General Microbiology Lab

Antimicrobial Susceptibility Test

Lab 6

2021-2022

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Medical Application

New antibiotics are continuously being developed

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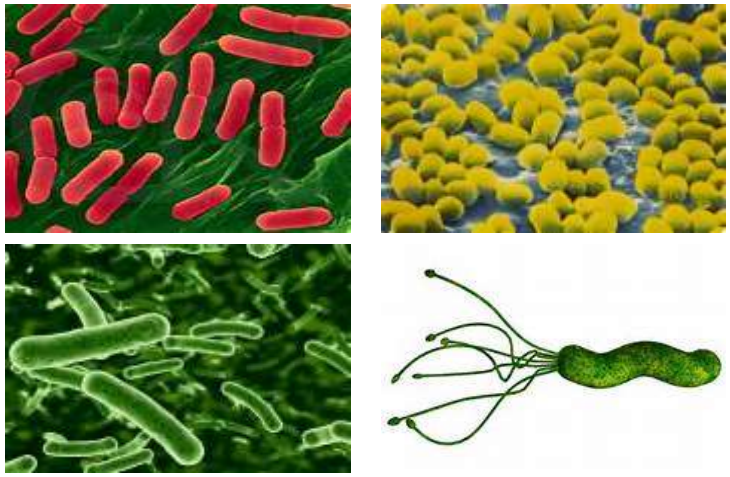


different bacteria acquire new resistant genes to the available antibiotics

determine the antibiotic susceptibility or resistance is required to determine most suitable antibiotic therapy

Methods of Antimicrobial Susceptibility Testing

1. Standardized filter-paper disc-agar diffusion (Kirby-Bauer method)

Qualitative Antimicrobial Susceptibility Testing

2. Minimum Inhibitory concentration (MIC)
   & Minimum lethal concentration (MLC)

3. Epsilometer test (E-test)

Quantitative Antimicrobial Susceptibility Testing

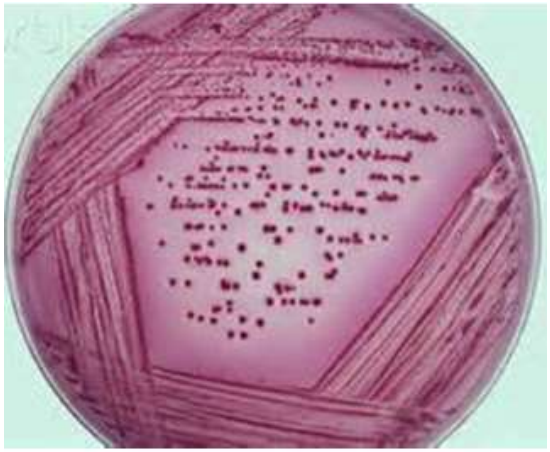
Standardized filter-paper disc-agar diffusion

Procedure

Urine sample

→ MacConkey agar →

Gram negative bacilli
Lactose fermenter



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Biochemical reactions

Glucose	A, G
Lactose	A, G
Maltose	A, G
Mannitol	A, G
Sucrose	A, G

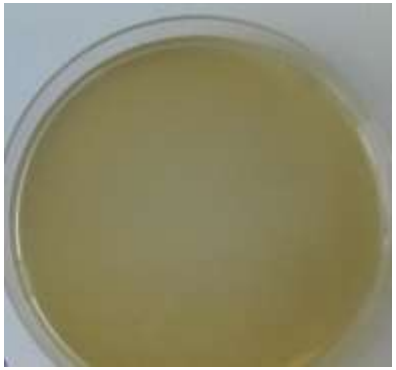
indole	+ve
MR	+ve
VP	-ve
Citrate	-ve
Urease	-ve
H2S	-ve

← *E. coli* ←

Antibiotic susceptibility test

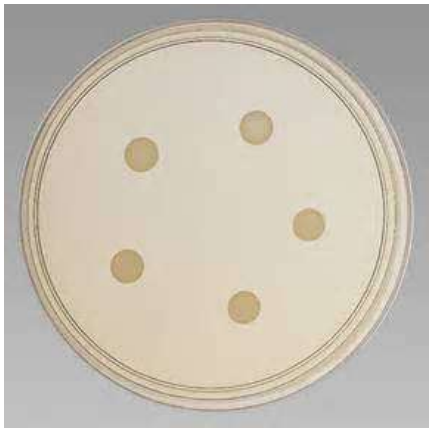
Standardized filter-paper disc-agar diffusion

Principle



Mueller Hinton agar

→ Confluent growth → Applying antibiotic disks



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Incubation 24h at 37°C

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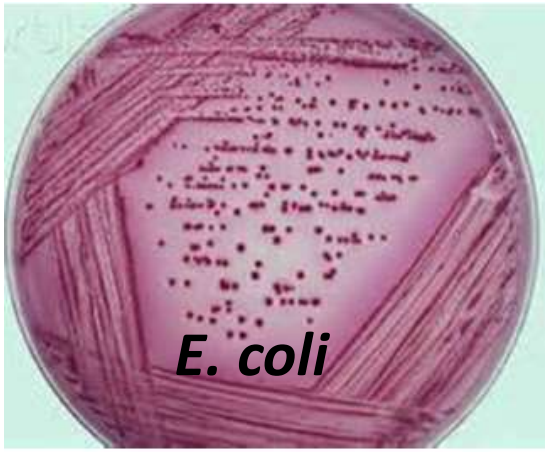
Read the diameter of the inhibition zone

Standardized filter-paper disc-agar diffusion

Procedure



1

Transfer at least three to five well-isolated colonies of the same morphological type into nutrient broth tube

2

Incubated between 2 to 6 hrs

3

4

Compare the turbidity of the nutrient broth to the 0.5 McFarland standards by either a photometric device or visually.

Standardized filter-paper disc-agar diffusion

Procedure

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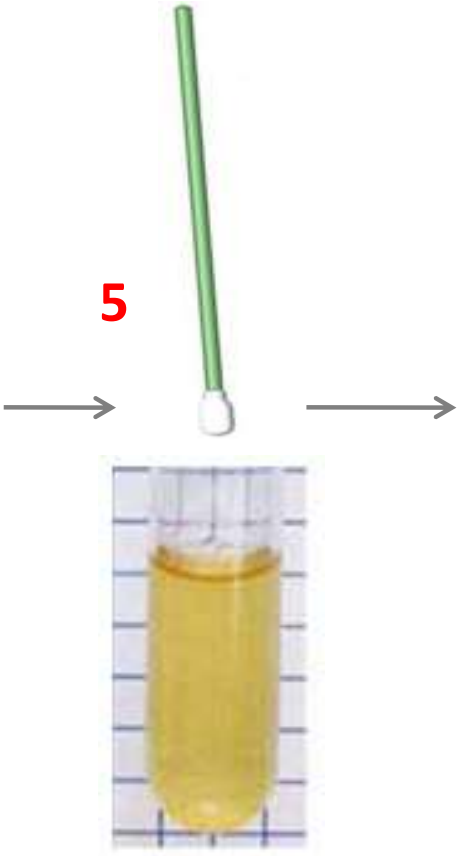


Dip a sterile cotton swab into a well-mixed saline test

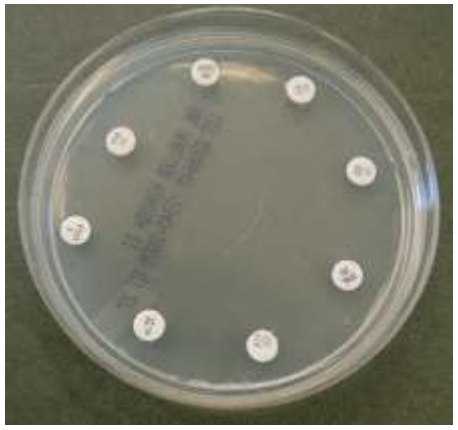
6

streak the entire agar surface horizontally, vertically, and around the outer edge of the plate

Confluent streaking

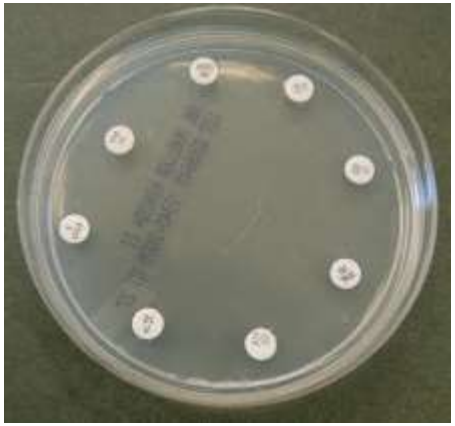
7

Carefully place the provided antibiotic discs onto the plate at equal distances using a sterile forceps and lightly touch each disc to make sure it will stay in place

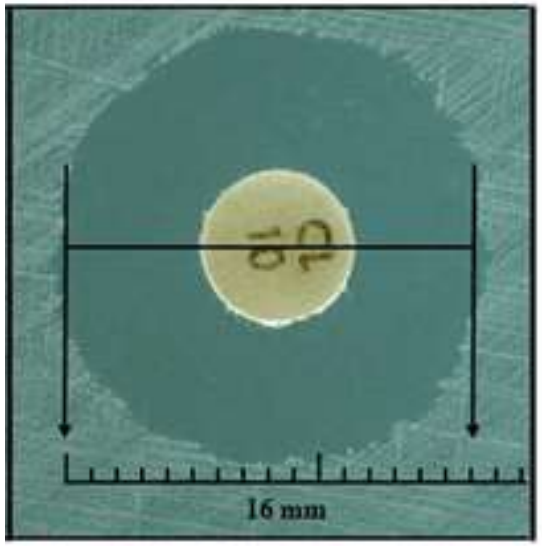


Standardized filter-paper disc-agar diffusion

Procedure



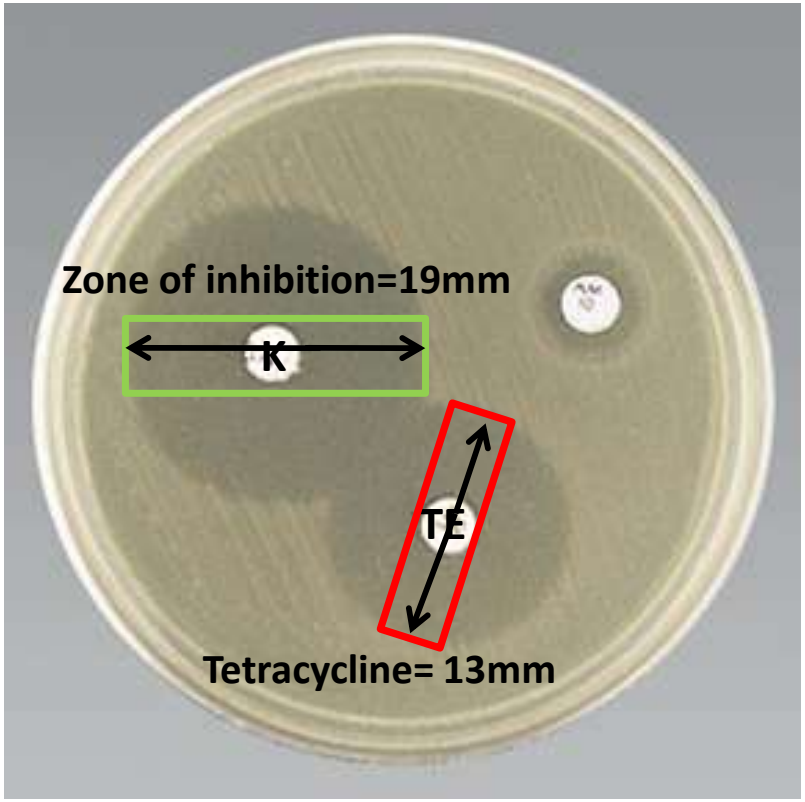
Incubation 24h
At 37°C



Standardized filter-paper disc-agar diffusion

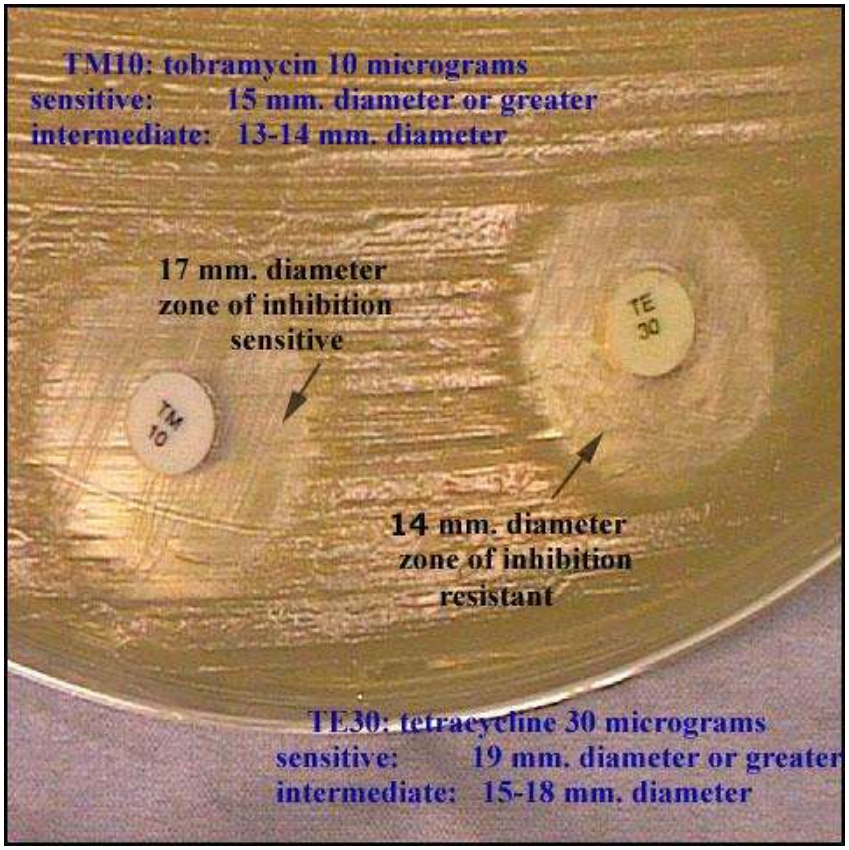
Results

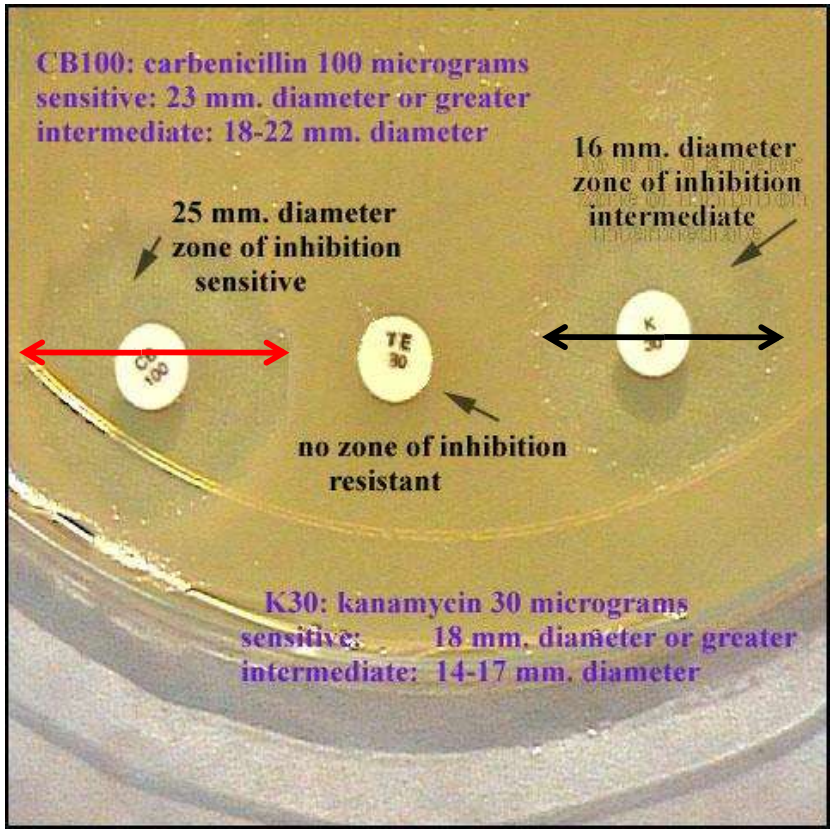
Antibiotic	Disk Conc.	Diameter of zone of inhibition (ZOI)		
		Resistant	Intermediate	Susceptible
Amikacin	10 μg	≤11	12-13	≥14
Ampicillin	10 μg	≤11	12-13	≥14
Bacitracin	10 units	≤8	9-11	≥13
Cephalothin	30 μg	≤14	15-17	≥18
Chloramphenicol	30 μg	≤12	13-17	≥18
Clindamycin	2 μg	≤14	15-16	≥17
Erythromycin	15 μg	≤13	14-17	≥18
Gentamicin	10 μg	≤12	13-14	≥15
Kanamycin	30 μg	≤13	14-17	≥18
Lincomycin	2 μg	≤9	10-14	≥15
Methicillin	5 μg	≤9	10-13	≥14
Nalidixic acid	30 μg	≤13	14-18	≥19
Neomycin	30 μg	≤12	13-16	≥17
Nitrofurantoin	0.3 mg	≤14	15-16	≥17
Penicillin				
vs. staphylococci	10 units	≤20	21-28	≥29
vs. other organisms	10 units	≤11	12-21	≥22
Polymyxin	300 units	≤8	9-11	≥12
Streptomycin	10 μg	≤11	12-14	≥15
Sulfonamides	0.3 mg	≤12	13-16	≥17
Tetracycline	30 μg	≤14	15-18	≥19
Vancomycin	30 μg	≤9	10-11	≥12



Standardized filter-paper disc-agar diffusion

Results



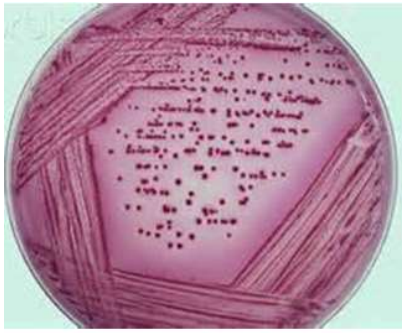


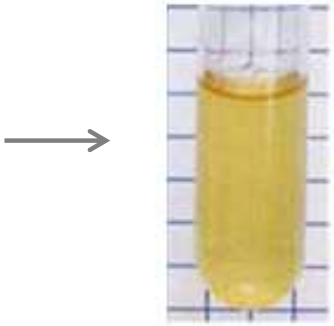
Minimum Inhibitory concentration (MIC)
&
Minimum lethal concentration (MLC)

MIC: is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation

MLC (MBC): Is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates that do not contain the test agent.

Minimum Inhibitory concentration





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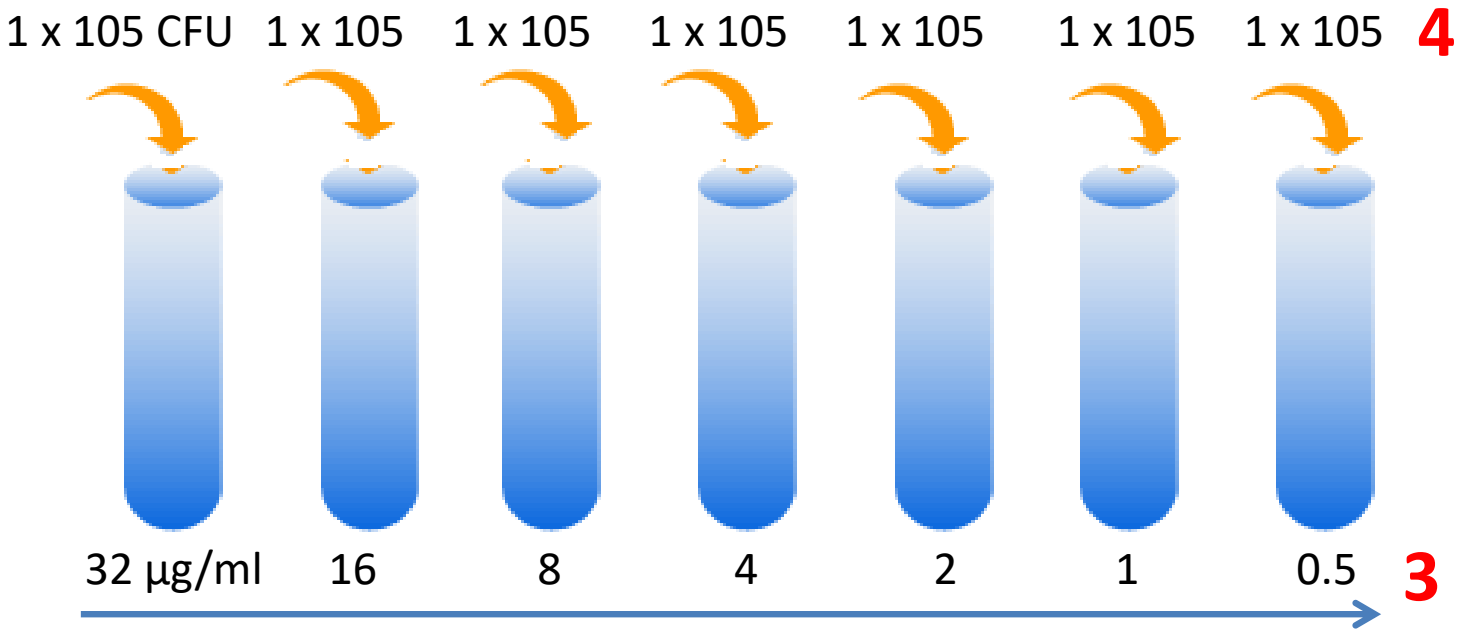
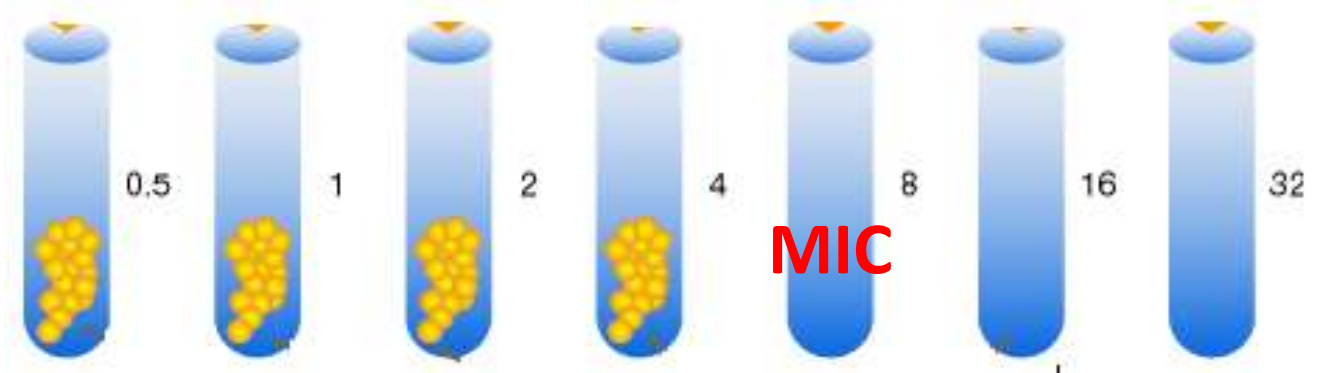
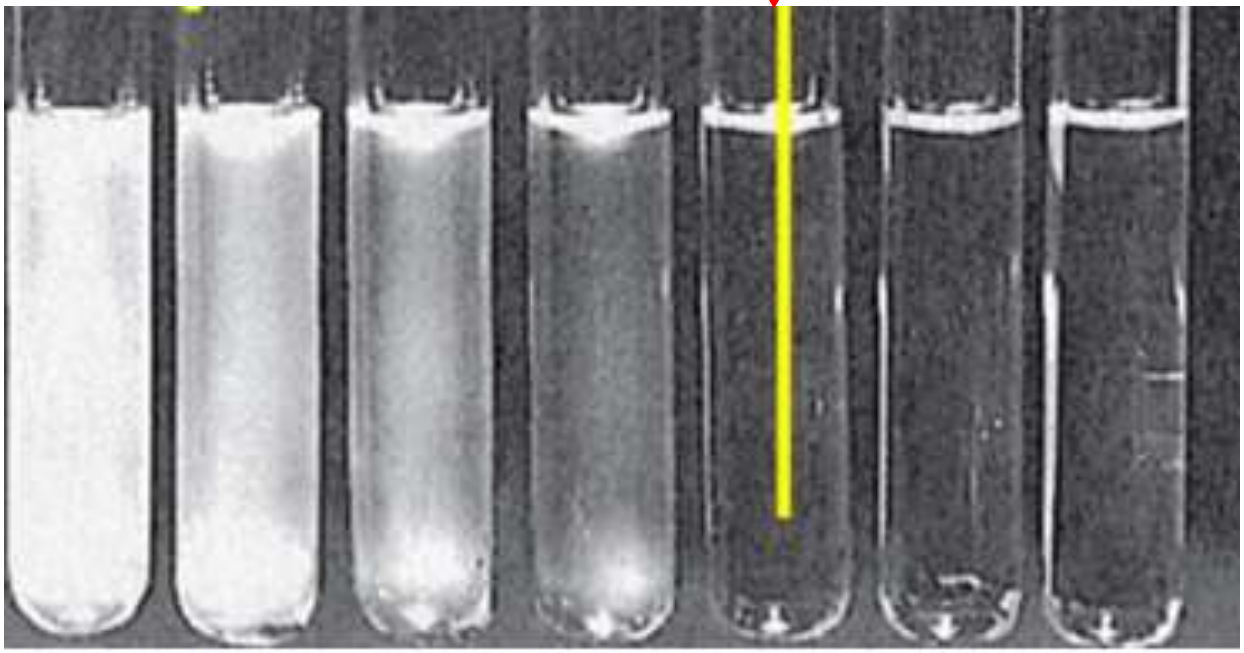




Minimum Inhibitory concentration

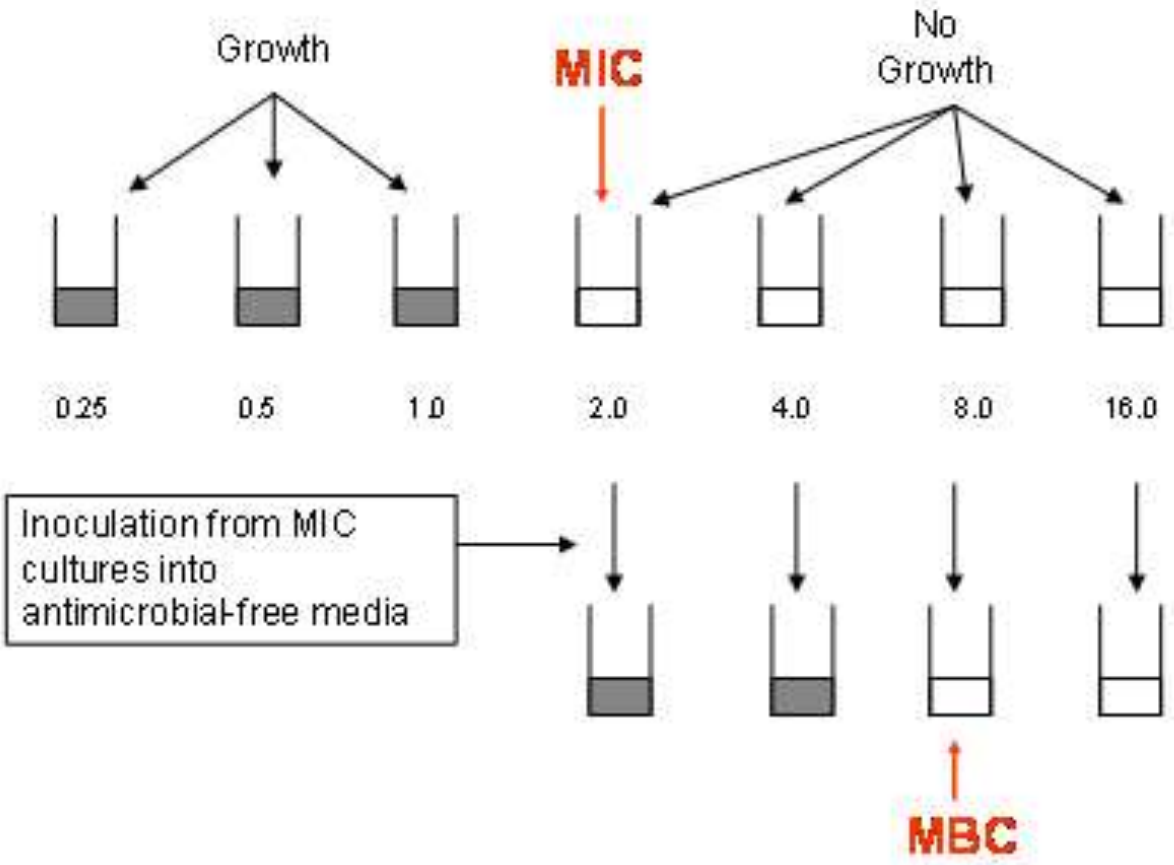
Minimum Inhibitory concentration



0.5 1 2 4 8 16 32 µg/ml

Sub-culture to agar medium

Serial Dilution Susceptibility Testing



Clinical applications for the Qualitative Antimicrobial Susceptibility Testing

MICs can also be used to reduce drug dosage and cost of antimicrobial therapy for very susceptible organisms; therefore, drugs with lower MIC scores are more effective antimicrobial agents.

This is important because populations of bacteria exposed to an insufficient concentration of a particular drug or to a broad-spectrum antibiotic (one designed to inhibit many strains of bacteria) can evolve resistance to these drugs. Therefore, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains

MIC is used for determining treatment for patients suffering from infections such as sepsis, pneumonia, meningitis, endocarditis or osteomyelitis or managing the treatment of high-risk patients such as those suffering from cystic fibrosis or immunocompromised individuals.

Epsilometer test (E-test)

Used as a substitution for the MIC test

Plastic strips with a predefined gradient of

One antibiotic

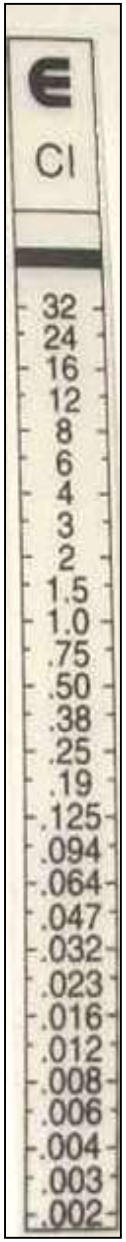
One antifungal

One strip per antibiotic

Easy to use

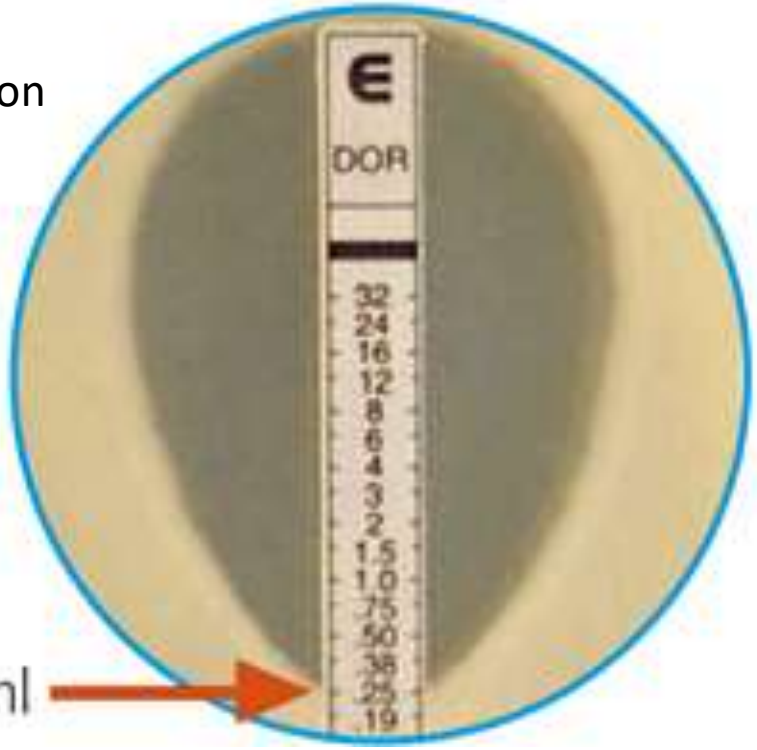
Storage at -20°C

Short shelf life, expensive



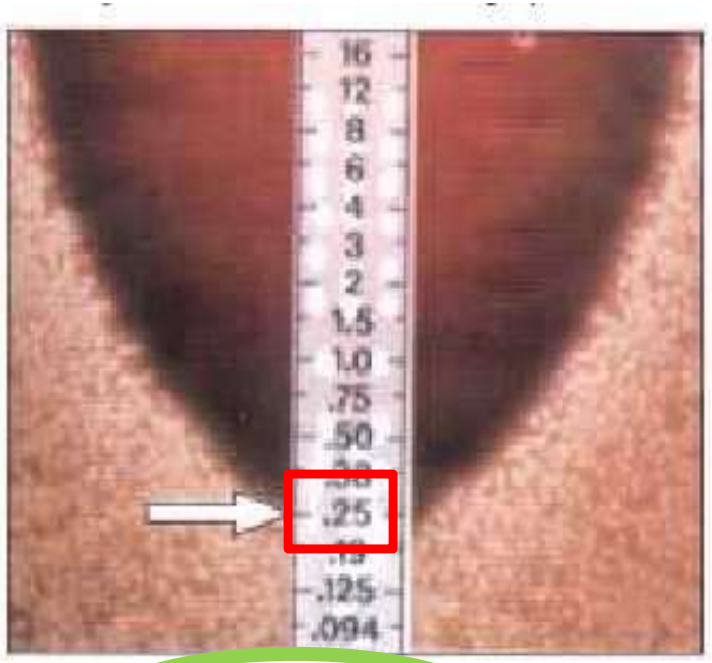
Epsilometer test (E-test)

Elliptical zone of inhibition



MIC 0.25 µg/ml

Reading E-tests



Ciprofloxacin

Resistant ≥ 4 ug/ml

Intermediate 1-4 ug/ml

Susceptible ≤ 1