the permeability** of cell membrane.
   b) **Actively exporting drugs** through **M**ulti **D**rug **R**esistant pump ('MDR' OR **EFFLUX** pump).

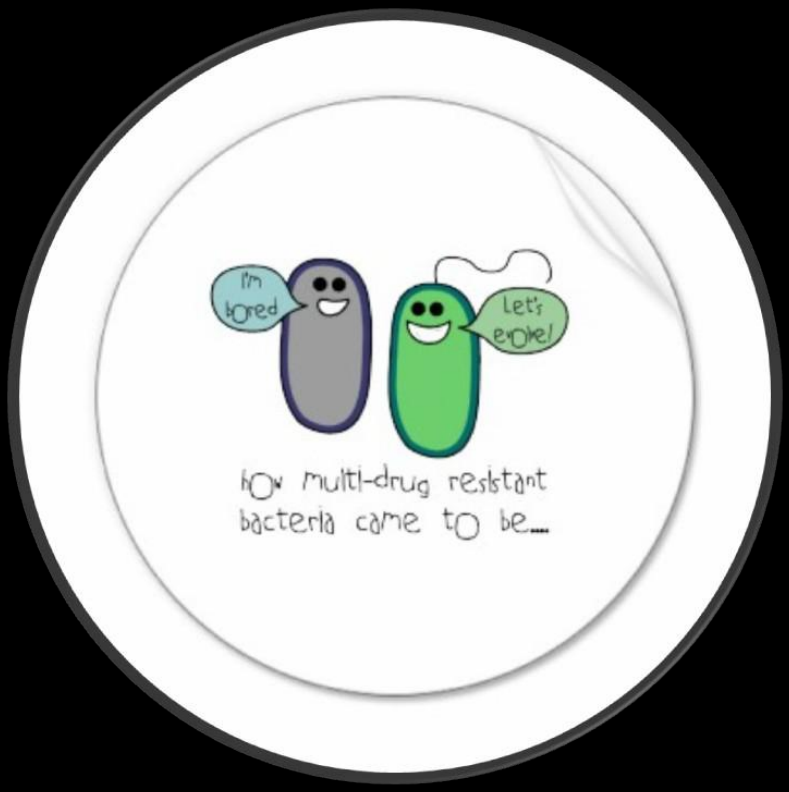

(a) Sensitive bacteria
(b) Resistant bacteria
Drugs
Porin
Efflux pump
4
Outer membrane
Cell wall
Drug target
5
3
Periplasm
Cytoplasmic membrane
Cytoplasm
3
Enzyme
5
1
2
Modified targets
GB.C

4. Altering the metabolic pathway so that the reaction inhibited by the drug can be bypassed.

5. Developing an altered enzyme that is less inhibited by the drug but can still perform its metabolic function.

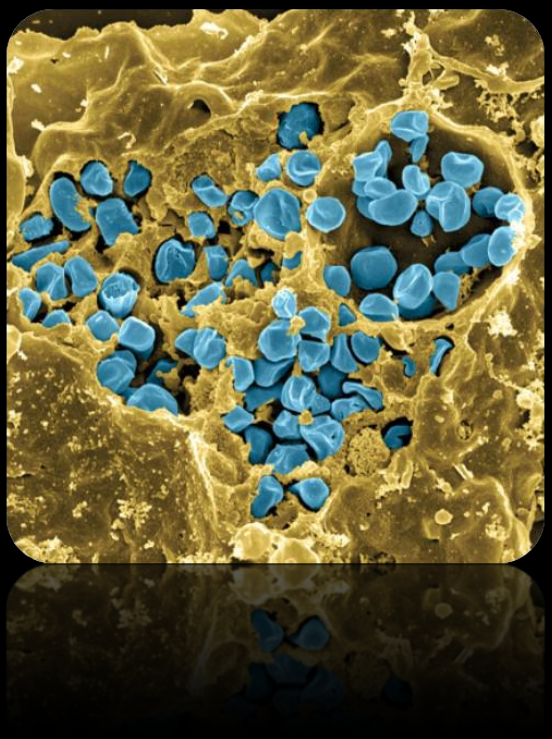


TYPES OF DRUG RESISTANCE



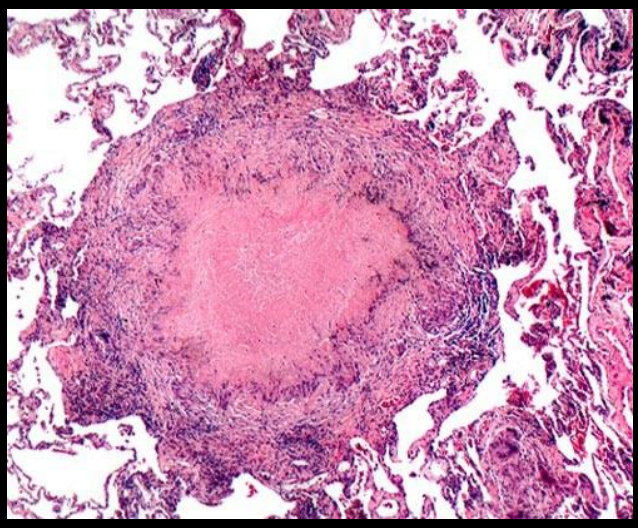
PRIMARY/NATURAL/NON GENETIC ORIGIN OF RESISTANCE:

Bacteria possess an innate property to resist drug.

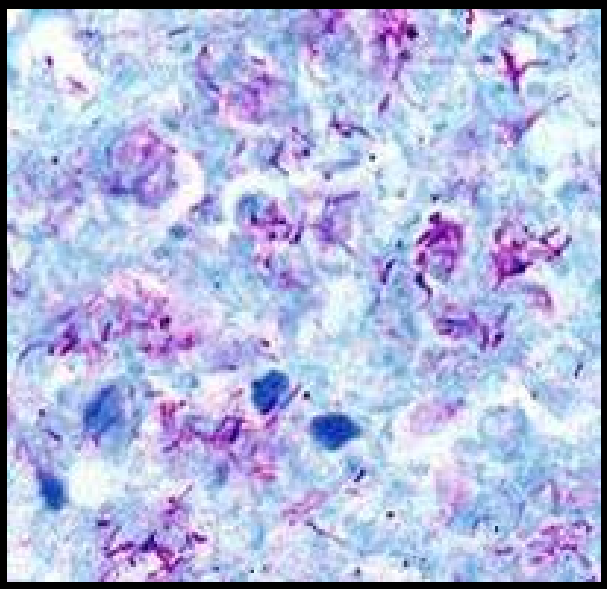


- EXAMPLES
- The bacteria may infect host at sites where drugs are inaccessible or not active seen in Salmonellae.
- The cell wall may be covered with an outer membrane that establishes a permeability barrier against the antibiotic as seen in Gram negative bacteria.

- Bacteria may remain in dormant resting state without multiplying and become phenotypically resistant to drugs as seen in M. tuberculosis.



- Micro-organisms may lose the specific target structure for a drug for several generations and become resistant.

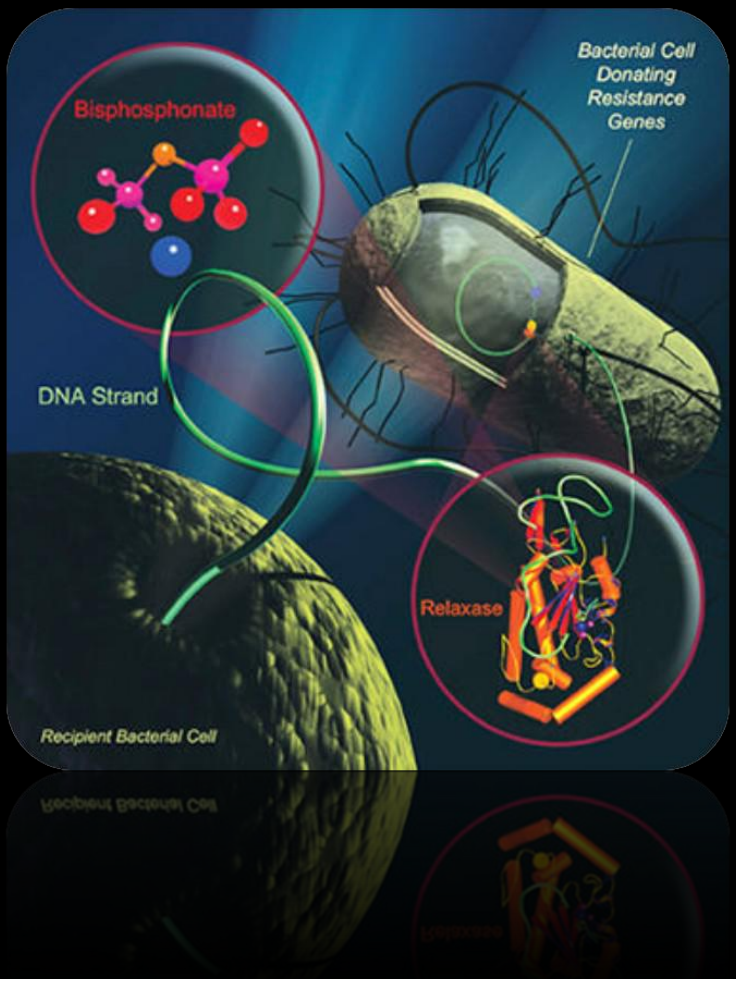


An acid fast stain (Ziehl-Neelsen) shows numerous mycobacterium bacilli.

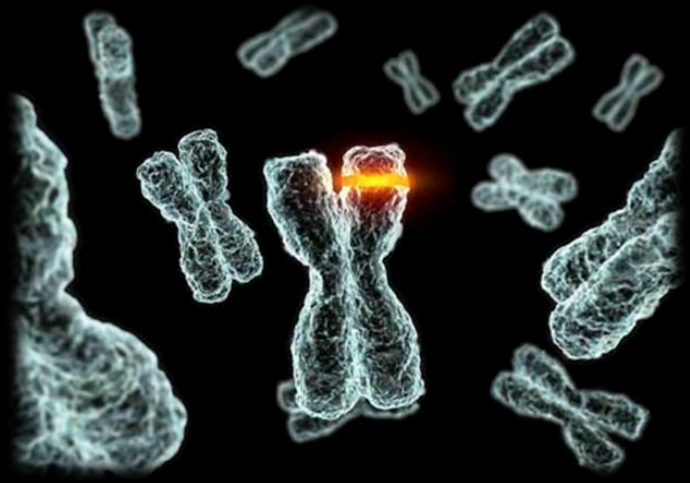
ACQUIRED/GENETIC ORIGIN OF DRUG RESISTANCE

Bacteria acquire/develop resistance to antibiotics either through the modification of existing genetic material **(mutation)** or the acquisition of new genetic material from another source **(plasmid/gene transfer).**

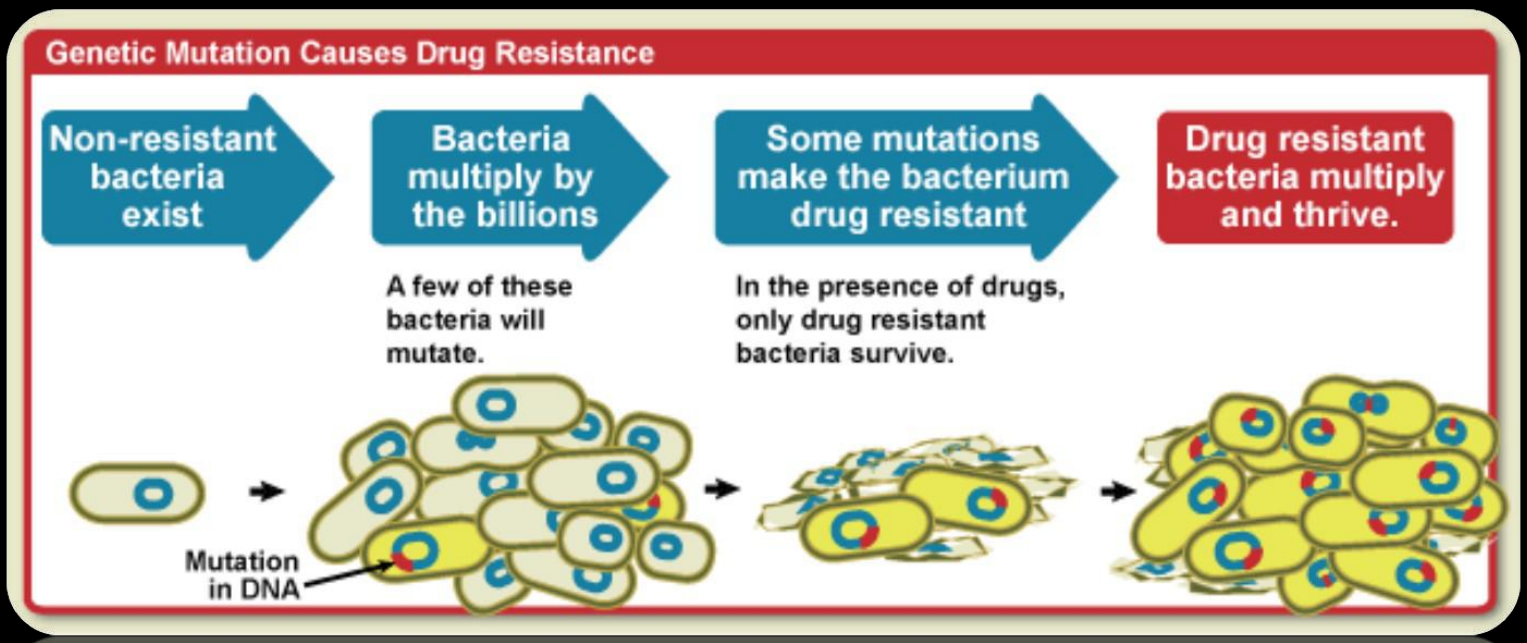
Further classified into:

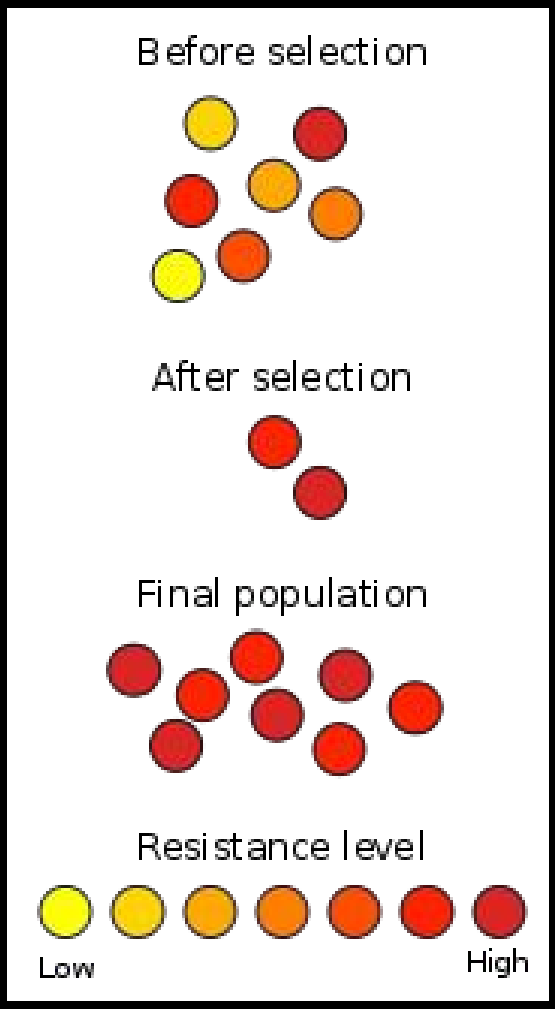


1. CHROMOSOME MEDIATED RESISTANCE:

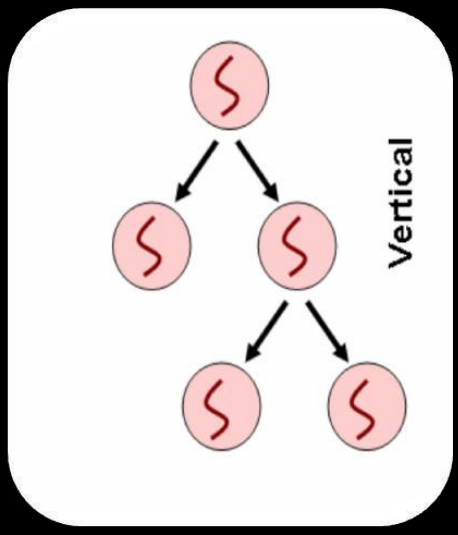


- Resistance acquired due to **spontaneous mutation** of gene that controls the susceptibility to a given antimicrobial drug.
- **Structurally alters the target** of the drug or the **transport system** that controls the uptake of the drug.
- 2 types:
  - **Stepwise mutation:** Penicillin
  - **One step mutation:** Streptomycin


Genetic Mutation Causes Drug Resistance
Non-resistant bacteria exist
Bacteria multiply by the billions
Some mutations make the bacterium drug resistant
Drug resistant bacteria multiply and thrive.
A few of these bacteria will mutate.
In the presence of drugs, only drug resistant bacteria survive.
Mutation in DNA



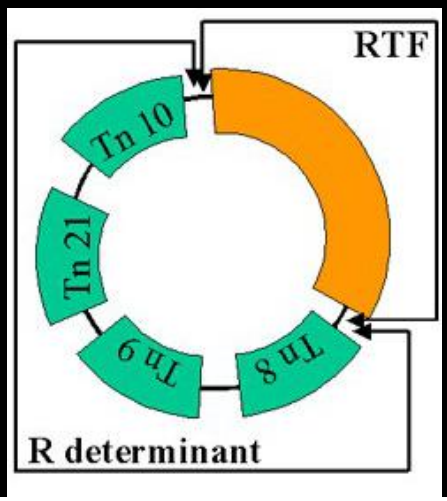
- **Vertical gene transfer** of resistant genes to progeny.
- Growth of resistant mutants.
- Seen in **M. tuberculosis** and streptomycin.

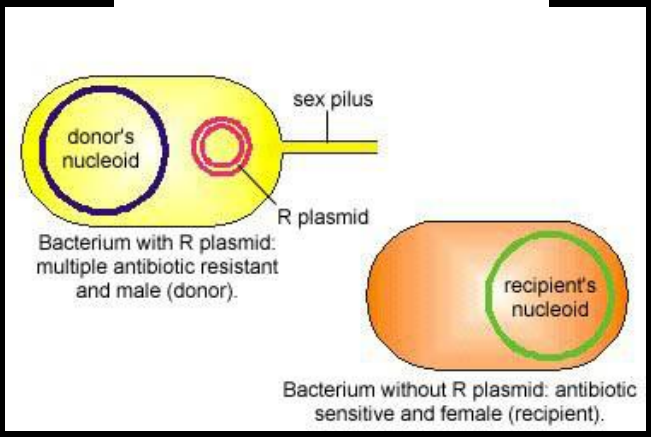


2. TRANSFERABLE DRUG RESISTANCE

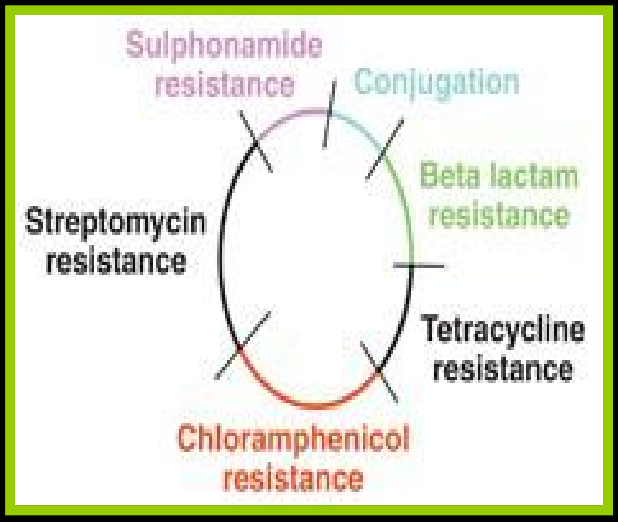
A. PLASMID MEDIATED RESISTANCE:

- Resistance acquired through the transfer of extrachromosomal resistance plasmids(R factors)
- R factor = RTF (Resistance Transfer Factor) + r determinant
- Main features:
  - Frequency of resistance transfer is high.
  - Resistance transfer can occur to cells of different species



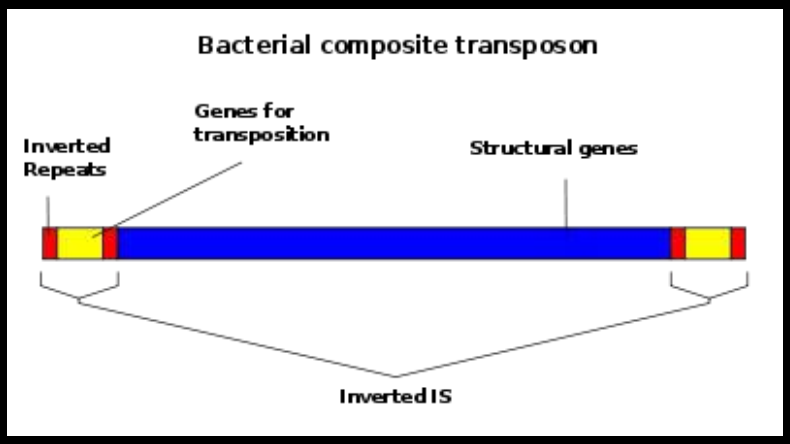


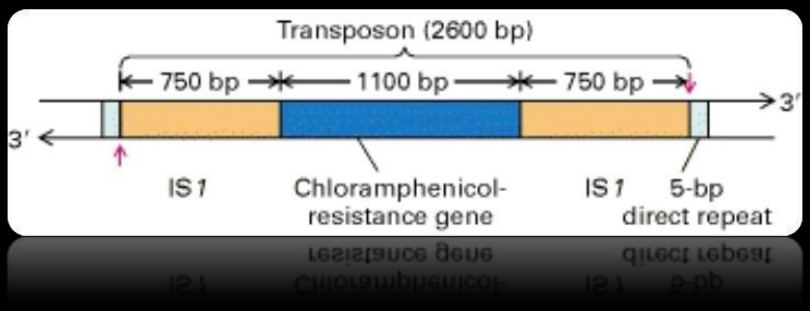
- Plasmids can mediate resistance to multiple drugs.
- R factors provide resistance to metal ions and bacterial viruses/bacteriophages.
- R factors **code for enzymes** causing inactivation of drug.

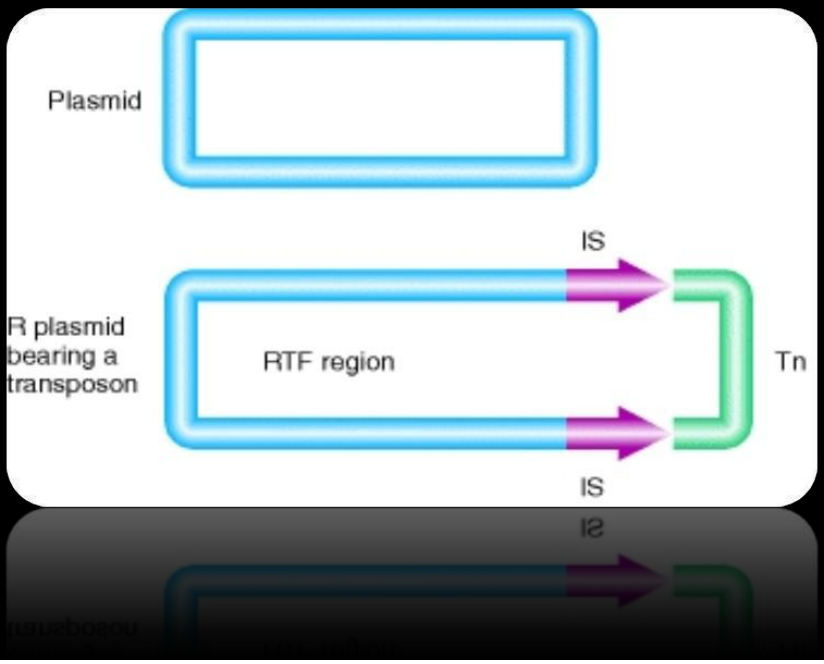


b) TRANSPOSON MEDIATED RESISTANCE:

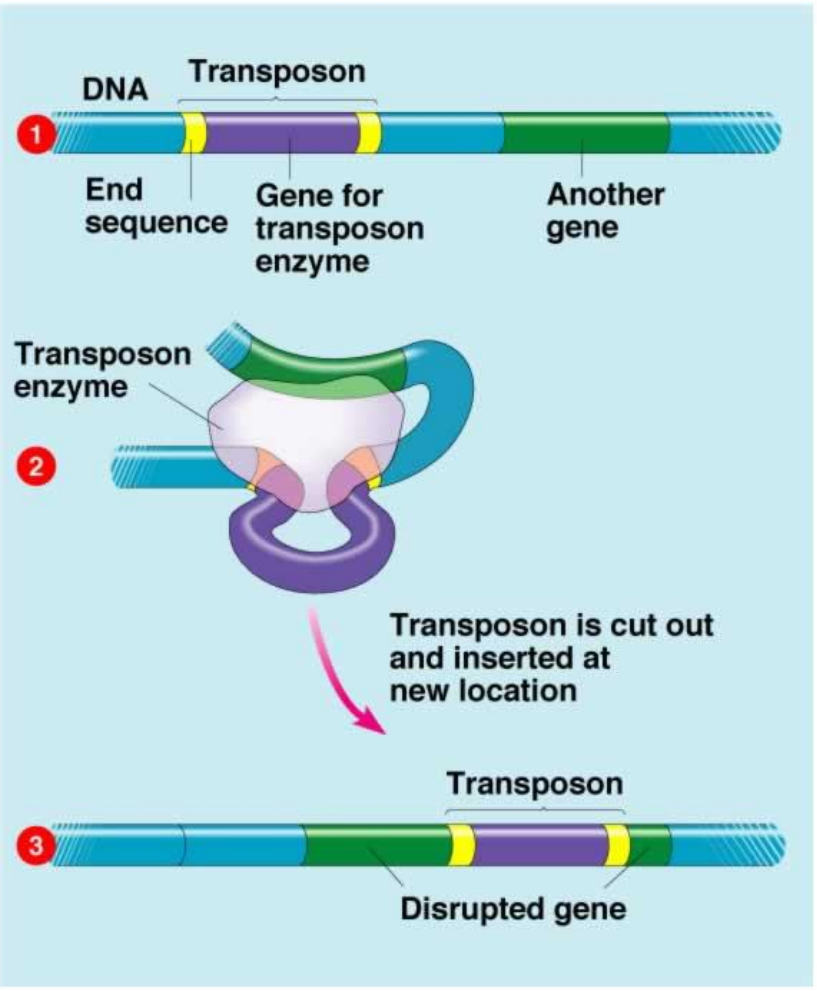
- Transposons are genes/segments of DNA that are transferred within themselves or between chromosomes and extrachromosomal plasmids.







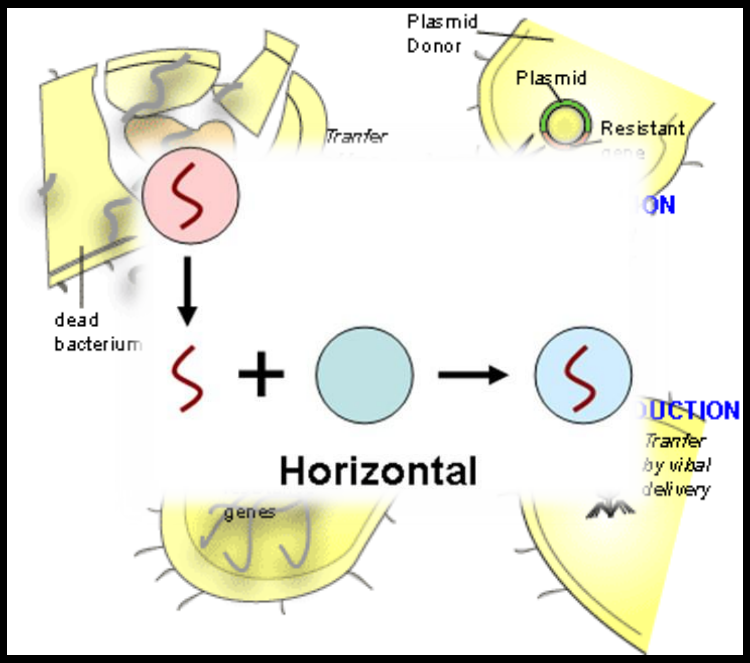
- They are also known as **jumping genes** and this mode of genetic transfer as **transposition.**
- Transposons attach themselves to chromosomal, plasmid or phage DNA molecule and confer resistance to drugs under suitable environmental conditions.
- Transposons are not self replicating.
- R determinant segments of R Factors are said to be collections of Transposons.



Transposition

METHODS OF TRANSFER OF RESISTANCE:

- **Horizontal gene transfer** (HGT) is a process whereby genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species.
  a) Conjugation
  b) Transduction
  c) Transformation
- Transposition



ANTIBIOTIC SENSITIVITY TESTS

- **Antibiotic sensitivity** is a term used to describe the susceptibility of Bacteria to antibiotics.

- Uses:
  - **To determine the potency of drug in solution and sensitivity of organism to drug. on ,its concentration in body fluids**
  - **Choosing the right drug.**
  - To determine the **Inhibitory Concentration].MIC [Minimum**
  - To determine the **MBC [Minimum Bactericidal Concentration].**

DISC DIFFUSION METHODS

1.KIRBY-BAUER METHOD

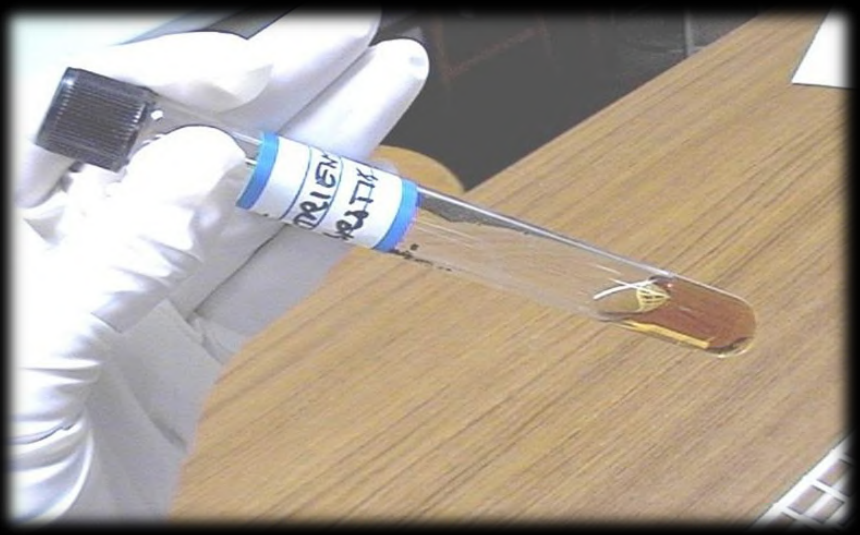
- It is a means of measuring the effect of an antimicrobial against bacteria grown in culture using antibiotic impregnated filter paper discs.

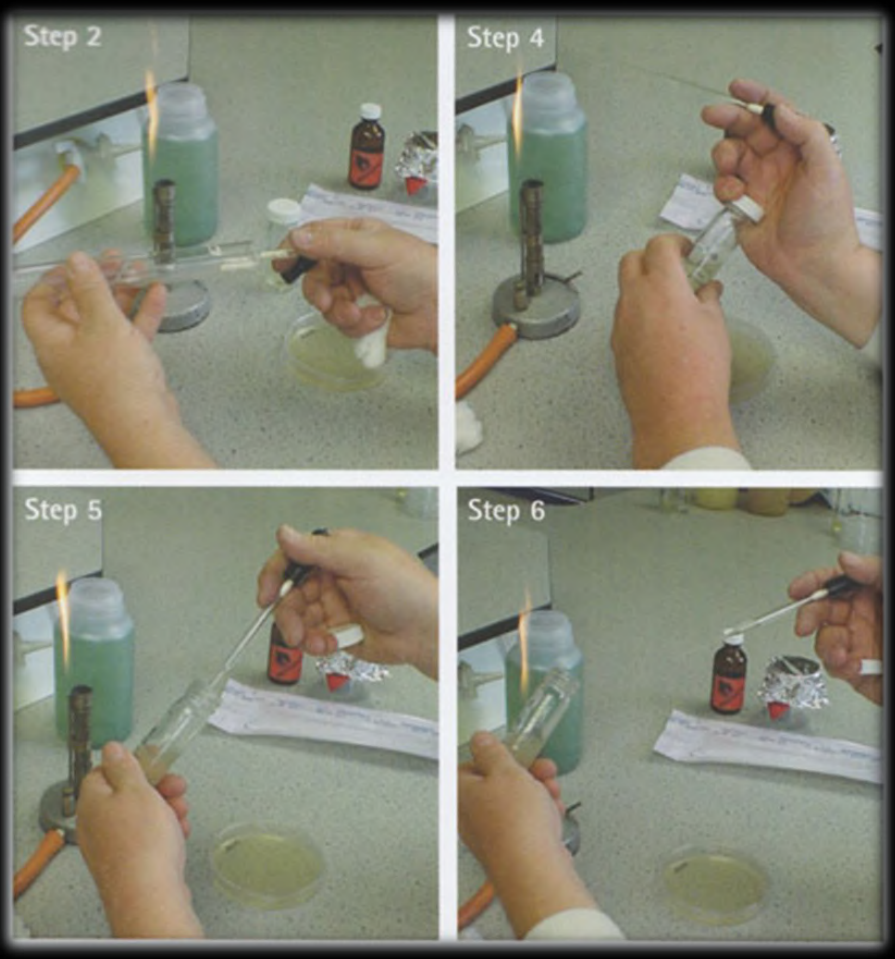
PROCEDURE

- Filter paper discs about 6mm in diameter are charged with appropriate concentrations of drugs, dried in the incubator and stored in the refrigerator.

- The test bacterium is isolated from its culture plate

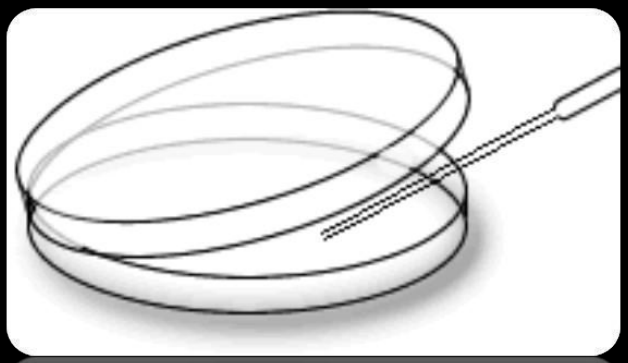
- A liquid culture of the test bacterium in a suitable broth is prepared in a test tube.





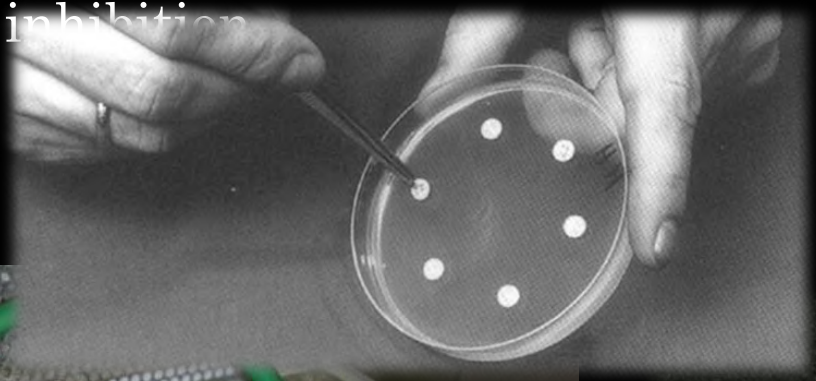
- This is then poured on to a suitable solid agar medium (nutrient/Mueller-Hinton) in a Petri-dish which is tilted to ensure uniform spreading.

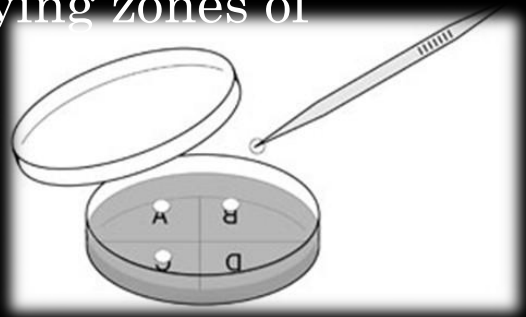
- Excess broth is pipetted off.

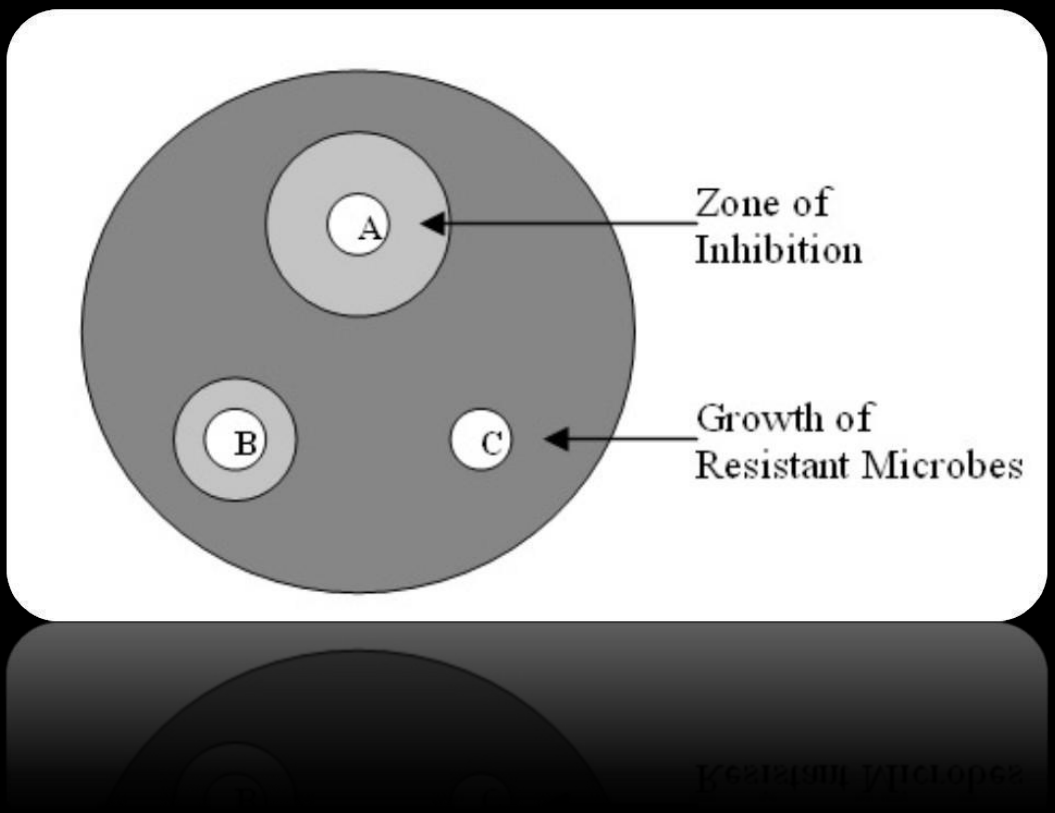


- Alternately, a sterilized cotton swab may be dipped in the liquid bacterial suspension and streaked across the solid agar medium in different angles to ensure uniformity.

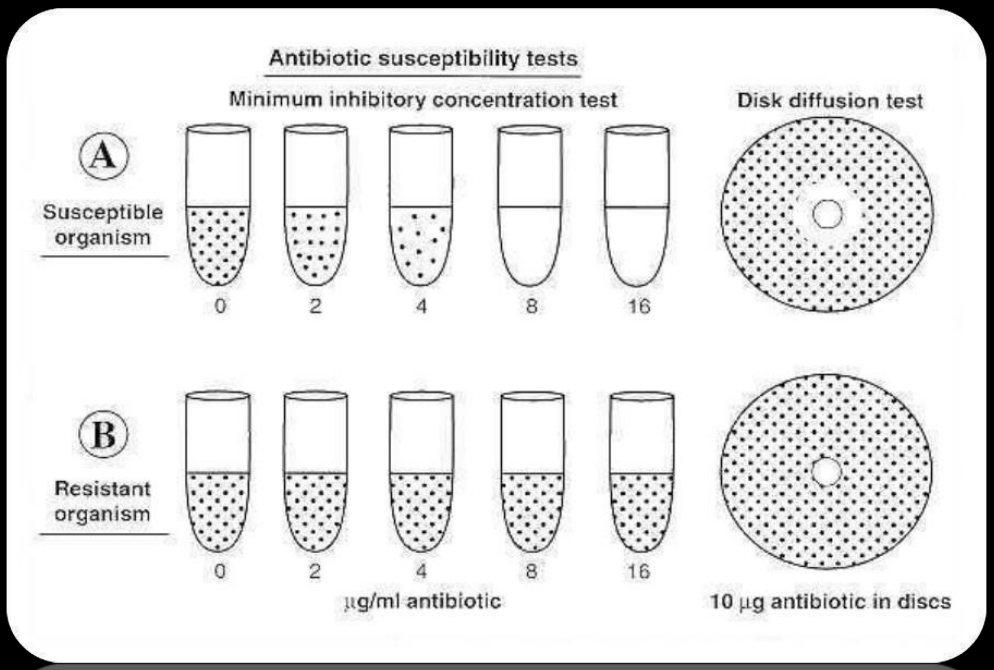
- The plate is dried at 37 degrees Celsius for 30 minutes.
- The antibiotic filter paper discs(4/5 per 10cm dish) are placed with sterile forceps and incubated overnight.
- A ‘lawn’ culture develops with varying zones of inhibition.

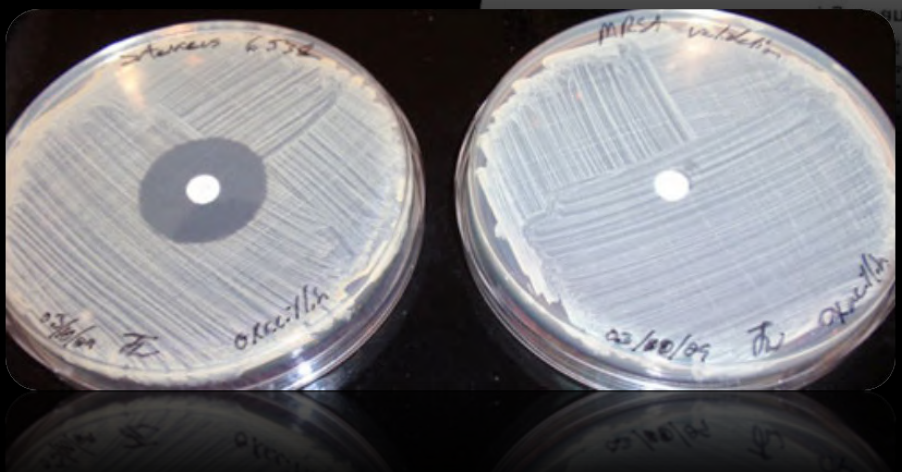





A
B
C
Zone of
Inhibition
Growth of
Resistant Microbes

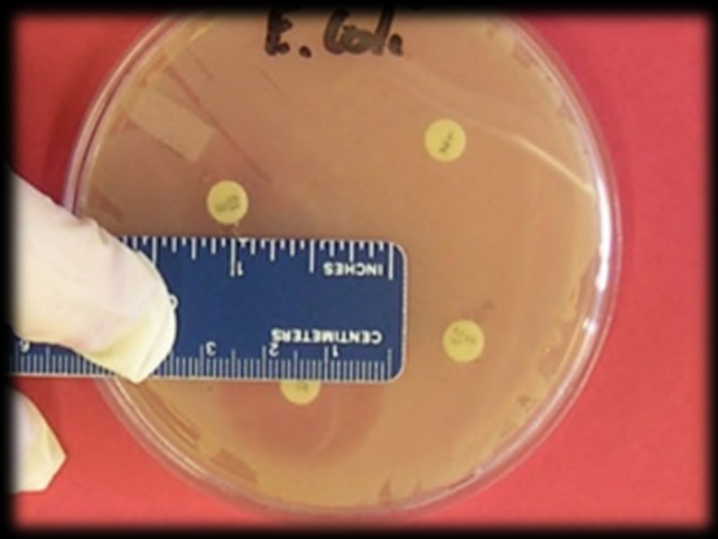
- If the bacterium is susceptible to the drug, growth is inhibited , but if it is resistant , no zone of inhibition will be seen.

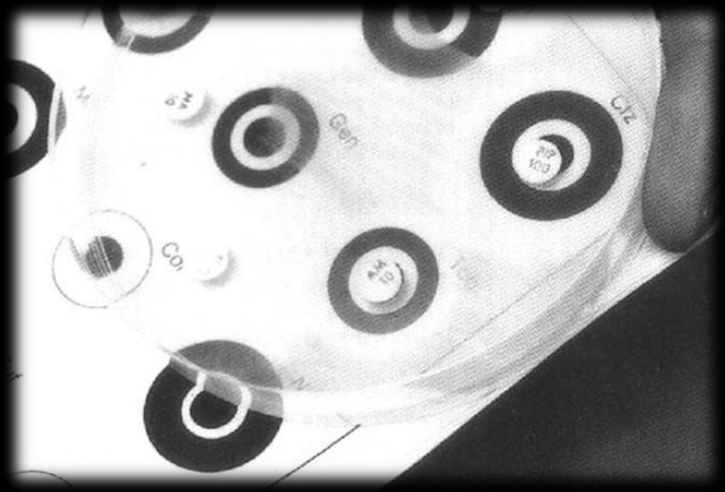




- The Petri-dish is inverted with the lid placed and the zones of inhibition are measured using a ruler or template and compared with the critical diameters for a given bacteria and antibiotic.

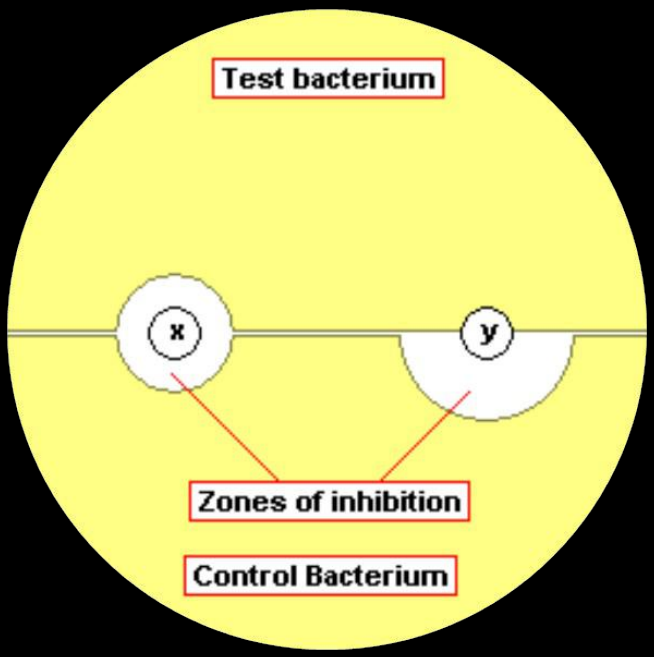
- The results are reported as ‘sensitive’, ‘intermediate’ or ‘resistant’.





STOKE'S METHOD

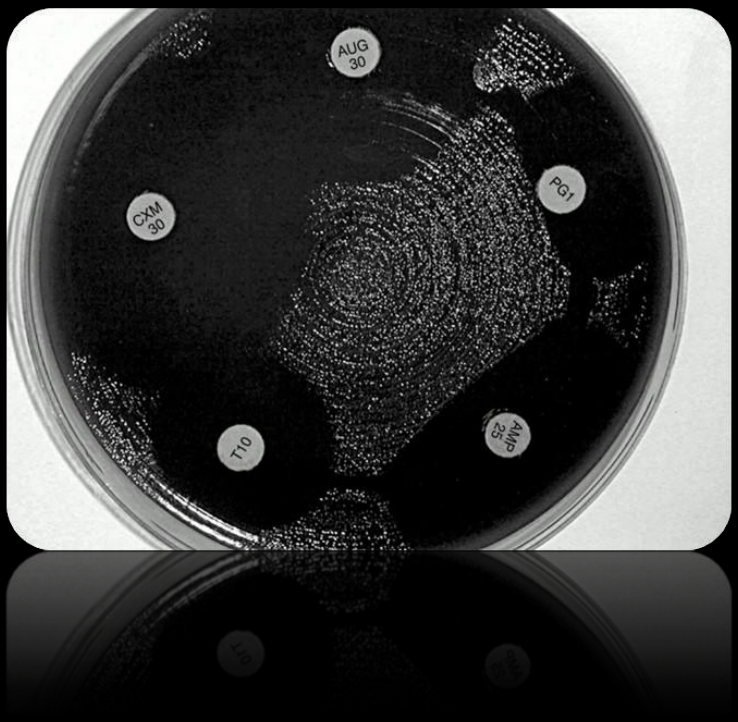
- Similar to the Kirby-Bauer method, however a control organism is used.
- The control organisms used are:
  - Staph. Aureus ATCC 25923
  - E. coli ATCC 25922
  - Ps. aeruginosa ATCC 27853
- A standard sensitive control organism is inoculated on one side of the plate.
- The test bacterium is inoculated on the other side of the plate.



- Antibiotic discs are placed at the junction of the two layers.
- Comparison of the zones of inhibition indicates the susceptibility of the test bacterium with respect to the Std. bacterium

Advantage

- The use of a sensitive control shows that the antibiotic is active, so that if the test organism grows up to the disk it may safely be assumed that the test organism is resistant to that drug.



- **Primary disc diffusion test:**
  - Urine or fluid specimen is directly swabbed or inoculated on the solid medium
  - For faster detection.

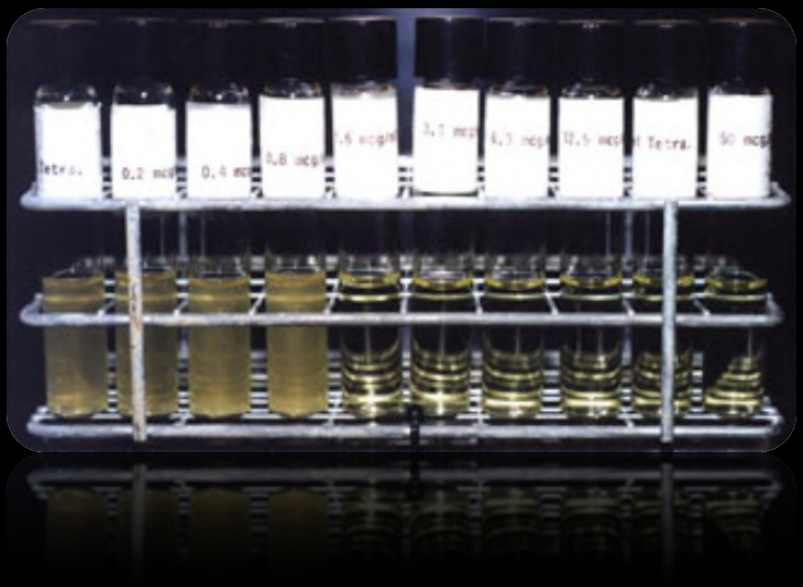
3.E-TEST

- E test is the **Epsilometer** test .
- Mainly used to measure the MIC.
- Similar to Kirby-Bauer method but uses an thin inert plastic strip impregnated with a known gradient of varying drug concentrations along its length.
- Here, **a symmetrical inhibition ellipse** is produced.
- The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value.

ADVANTAGE:

- It is faster, precise and accurate.
- Results are read directly on the strip where the zone of inhibition intersects with the strip.

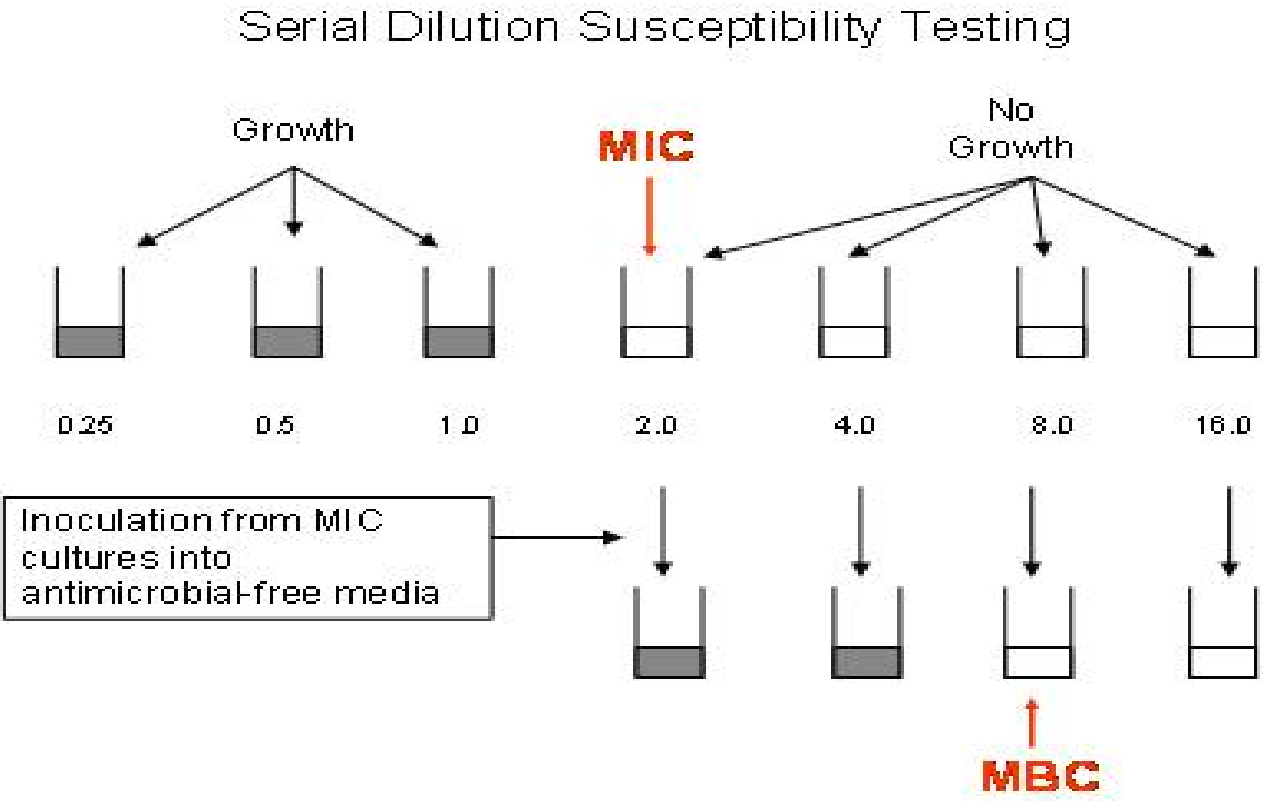
DILUTION TESTS



TUBE DILUTION METHOD

- Here serial dilutions of the drug in broth are inoculated with test bacterium isolated from patient and incubated at 35 degree Celsius for 18 hours (overnight).
- It is used to determine the MIC and MBC.
- This is a laborious procedure and time consuming.

PROCEDURE


Serial Dilution Susceptibility Testing
Growth
MIC
No Growth
0.25
0.5
1.0
2.0
4.0
8.0
16.0
Inoculation from MIC cultures into antimicrobial-free media
MBC

AGAR DILUTION METHOD

- Similar to tube dilution method.
- Here serial dilutions of antibiotics are prepared on agar, and poured into plates.
- Advantage:
  - Several strains of bacteria can be inoculated for each plate of antibiotic dilution.

ANTIBIOTIC ASSAY

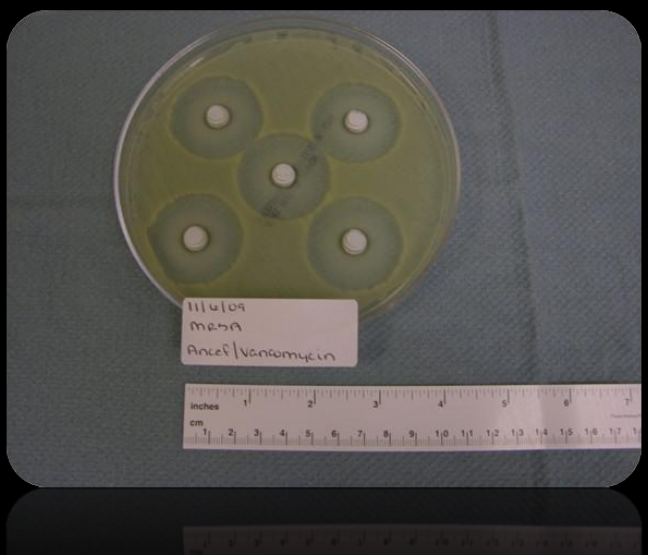
- It measures the amount of antibiotic in the blood or other body fluids of patients

- Assay is done:
  1. To ensure adequate therapeutic concentrations are reached in serious infections.
  2. To avoid toxic concentration in blood.

- Patients blood and body fluid are collected before and after administration of dose of a particular drug.
- The trough and peak levels of drug are estimated by 2 ways:
  - Bioassay
  - Immunochemical assay

1.BIOASSAY

- In an agar media, small wells are cut out.
- The agar media is inoculated with suitable bacterial culture.
- Serum or body fluid before and after drug administration is placed in the well and incubated overnight at 37 degree Celsius
- The zone of inhibition produced by serum or fluid is compared with the standard concentration of the drug.
- The level of drug in the serum is calculated.

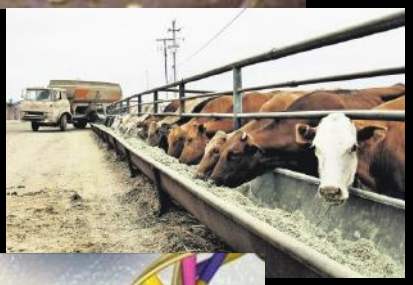


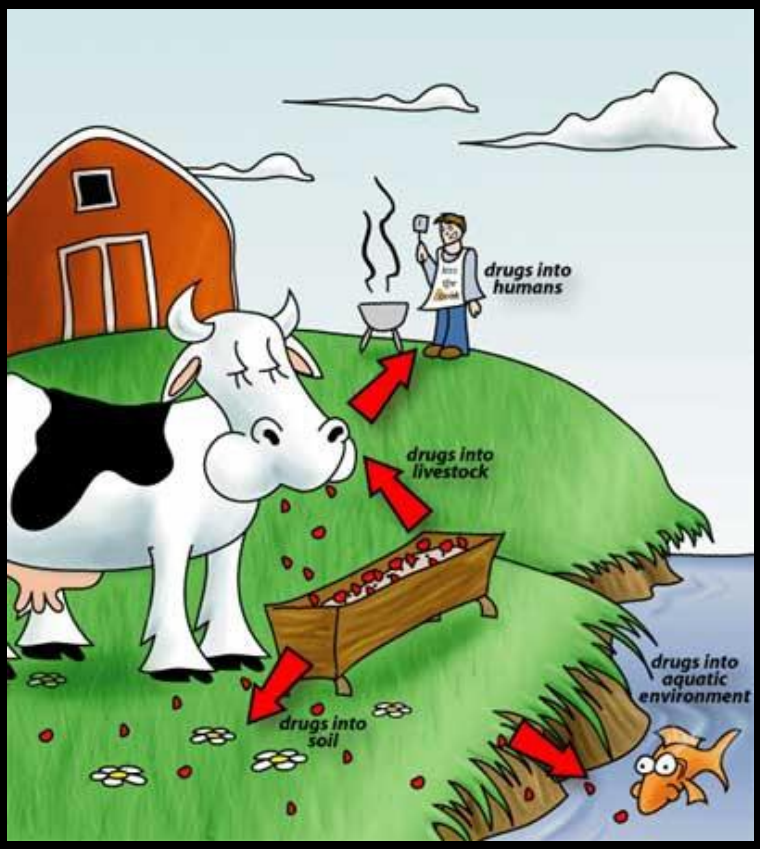
2.IMMUNOCHEMICAL ASSAY

- Assay is made by computerized equipment with commercially available kits for drugs like amino glycosides etc
- Rapid and accurate but available only in limited centers.

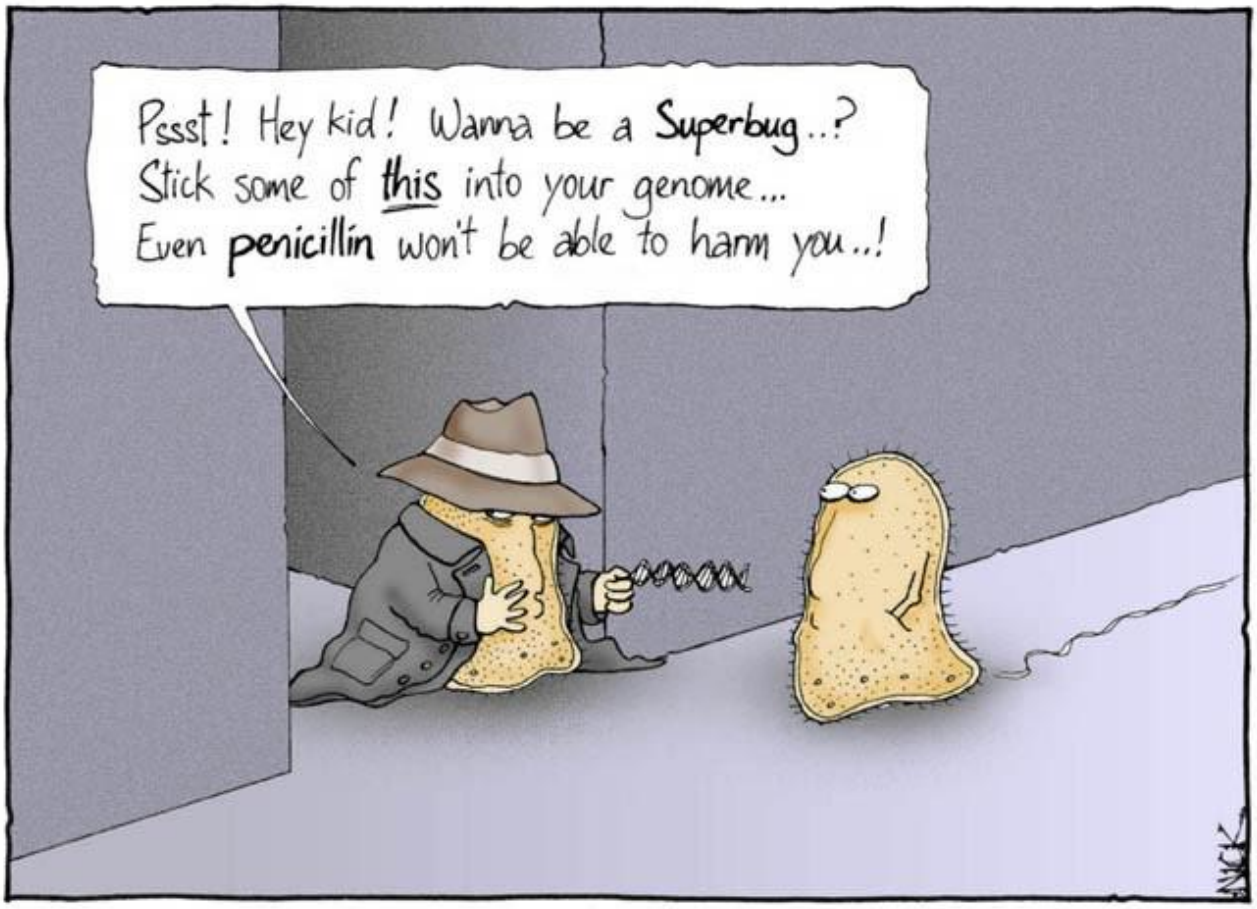
PROMOTERS OF DRUG RESISTANCE

- **Indiscriminate use of antibiotics in**
  a) Agriculture and veterinary practice which can accumulate in food and water.
  b) Genetically modified crops.
  c) Hospital environment and infections.
  d) Inappropriate selling of antibiotics over the counter to the general public .




drugs into humans
drugs into livestock
drugs into soil
drugs into aquatic environment


The problem beyond the peanuts.
Feeding human antibiotics to hogs makes salmonella harder to cure.



It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

PREVENTION OF DRUG RESISTANCE

- Patients must stop taking antibiotics for self limiting infections.
- Doctors have to **stop giving unnecessary antibiotic prescriptions.**
- Patients must **follow and complete antibiotic prescriptions.**
- Using the **right antibiotic** determined by antibiotic sensitivity testing.
- **Stop** the use of antibiotics as **growth-promoting substances** in farm animals.

CONTROL

Search for new antibiotics.

Biotechnology and pharmaceutical companies must constantly research, develop and test new antimicrobials in order to maintain a pool of effective drugs in the market against the rise of resistant bacteria.