Microbiology

Lab 6

Antimicrobial susceptibility Test

General Microbiology Lab

Antimicrobial Susceptibility Test

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Medical Application

New antibiotics are continuously being developed

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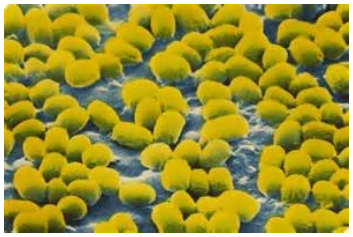
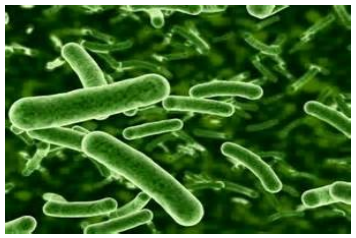
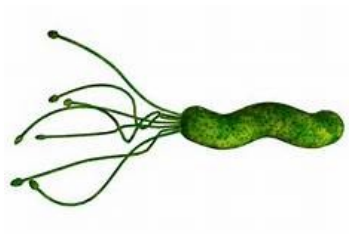






different bacteria acquire new resistant genes to the available antibiotics

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

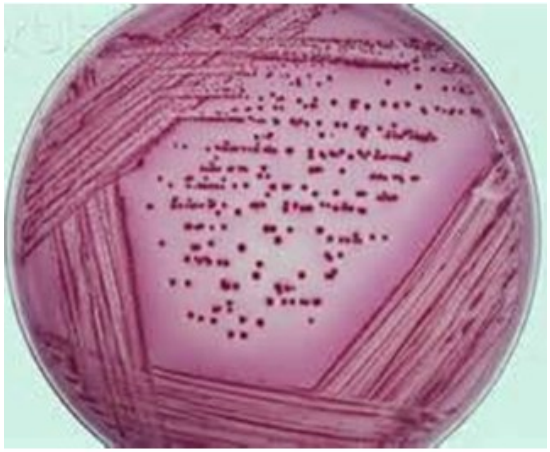
Standardized filter-paper disc-agar diffusion

Procedure

Urine sample

→ MacConkey agar →

Gram negative bacilli
Lactose fermenter



↓

Biochemical reactions

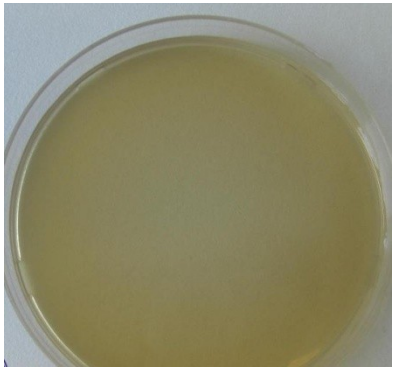
Glucose	A, G
Lactose	A, G
Maltose	A, G
Mannitol	A, G
Sucrose	A, G

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

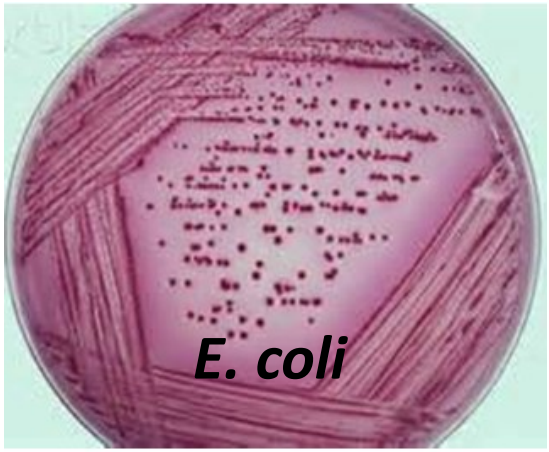
→ Confluent growth → Applying antibiotic disks

↓ Incubation 24h at 37°C

← Read the diameter of the inhibition zone

Standardized filter-paper disc-agar diffusion

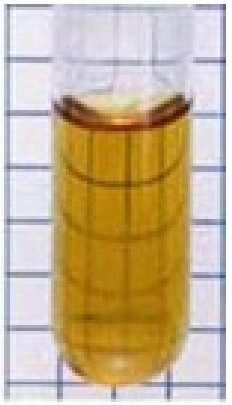
Procedure



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

حتى اعطيه مجال للانقسام

3

4

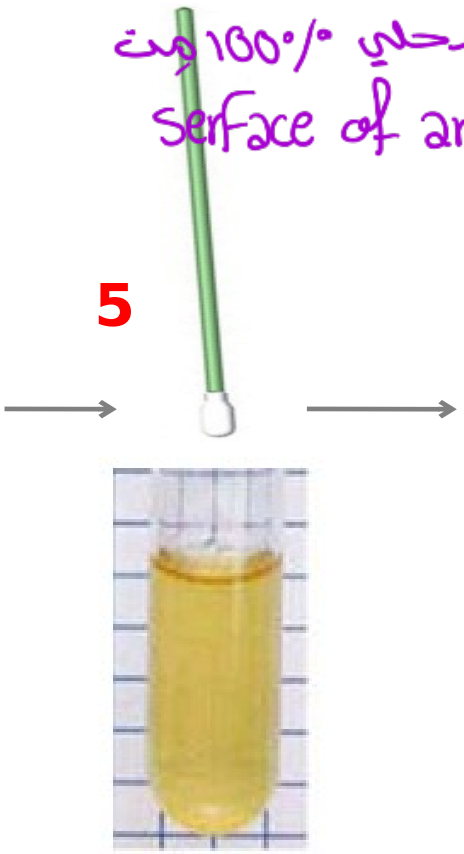
Compare the turbidity of the nutrient broth to the 0.5 McFarland standards by either a photometric device or visually.

Standardized filter-paper disc-agar diffusion

Procedure

* يحاول إنه يمسحلي 100% من surface of area.

5



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

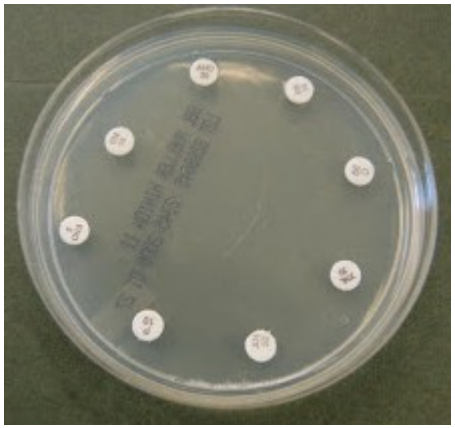
Standardized filter-paper disc-agar diffusion

Procedure

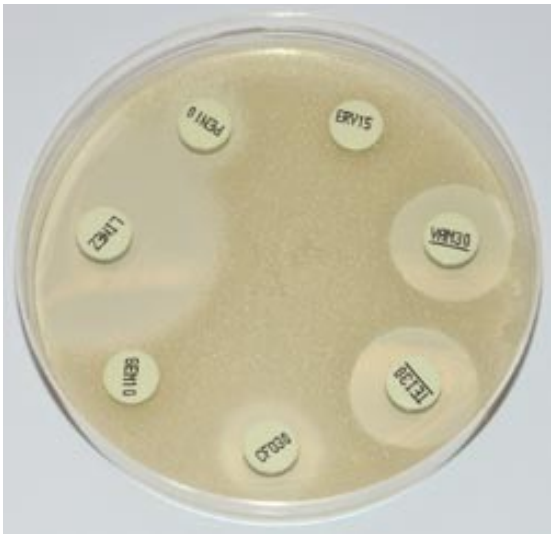
* قاعدة ← حش كل Halo zone حول البكتيريا

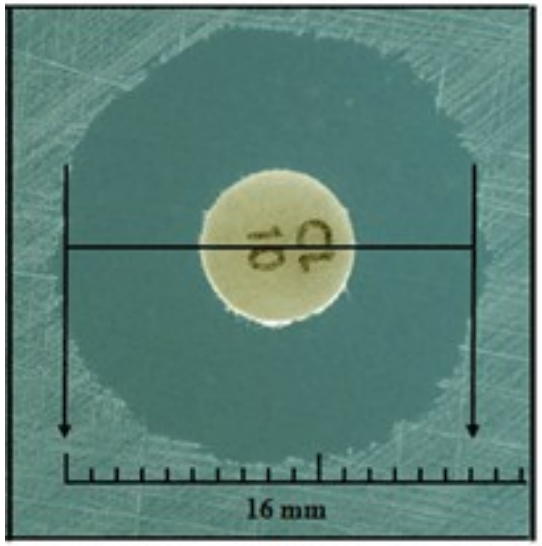
يعني ان البكتيريا ← Sensetive

فاستخدمو مسطرة لقياس Diamitar of inhiption Zone.



Incubation 24h
At 37°C

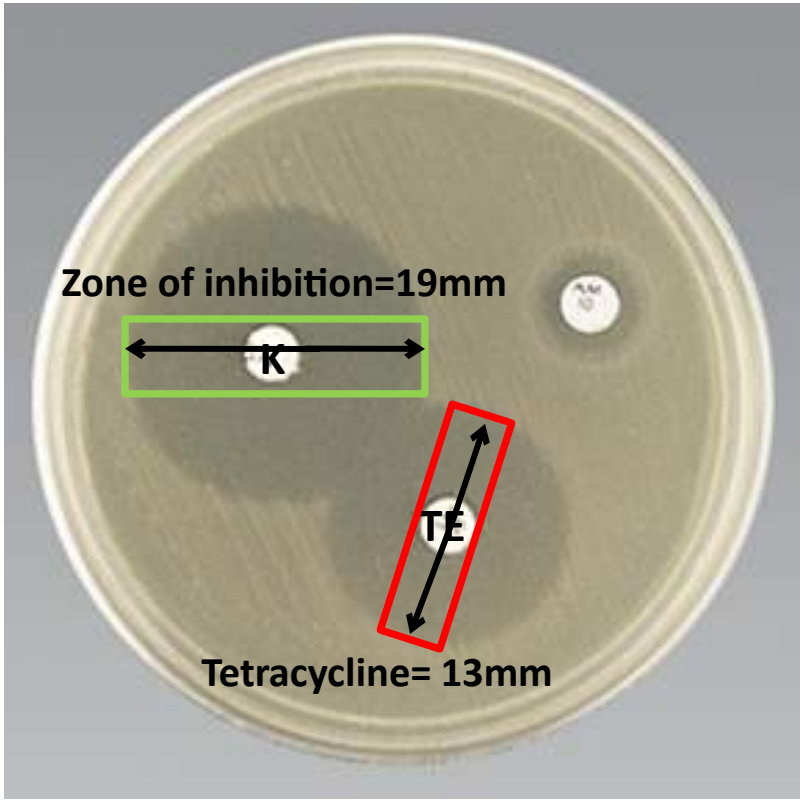




Standardized filter-paper disc-agar diffusion

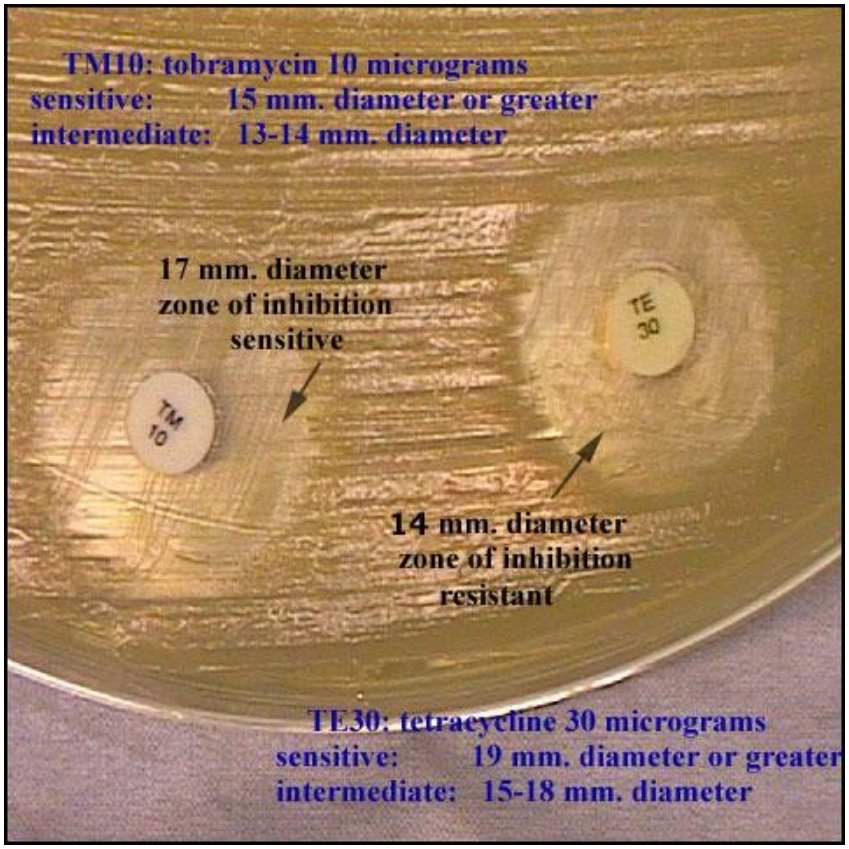
Results

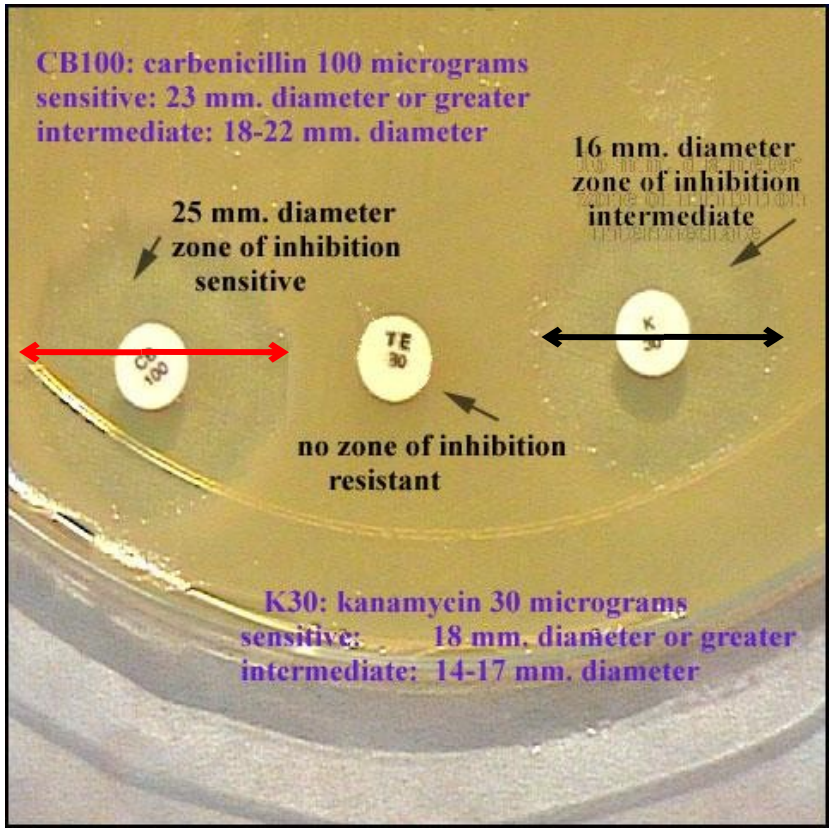
Antibiotic	Disk Conc.	Diameter of zone of inhibition (ZOI)		
		Resistant	Intermediate	Susceptible
Amikacin	10 μg	≤11	12-13	≥14
Ampicillin	10 μg	≤11	12-13	≥14
Bacitracin	10 units	≤8	9-11	≥13
Cephalothin	30 μg	≤14	15-17	≥18
Chloramphenicol	30 μg	≤12	13-17	≥18
Clindamycin	2 μg	≤14	15-16	≥17
Erythromycin	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





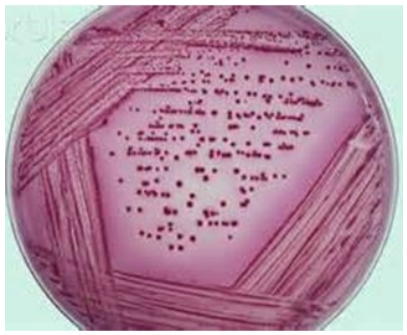
* بقيس وبعمل مقارنة مع الـ standerd من الشركة .

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



2

1

x 105 CFU 1 | x 105 1 | x 105 1 | x 105 1 | x 105 1 | x 105 1 | x 105 1

4

why we use serial dilution ?

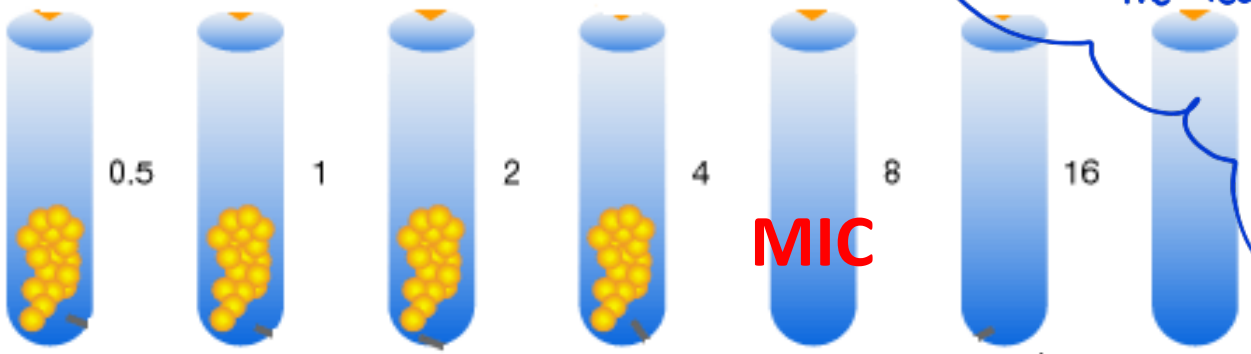
µg/ml 32 | 16 | 8 | 4 | 2 | 1 | 0.5

3

Antibiotic serial dilution

To determine how much the least concentration able to prevent bacterial growth by naked eye.

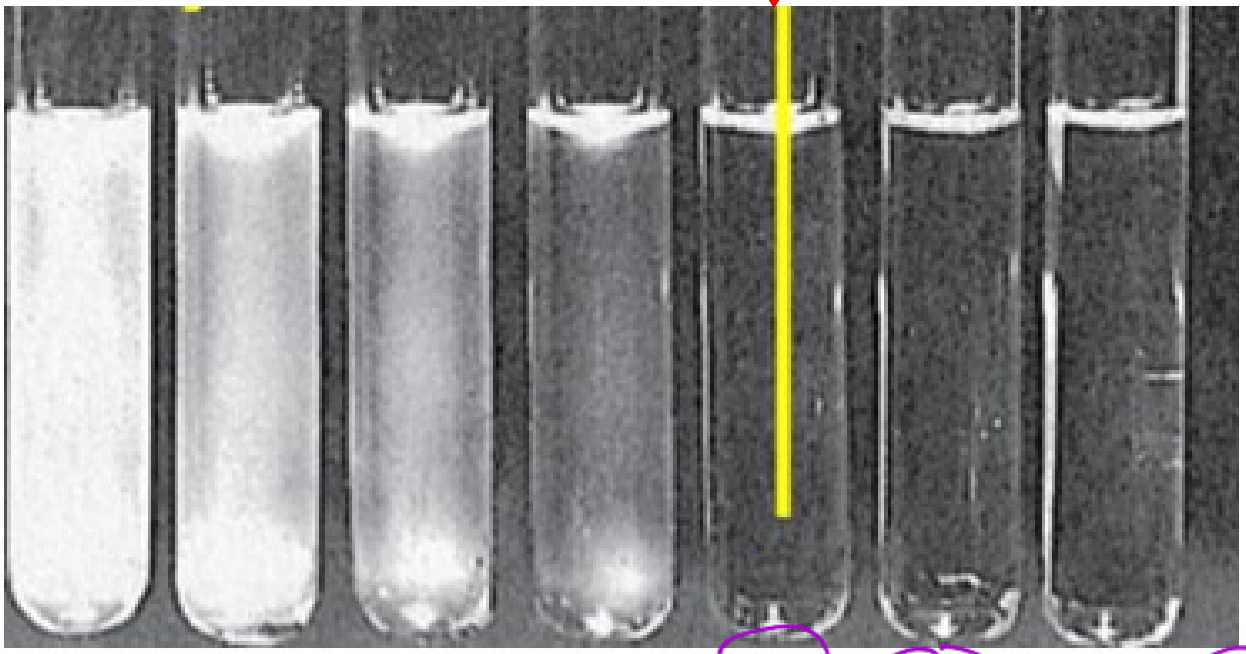
5



MIC

Minimum Inhibitory concentration

Minimum Inhibitory concentration

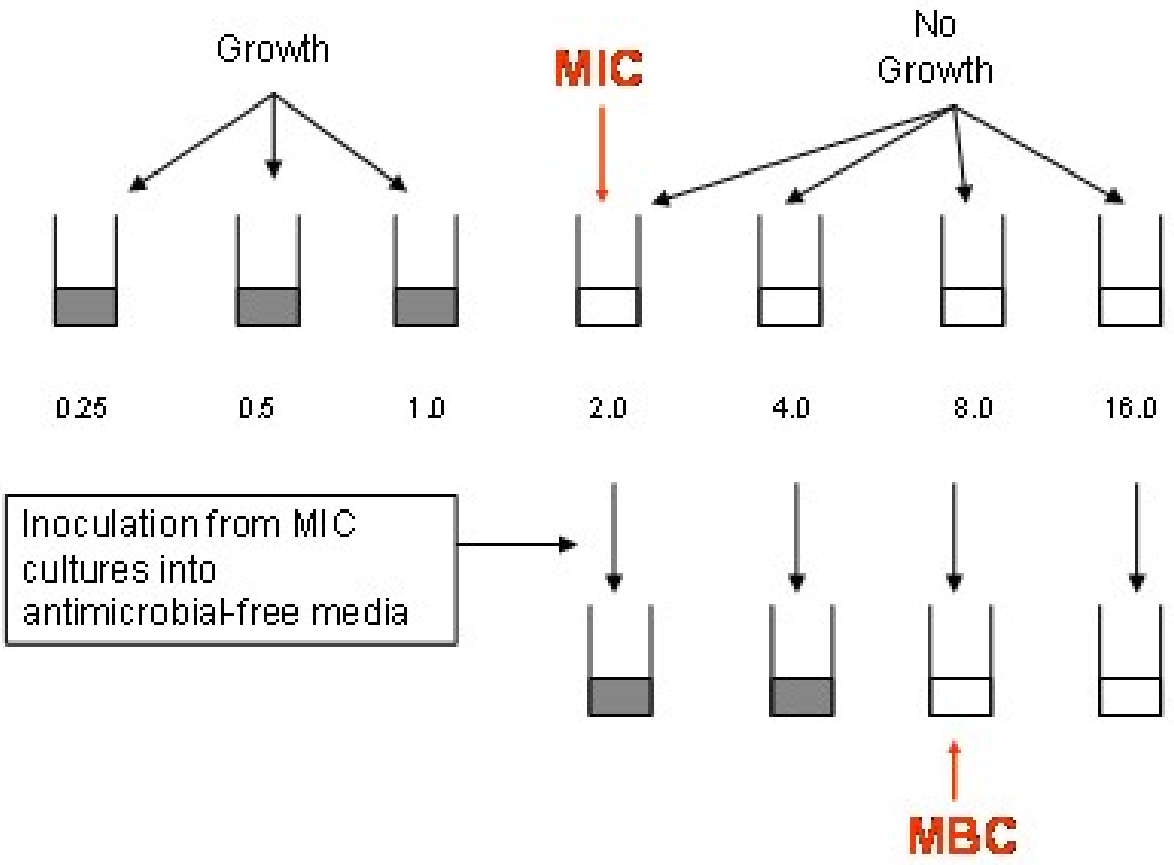


I can't see bacterial growth by naked eye

Sub-culture to agar medium

* ثاني يوم بشوف مين البكتيريا اللي ما اعطاني Growth in the surface of area → MLC

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

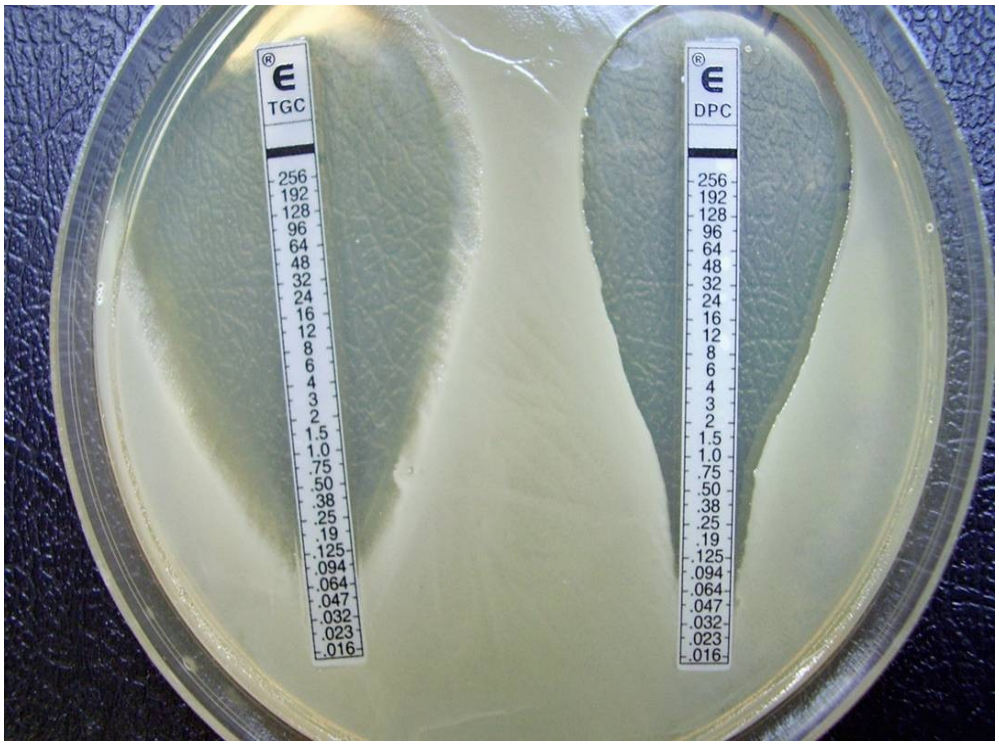
One antifungal

One strip per antibiotic

Easy to use

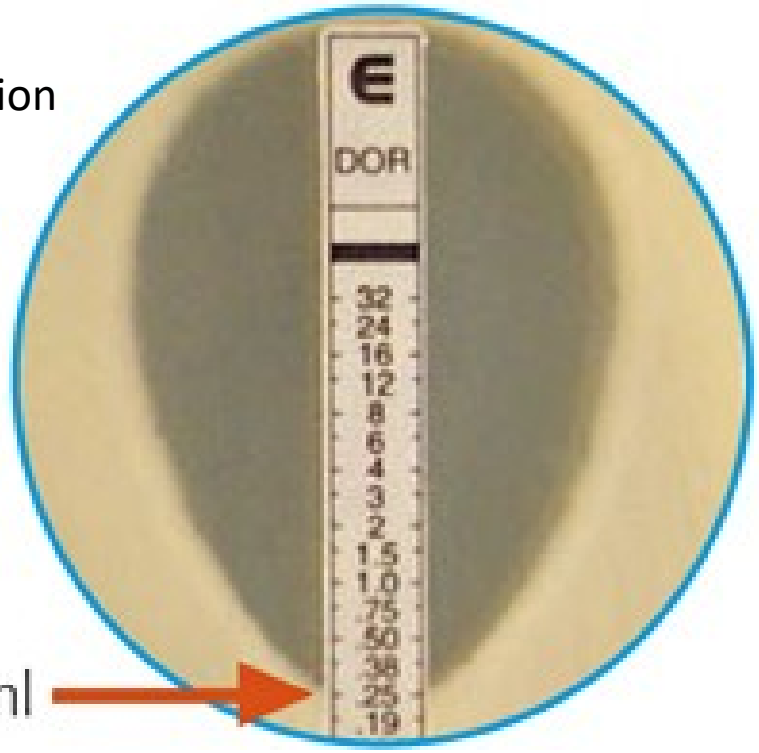
Storage at -20°C

Short shelf life, expensive



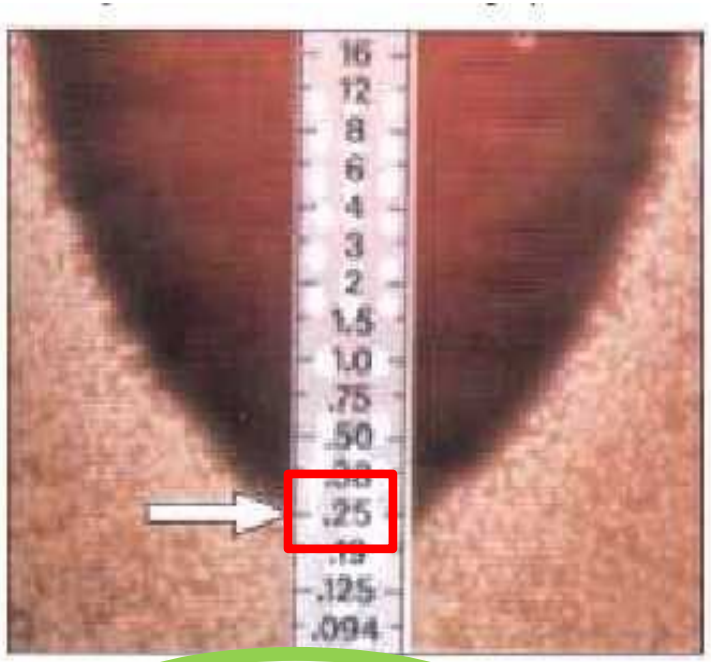
Epsilometer test (E-test)

Elliptical zone of inhibition



MIC 0.25 µg/ml

Reading E-tests



Ciprofloxacin

Resistant ≥ 4 ug/ml

Intermediate 1-4 ug/ml

Susceptible ≤ 1

Subject : Lab 6

* Antimicrobial Susceptibility Test :

↷ New antibiotics are continuosly being developed

↷ Different bacteria acquire new resistant genes to the available antibiotics.

> Determine the antibiotic susceptibility or resistance is required to determine most antibiotic

* Methods of antimicrobial Susceptibility Testing.

- Standardized Filter-peper disc agar diffusion (Kirby-Bauer method)
  - Qualitative (sensitive or not)
- Minimum Inhibitory contraction (MIC)
  Minimum Lethal Contraction (MLC)
  - Qualitative + Quantitive
- Epsilometer Test (E-Test)

(1) Standardized Filter-peper disc agar diffusion :-

Urin sample → Macconkey agar → growth (Gram negative bacilli) Lactose Fermenter → Biochemical reaction → E-coli

E-coli → Antibiotic susceptibility test ← (1) Hinton agar ← confluent growth ← Antibiotic disk ← 24h ← Read the diameter of the inhibition zone.

* (1) E-coli in Macconkey agar.
(2) Transfar three to Four colony and put them in tube of nutrient broth —(3) put them in→ Incubater (2-6 hrs)
why ? حتى اعطيها مجال تتفعل

(4) 0.5 McFarland → solution نحله بالمختبر
Compare turbidity of the standerd with broth after 6 hrs

If the turbidity of bacteria (>) standered
↓
Add some broth to decreas turbidity

the turbidity of bacteria (<) standereel
↓
بزيد سا عتين لل broth

(5) Dip swab in borth.
(6) Streak the antire agar (hinton agar) surface horizontally, around the outer edge → حتى يطلع عندي

« Confluent streaking »

(7) Carefully place the provided antibiotic disk on the plate at equal distances.

* halo zone antibiotic in disk → diffusion in serface of agar
If this bacteria is senseltive → بيموت the antibiotic will kill it
← فراح يصير على disk نفو حواليه.

Subject :

* Minimum Inhibitory concentration & Minimum Lethal concentration.

MIC → Lowest concentration antibiotic that will inhibit the visible growth
- we can determine by naked eye.

MLC → Lowest concentration of an antibacterial agent required to kill aparticular bacterium.
- we can determine by subculturing to agar plates that don't contain the test agent.

1. E-coli in Macconkey agar.
2. Add 3-4 colony in nutrient growth
3. Antibiotc serial dilution in different concentration.
4. Add Fixed number of bacteria in a tube.
5. In the next day → see where the least concentration that I can't see the Growth.

Growth وبنأخذ أقل تركيز ما شفنا فيه

* كل ما زاد antibiotic قل growth

concentration

tube في ← اللي ما فيه Growth بنأخذ منهم

↳ Sub culture to agar medium

وبنشوف منهم

Specific No Growth

MLC

▸ Subject : .....

③ Epsilometer test (E-test) :

Used → ~~Study~~ substitution For the MIC test.

↳ plastic strips with apredefined gradiant
- One antibiotic ← ~~a~~
- One antiFungel

→ From low concentration to higher.

- One strip per antibiotic
- Easy to use
- Storge at 20°c
- Short shelf life, expensive.

Clinical application for the Quantitative Antimicrobial Susceptibility Testing.. MIC & MLC

- MIC used to reduce drug dosage and cost of antimicrobial therapy For very susceptible Organisms
  ↓
  Drug with lower MIC scores are more effective antimicrob
- MIC scores aid in improving outcomes For patient and preventing evolution of drug-resistant microbial agent.
  ↓
  populations of bacteria exposed to an insufficient concentration of aparticular drug (one designed to inhibit many stains)
- MIC is used For determining treatment For patients suffering From infection Like 1. sepsis 2-meningits 3-pneumonia 4-endocarditis or osteomyelitis or manging the treatment of high-risk patint