بسم الله الرحمٰن الرحيم

اللهم علمنا ما ينفعنا ، وانفعنا بما علمتنا ،
وزدنا علمًا

Experiment 7

تثبيط نشاط البكتيريا دون قتلها عن طريق الدواء

Qualitative methods used for the evaluation of bacteriostatic activity of different antimicrobial agents

❖ Objectives:

- Evaluate the different methods of antimicrobial susceptibility testing. ✓ (sensitivity)
- Specifically differentiate between the **qualitative methods** of antimicrobial susceptibility testing.

❖ Introduction:

أشياء عادية في حياتي الفترة

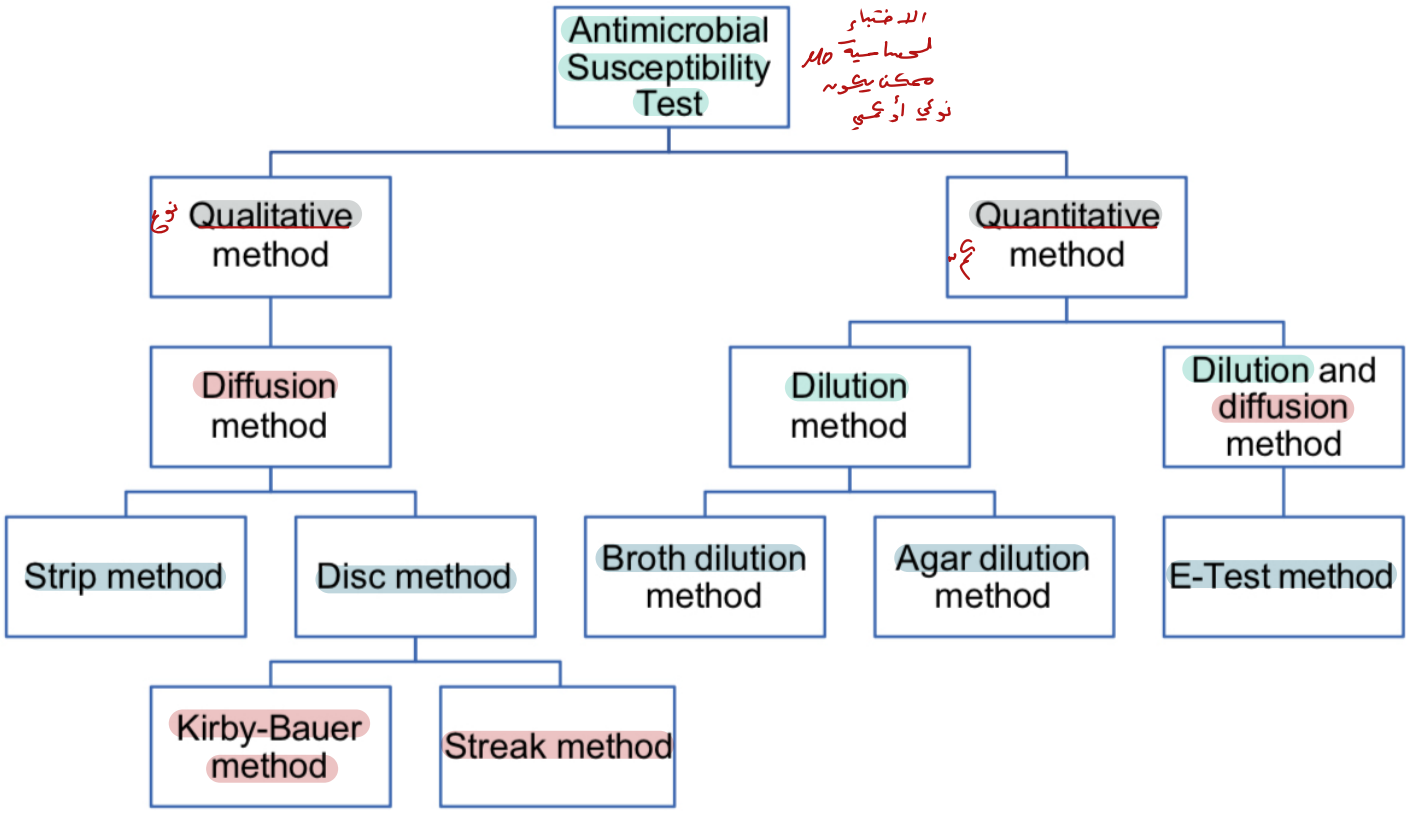
Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem. The results of *in-vitro* antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and drugs used for individual patients in specific situations (although sensitivity tests measure antimicrobial activity against bacteria under laboratory conditions it cannot be assumed that the results of *in vitro* tests will be necessarily the same *in vivo*). The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

التجارب في المختبرات مش شرط نتائجها تكون بالضبط زي الجسم

اللهم ارحم أيتهم واغفر له
وعافه واعفُ عنه وتجاوز عن
سيئاته وباركْ وفاعفُ في جسمانة
وادخله أعلى الجنة والحقين

ربِّ اغفر لي ولوالدي
وللمسلمين والمسلمات
والمؤمنين والمؤمنات
الأحياء والأموات

Methods of Antimicrobial Susceptibility Testing:



الاختبار لحساسية MD ممكن يكون نوعي أو كمي

هو وسط لاختبار حساسية MD ضد العقاقير ← باستخدام diffusion

* Mueller Hinton Agar (MHA):

Mueller and Hinton developed Mueller Hinton Agar (MHA) it is commonly used for the routine susceptibility testing of microorganism by the diffusion technique.

محتويات هذا الوسط هي:

Mueller Hinton Media contains Beef Extract, Acid Hydrolysate of Casein, Starch and Agar. **Beef Extract** and **Acid Hydrolysate of Casein** provide nitrogen, vitamins, carbon, amino acids, Sulphur and other essential nutrients. **Starch** is added to absorb any toxic metabolites produced. **Starch** hydrolysis yields dextrose, which serves as a source of energy. **Agar** is the solidifying agent.

Why MHA is used for antibiotic susceptibility testing?

هاد معناه بنقدر نزرع في هذا الوسط أنواع كثيرة من MD عشان نختبر حساسيتها ضد العقاقير

1. It is a non-selective, non-differential medium. This means that almost all organisms plated on here will grow.
2. It contains starch. Starch is known to absorb toxins released from bacteria, so that they cannot interfere with the antibiotics. It also mediates the rate of diffusion of the antibiotics through the agar.
3. It is a loose agar. This allows for better diffusion of the antibiotics than most other plates. A better diffusion leads to a truer zone of inhibition.
4. MHA shows acceptable batch-to-batch reproducibility for susceptibility testing.
5. MHA is low in sulfonamide, trimethoprim, and tetracycline inhibitors (i.e., concentration of inhibitors thymidine and thymine is low in MHA).

قدرة النشأ على إنها ما بها السموم يمنع تأثير العقاقير بشكل سلبي + تنظم سرعة انتشار العقاقير

بيئة جافة نوعا ما مفيدة كانتشار العقار + تكوين منطقة تثبيط بشكل أوضح من البيئات الأخرى

الذي يساعد على تقليل التداخلات في نتائج الاختبار هو وجود هاي المثبطات بشكل قليل

لا إله إلا أنت سبحانك

إني كنت من الظالمين !

أساسيات لتكوين معظم DNA في البكتيريا تستخدمها البكتيريا لإنتاج حمض الفوليك للنمو

6. Both the para-aminobenzoic acid (PABA) and thymine/thymidine content in Mueller Hinton Agar are reduced to a minimum, thus markedly reducing the inactivation of sulfonamides and trimethoprim when the media is used for testing the susceptibility of bacterial isolates to these antimicrobics.

• فالوكالة عندي كمية PABA + ثايمين في الوسط رح تتجاور البكتيريا تأثير المضاد فا رح تشوف نتائج ضايجة بالك رح تشوفها مقاومة (Resis) مع إنها في الحقيقة حساسة (Sens)

* المختصر: لازم يكون PABA + ثايمين قليلة ت في الوسط MHA عشان دقة النتائج وما تتأثر فعالية المضاد مثل trimetho prim/sulfonamine

سبحان الله وبحمده
سبحان الله العظيم

1 Measure length of inhibition zone

2 More than One MO

3 incubation for 24 h

جدول هم الفرق مع الطريقة اللي بعد هاي

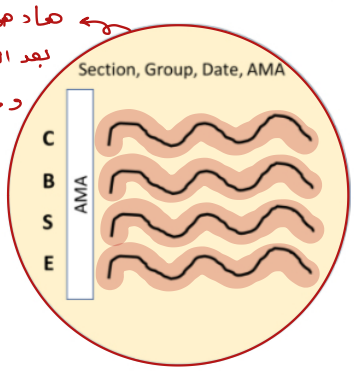
Determination of the Susceptibility of More Than One Organisms to the Bacteriostatic Agent Provided (Strip method)

Experimental:

1. Pour an agar plate and allow to set firm
2. With a sterile forceps pick a sterile paper strip from the Petri dish and dip it into the bacteriostatic solution (Ethanol followed by heating)
3. Place the soaked strip onto the surface of nutrient agar at one side
4. Transfer a loopful from each of the test organisms to be tested and streak across the agar surface starting at the margin of the strip and at right angle to it (mark each streak on the bottom of the plate to identify)
5. Incubate at 37 °C for 24 hr.
6. Measure the length of the inhibition zones for each organism

• شريط فيه مضاد حيوي بنثبته على أحد الجوانب في Petri dish بعدين بناخذ عينات MO مختلفة بدنا نعرف مدى حساسيتها / مقاومتها لهاد الكمضاد عن طريق نقيس طول منطقة التثبيط و مقارنتهم

هاد هو الشكل بعد الزراعة و قبل الحضانة



AMA: Antimicrobial agent name

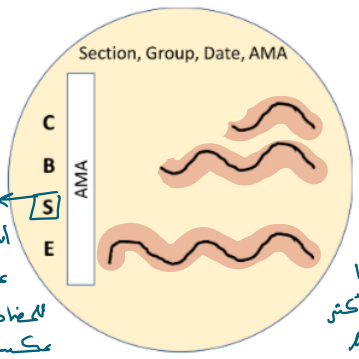
C: *Candida albican* Fungi

B: *Bacillus subtilis* Bacteria

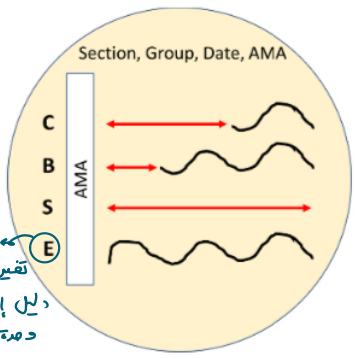
S: *Staphylococcus aureus* Bacteria

E: *Escherichia coli* Bacteria

Result after incubation: → بعد ٢٤ ساعة حضانة



كما لاحظوا اختفاء MO اي أسميها S هاد دليل على شي؟ على إنها أكثر وحدة حساسة للمضاد المستخدم وتم تثبيطها كليا عكسها E



وكمان اشي تقيس في طول منطقة التثبيط دليل إنه مقاومة أكثر وحدة من باقي MO

Result interpretation:

- Order according to the length of zone of inhibition:

$$\underline{S} > C > B > (E)$$

The most sensitive — The most resistance

- So, order of organism sensitivity towards the tested antimicrobial agent is:

The most sensitive microorganism is *Staphylococcus aureus*, then *Candida albican*, then *Bacillus subtilis* and the least sensitive (most resistant) is *Escherichia coli*.

1 Measure Diameter

2 One MO 3 incubation 18-24h

➢ Determination of the susceptibility of a selected organism against different antibiotics (Kirby-Bauer method)

Kirby–Bauer (K-B) antimicrobial sensitivity test allows for the rapid determination of the efficacy of a drug (antibiotic) against certain type of bacteria by measuring the diameter of the zone of inhibition that resulted from diffusion of the drug from its disc into the surrounding medium.

K-B Test Standards:

تؤخذ العينة من مكان حصاب infection في

A. Microorganisms are isolated from infected tissues. For example:
   - Septicemia: a blood sample is obtained
   - Upper respiratory tract infections: a throat swap or a sputum sample.

المهم عشان نحصل على نتائج صحيحة + عادية بدنا نوحّد كل اشي لـ MO المزروعة، كيف؟

① The obtained bacterial suspension should be **standardized** by comparing it with McFarland standard # 0.5 or # 1 (according to the type of bacteria). This would be equivalent to a bacterial concentration of $1.5 * 10^8$ cells/mL or $3 * 10^8$ cells/mL.

هو معيار وطريقة قياس كثافة البكتيريا بصريًا (دقيقة)

② توحيد الوسط المزروع عليه

B. The used medium should be **standardized**. Mueller-Hinton Agar is used. This medium with a pH of 7.2 to 7.4 contains high percent of proteins, which will facilitate diffusion from the antibiotic disc and improve bacterial growth.

تسهل انتشار المضادات من الأقراص وتدعم نمو البكتيريا

③ توحيد الأقراص إلي بنا نختبر حساسية MO تجاهم

C. The antibiotic disc is made from filter paper. The concentration of certain antibiotic (antibiotic amount per disc area) should be **standardized** to ensure consistency in results. Knowing that antibiotic concentration is a factor affecting the diffusion rate from disc into the surrounding medium.

④ توحيد حرارة الحاضنة عشان دقة النتائج

D. The incubation temperature should be **standardized** to be 37 °C. Since temperature is also a factor affecting rate of diffusion (proportional relationship).

↑ Temperature ↑ Diffusion

E. The incubation time should be **standardized** to be from 18 to 24 hours (overnight incubation).

⑤ توحيد مدة الحضانة [18-24]h

← بمناسبة كلمة توحيد، بنهمكم تدرسوا أو تقرأوا كتاب التوحيد في الإجازة

بنغطي سطح agar بالبكتيريا.
إلي بدي أقيس حساسيتها لأكتر من المضاد
وبعدين بشبت الأقراص على السطح و بشوف منطقة التثبيط و بقيس قطرها

Experimental:

1. Prepare a bacterial lawn using MHA.
2. Mark the position for the discs with a marker pen on the bottom of the plate
3. With a sterile forceps (by ethanol followed by heating) pick a disc from the container.
4. Place the disc onto the surface of the agar at the marked position. The discs must be distributed evenly and not too close to the plate edge.
5. Use a sterile loop to help lacing the disc on the agar.

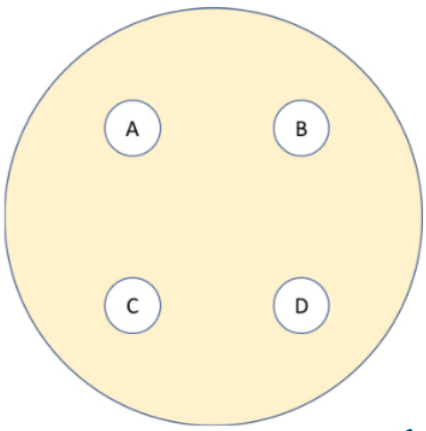
لازم نخلي مسافة حلوة بين الأقراص و مابكونو الأقراص قريبة من الأطراف للطبق (Petri dish) ← إذا كانت 6 أقراص نستعمل Petri dish قطره 9cm

Note:

a. The minimum distance between disc centers to neighboring disc center is 24 mm, and also no closer than 10-15 mm from the edge of Petri-dish). A maximum of 6 discs may be placed on a 9-cm Petri-dish, and 12 discs on a 15-cm dish. Reduce the number of applied discs per plate if overlapping zones of inhibition are encountered.

b. A disc should not be relocated once it has come into contact with the agar surface because some of drug diffuses instantaneously. Instead, place a new disc in another location on the agar.

6. Repeat steps 3 and 4 with other antibiotics disc
7. Incubate the plate inverted at 37 °C for 24 h



اللهم يا جماعة
هون عندكم وسط
مزروع فيه بكتيريا
و ثبتنا عليه 4 أقراص
مضاد و بعدين حضانة

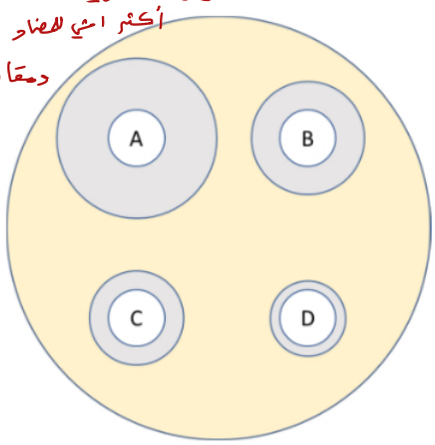
بعد الحضانة كل ما زادت
المنطقة الشفافة حول القرص ← كل ما كانت
عدد البكتيريا الحساسة أكتر ← يعني هاي
البكتيريا حساسة لهاد المضاد (القرص)

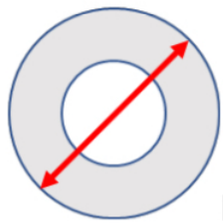
اللهم اشفي مرضى المسلمين
وارحم موتاهم

Zone of inhibition measurement and interpretation

1. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily prepared, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted Petri plate

   Note:
   If there is NO zone at all, report it as 0, even though the disc itself is around 7 mm.





Inhibition zone diameter

2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subculture, re-identified, and retested.

3. These zones of inhibition are then recorded for size; the diameter of the zone is proportional to the amount of anti-microbial agent, the solubility of the agent, the diffusion coefficient, and the overall effectiveness of the agent. Zones observed on the plates are then compared to standard data to determine if the isolate is truly sensitive to the antibiotic.

4. The sizes of the zones of inhibition are interpreted by referring to: **Zone size interpretive table for the Kirby Bauer test (CLSI table).**

هاد جدول فيه بـ E.coli أنواع مع
مختلفة من المضادات

Table 1: Panel of antibiotics used, their concentrations and zone diameter interpretative standards for *E.coli* (CLSI, 2007)

Group of Antimicrobial agents	Antimicrobial agents	Disk contents	Zone diameter, nearest whole (mm)			Manufacturer
			R	I	S	
Penicillin	Ampicillin	10 µg	≤ 13	14–16	≥ 17	Oxoid Ltd. Basingstoke, Hampshire, England
β-lactamase inhibitor combination	Amoxicillin-clavulanic acid	20/10 µg	≤ 13	14–17	≥ 18	
Cephems	Ceftriaxone	30 µg	≤ 13	14–20	≥ 21	
Amino glycosides	Gentamicin	10 µg	≤ 12	13–14	≥ 15	
Tetracycline	Tetracycline	30 µg	≤ 11	12–14	≥ 15	
	Doxycycline	30 µg	≤ 10	11–13	≥ 14	
Fluoroquinolones	Ciprofloxacin	5 µg	≤ 15	16–20	≥ 21	
Quinolones	Nalidixic acid	30 µg	≤ 13	14–18	≥ 19	
Folate pathway inhibitor	Trimethoprim-sulfamethoxazole	1.25/23.7 µg	≤10	11–15	≥ 16	
Phenicoles	Chloramphenicol	30 µg	≤ 12	13–17	≥ 18	

Classification of microorganisms according to susceptibility test results

1. **Susceptible (S):** an organism is called susceptible to a drug when the infection caused by it is likely to respond to treatment with this drug, at the recommended dosage.
   هي Sensitive
   يستجيب للمضاد

2. **Intermediate Susceptibility (I):** covers two situations
   ① حالة الاستجابة بين المقاومة والحساسية (عادي تعطيني واحد)
   ② ممكن خلي المضاد شغال في جسم المريض حتى بعد ما وقفه في التعطيني I و هاد لسببين تحت

   1. The organism cannot be classified as either susceptible or resistant, but is interpreted as being or intermediate (I) Susceptibility to a given drug.

   2. The clinical interpretation of this category is that the organisms tested may be inhibited by the antimicrobial agent provided that either:

      a. Higher doses of drug are given to the patient, or
      b. The infection is at a body site where the drug is concentrated.

3. **Resistant (R):** this term implies that the organism is expected not to respond to a given drug, irrespective of the dosage and of the location of the infection.

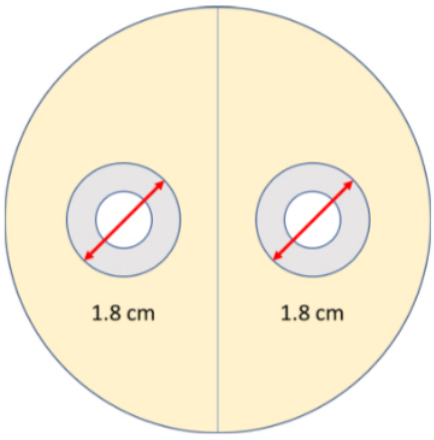
هو ده اختبرنا استجابة E.coli لهذا دين

Example: CLSI وطلعت نفس القيم !! هل نحكم مباشرة النتيجة تشبهها؟ أكيد عشان هو مرجعي

Suppose we test the sensitivity of E-coli against ampicillin (penicillin) and ceftriaxone (cepheme).

Suppose the two zone of inhibition were 1.8 cm = 18 mm.

Compere the antimicrobial activity of ampicillin and ceftriaxone against E-coli. And classify them as a susceptible, intermediate or resistant.



بعدما تشوفوا منطقة التثبيط
وتروحوا للجدول مع إنهم الأقطار = 18 mm
لا إنه واحدة عشان حالة E.coli S
والثانية I

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Phenicoles	Chloramphenicol	30 µg	≤ 12	13–17	≥ 18

★ R: resistant, I: intermediate sensitivity, S: sensitive

اللهم انصر عبادك تحت الأنقاض
اللهم أعز الإسلام والمسلمين
اللهم وحدنا بين ودائعك
ربِّ أسألك الجنة وأعوذ بك من النار