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3rd. Year

Module: Microbiological techniques

Semester: 6

Session : 3 (Practical)

Lecture Title: Antibiotic susceptibility MIC

Module staf

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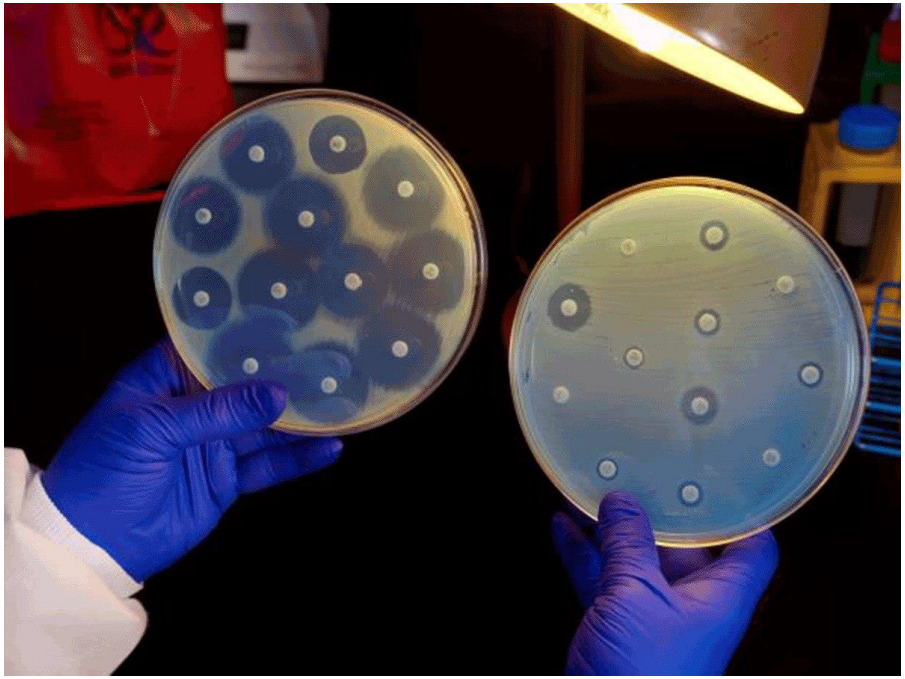
Introduction:

Antimicrobial susceptibility testing (AST) is a laboratory procedure performed by medical technologists (clinical laboratory scientists) to identify which antimicrobial regimen is specifically effective for individual.



Principle and Uses of Antibiotic Sensitivity Test

Principle:

- It is an antimicrobial susceptibility profile of a specific bacteria to a wide range of antimicrobial drugs in vitro.
- We transfer the battle between bacteria and antibiotics from the human body (in vivo) to a plate (in vitro).

Uses:

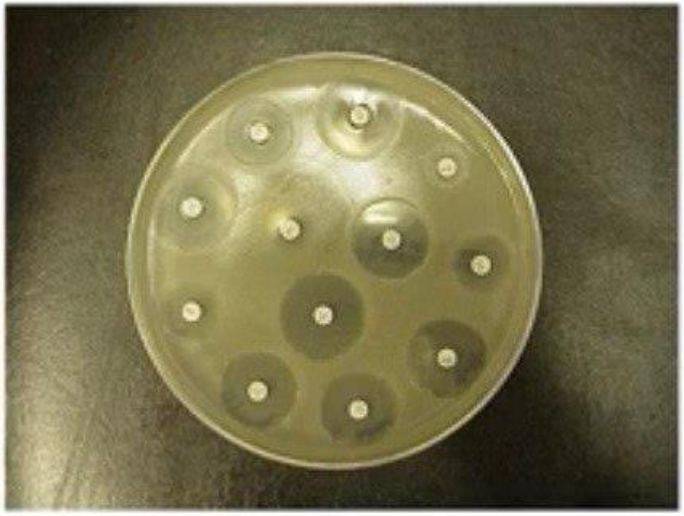
- It can help to find out which antibiotic will be most effective in treating bacterial infection.
- You may need this test if you have an infection that have antibiotic resistance or hard to treat.

Antibiotic Susceptibility test types

- Disk diffusion test
- Dilution method (MIC and MBC calculations)
- E test

The Kirby-Bauer test

The Kirby-Bauer test for antibiotic susceptibility (also called the *disc diffusion test*)



Scientific principle:

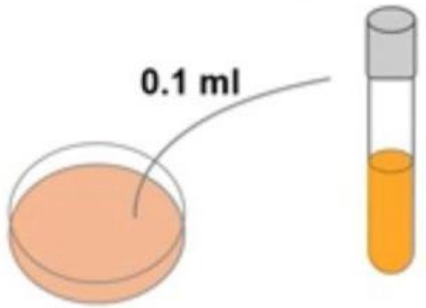
- The bacterium is swabbed on the agar and the antibiotic discs are placed on top.
- The antibiotic diffuses from the disc into the agar in decreasing amounts the further it is away from the disc.
- If the organism is killed or inhibited by the concentration of the antibiotic, there will be NO growth in the immediate area around the disc: This is called the zone of inhibition.
- The zone sizes are looked up on a standardized chart to give a result of sensitive, resistant, or intermediate.

Materials

- Medium: Muller hinton agar plates
- Culture: 24 hr old cultures (likely to be Staphylococcus aureus, E. coli, Bacillus subtilis, Enterococcus fecalis
- Antibiotics: commercial antibiotics discs
- Ethanol
- Forceps
- Chart guidelines

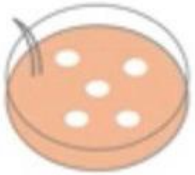
Steps:

1- pour Miller- Hinton agar into petri dishes. Leave it until solidify



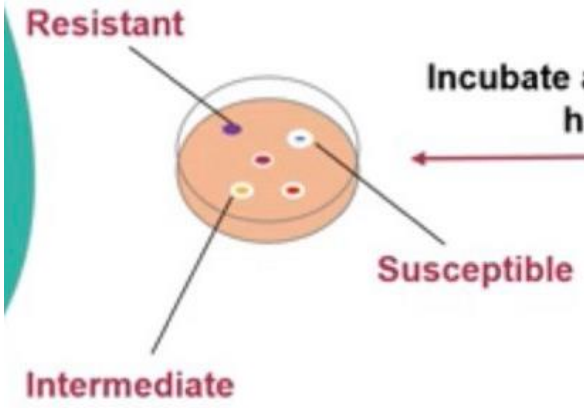
2- Add 0.1 ml of 24 hrs old of the studied bacterial culture

3- spread the bacterial inoculum onto the agar surface using sterilized spreader

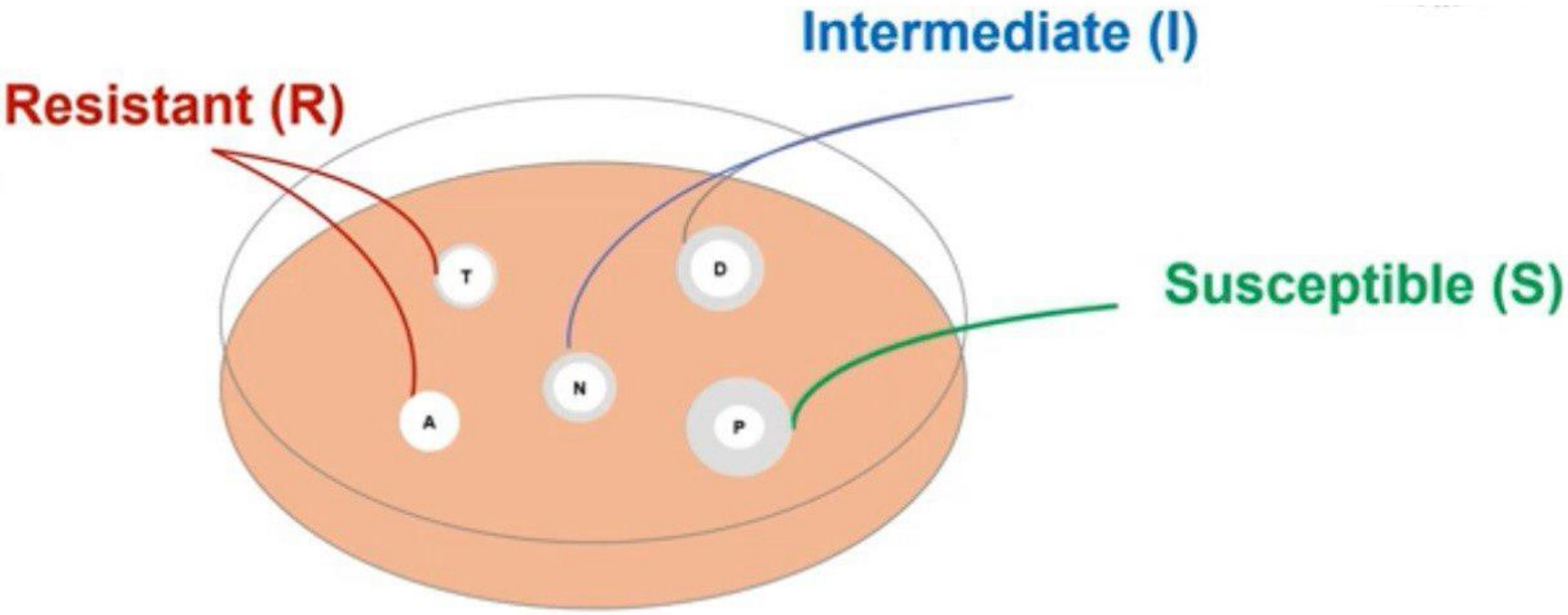


4- Using forceps, gently put the antibiotic discs onto the agar surface.

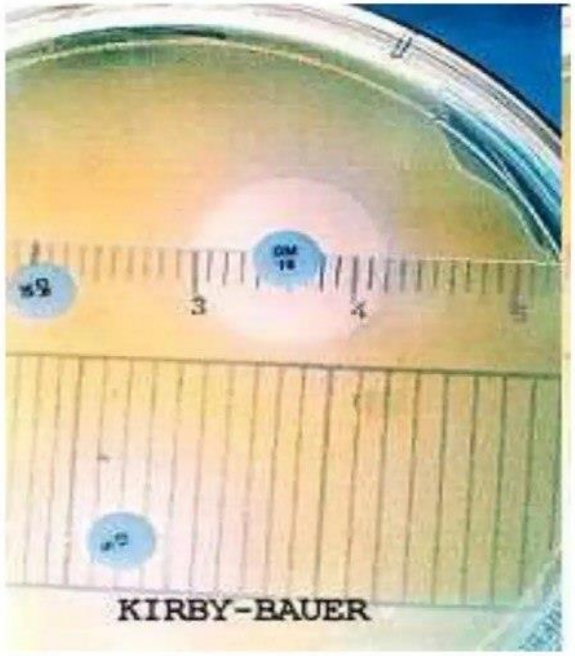
Incubate at 37°C for 24 hours.

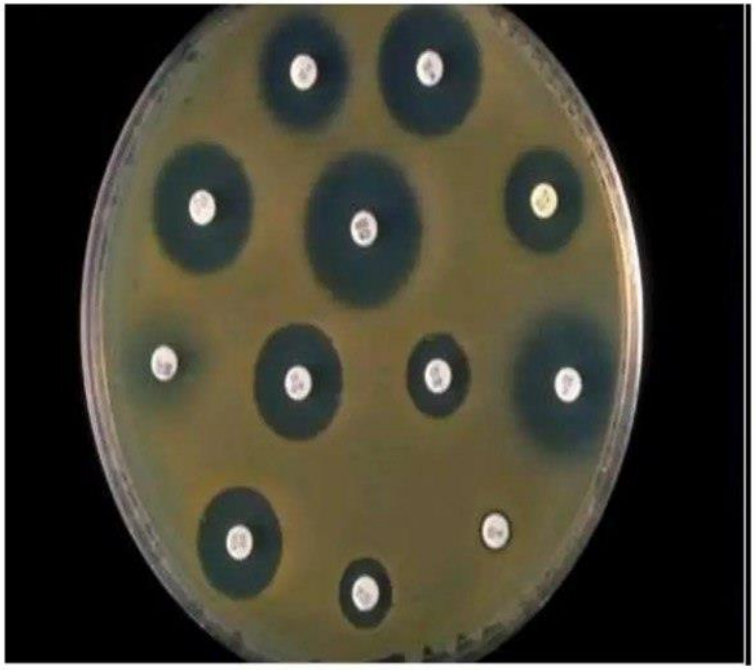


Antibiotic susceptibility results



Zone of inhibition- measurement





Guideline chart table

Antibiotic (Antimicrobial Agent)	DISC CODE	Resistant (< or = mm)	Intermediate (mm)	Susceptible (= or > mm)
Amoxicillin (other)	AMC	<13	14-17	>18
Amoxicillin (Staph)	AMC	19		>20
Ampicillin (other)	AM	11	12-13	>14
Ampicillin (Staph)	AM	28		29
Carbenicillin (other)	CB	17	18-22	>23
Carbenicillin (Pseudomonas)	CB	13	14-16	>17
Cefoxitin	FOX	14	15-17	>18
Cephalothin	CF	14	15-17	>18
Chloramphenicol	C	12	13-17	>18
Ciprofloxacin	CIP-5	15	16-20	>21
Clindamycin	CC-2	14	15-20	>21
Enoxacin (Fluoroquinolone, 2nd gen.)	ENX-10	14	15-17	>18
Erthromycin	E	13	14-22	>23
Gentamycin	GM	12	13-14	>15
Kanamycin	K-30	13	14-17	>18
Methicillin (Staph)	M(orDP)	9	10-13	>14
Oxacillin (Staph)	OX	10	11-12	>13
Penicillin G (Enterococcus)	P	14		>15
Penicillin G (Staph)	P	28		>29
Streptomycin	S-10	14	15-20	>21
Sulfamethoxazole-trimethoprim	SXT	10	11-15	>16
Tetracycline	Te-30	14	15-18	>19
Tobramycin	NN-10	12	13-14	>15
Vancomycin	Va-30	9	10-11	>12

Dilution tests:

Dilution tests are performed to determine the minimum inhibitory concentration (MIC) of an antimicrobial agent.

- MIC (minimal inhibitory concentration):

It is the lowest concentration of an antimicrobial agent that inhibit bacterial growth in vitro.

- MBC (minimum bactericidal concentration):

It is the lowest concentration of an antimicrobial agent that kills 99% of tested bacteria.

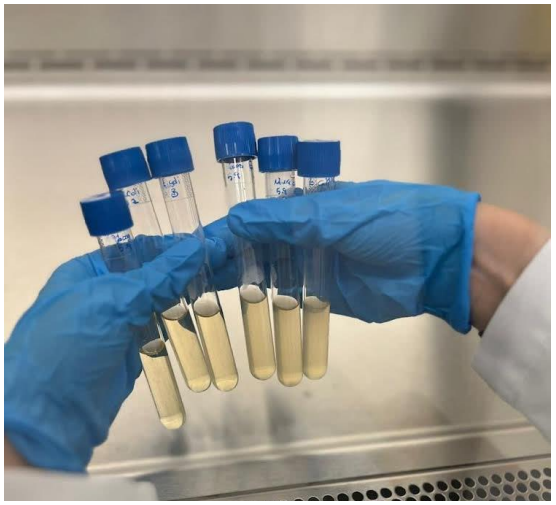
Principle and Uses of MIC and MBC

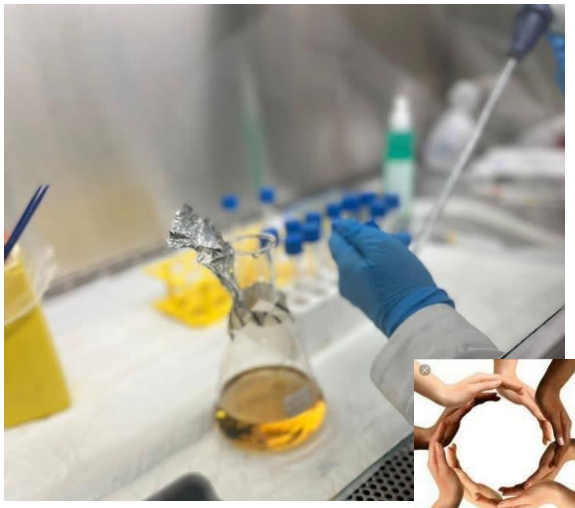
Principle:

Making serial two-fold dilution of antibiotics in a liquid growth medium dispensed in test tubes or microtiter plates against known susceptible bacteria.

Uses:

To measure the potency of certain antibiotic against know susceptible bacteria.

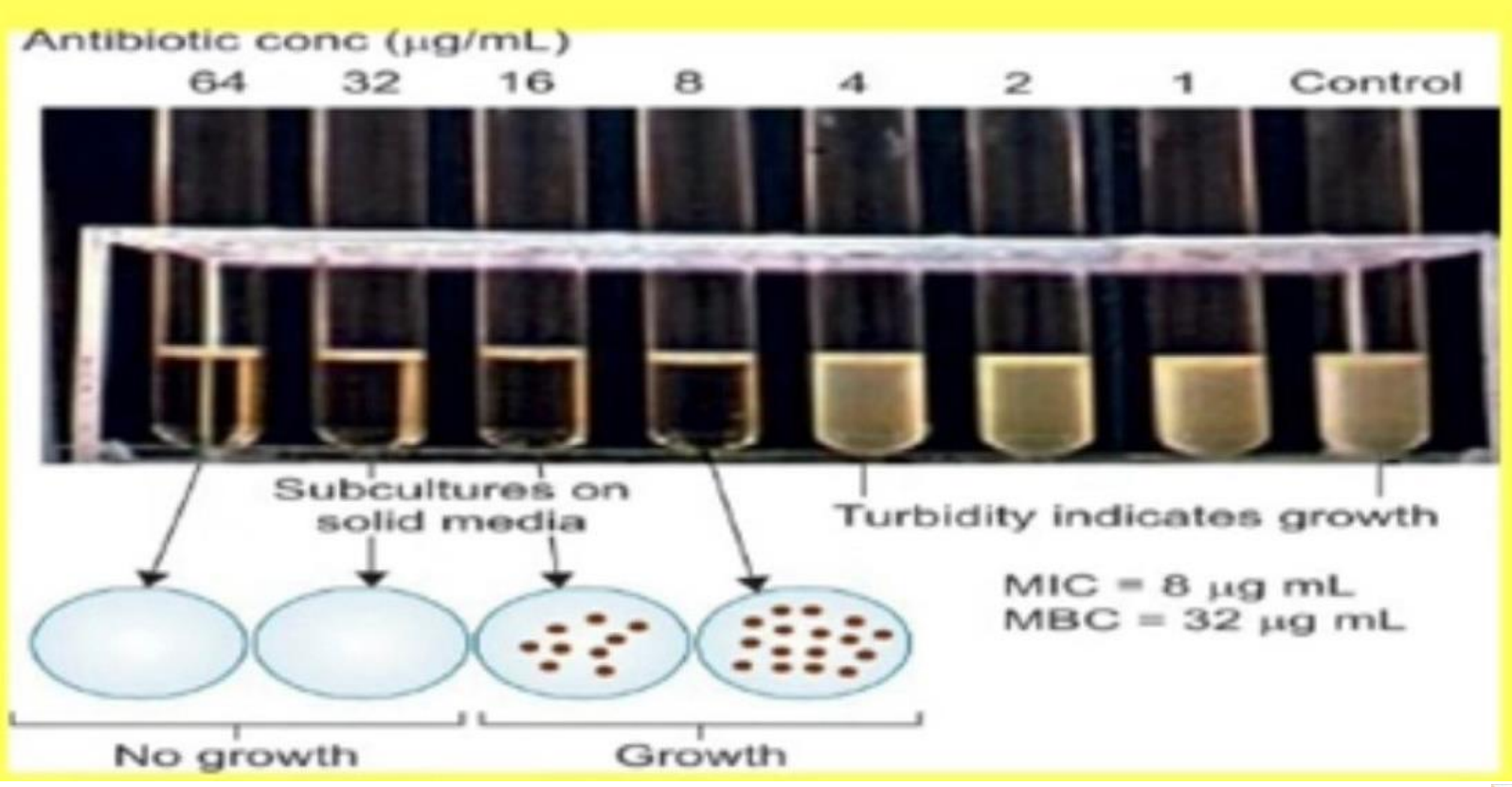




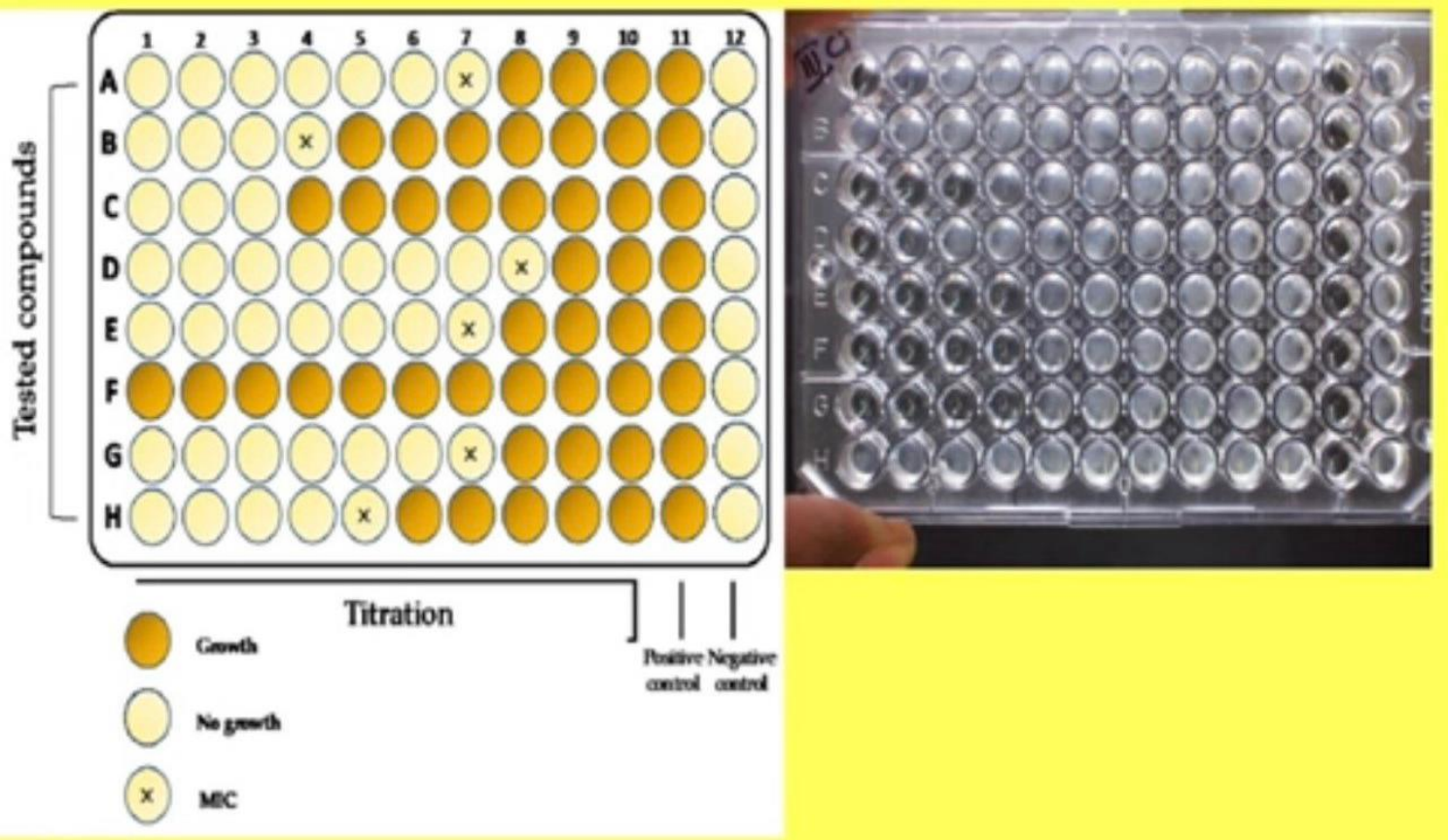
Broth Dilution Method

- This procedure involved preparing two-fold dilutions of antibiotics (eg, 64, 32, 16, 8, 4, 2, 1 ug/mL) in a liquid growth medium dispensed in test tubes.
- The antibiotic-containing tubes were inoculated with a standardized susceptible bacterial suspension of $1\text{-}5x10^5$ cfu/mL.
- Following overnight incubation at 37°C, the tubes were examined for visible bacterial growth as evidenced by turbidity.
- End point is the last tube showing no bacterial growth.
- Centrifuge tube of end point and discard the supernatant.
- Add fresh broth to the sediment, then incubate again.
- Results: MIC if growth appears or MBC if no growth appears

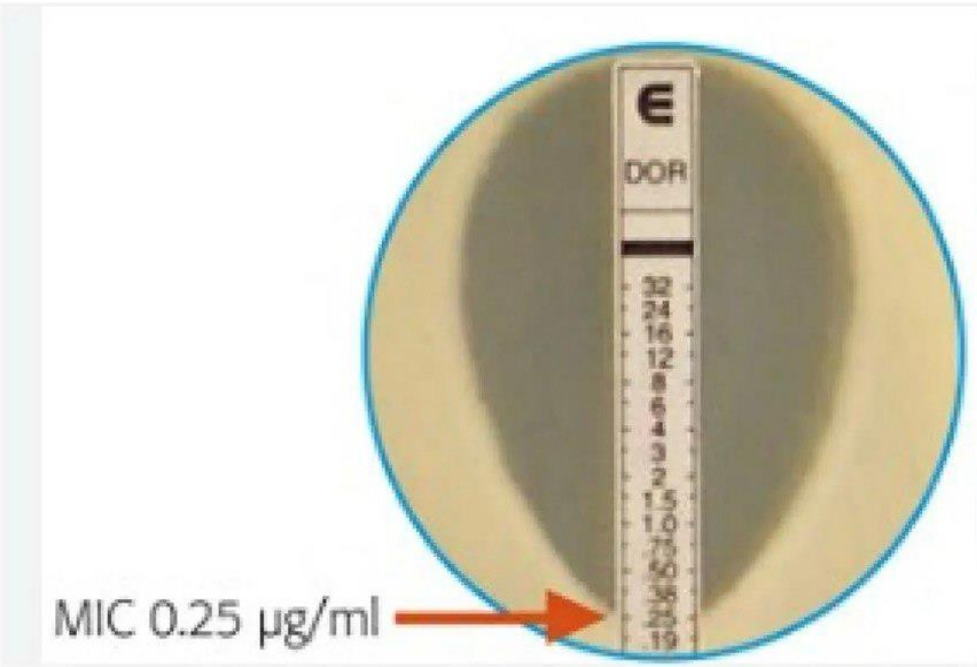
MIC Vs. MBC

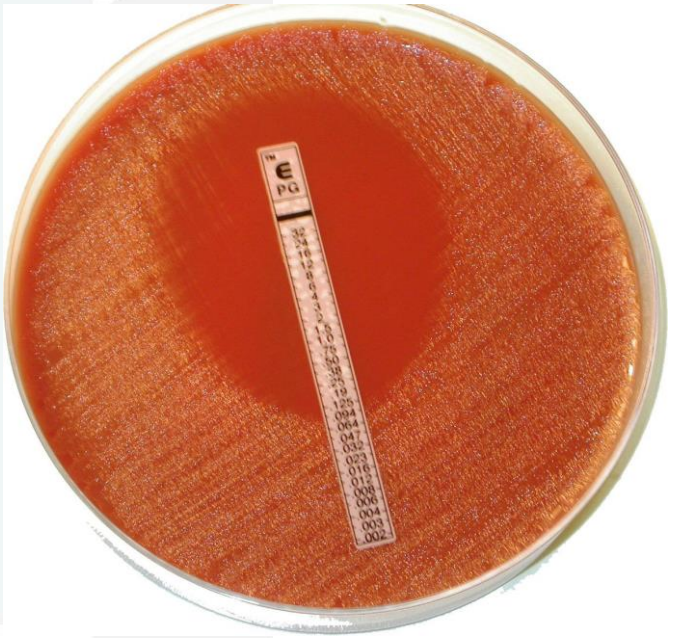

Antibiotic conc (µg/mL)
64
32
16
8
4
2
1
Control
Subcultures on solid media
Turbidity indicates growth
MIC = 8 µg mL
MBC = 32 µg mL
No growth
Growth

MIC (Microtiter plate)



E-test





E-test

- The E-test is a method of antimicrobial susceptibility testing that determines the minimum inhibitory concentration (MIC) of an antimicrobial agent against a particular bacterial strain.
- The E-test uses a plastic strip with a predefined gradient of antimicrobial agent, which is placed on a plate containing the bacterial culture.
- The strip is left on the plate for a specific amount of time, during which the antimicrobial agent diffuses out of the strip and into the agar, creating a concentration gradient.
- The bacterial growth is then observed around the strip, and the MIC is read as the point where the ellipse of inhibition intersects the strip.

automation

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Thank you  ...