


BCH 462- Biotechnology & Genetic engineering [Practical]

Lab (6) Kirby-Bauer test



Antibiotics

- **Antimicrobial drugs** interfere with the growth of microbes within a host.
- A true **antibiotic** is an **antimicrobial chemical** produced by microorganisms against other microorganisms.
- **Many drugs are now:**
 1. Completely synthetic.
 2. Natural drug is manipulated to change its structure somewhat called **semi-synthetics**.
- Bacteria respond in different ways to antibiotics and chemosynthetic drugs, even within the same species.

 Remember !!
Resistance **×** Sensitivity

Antimicrobial Susceptibility Testing

- **Antimicrobial susceptibility testing** is laboratory test which determines how effective antibiotic therapy is against a bacterial infections.
- **The goals of testing are:**
 1. To assure susceptibility to drugs of choice for particular infections.
 2. To detect possible drug resistance in common pathogens.
- Different automated and manual methods have been developed in order to screen the antimicrobial susceptibility.

Kirby-Bauer test

- First developed in the 1950s by **W. Kirby** and **A. Bauer**.
- Also called the **disc diffusion** test or **zone of inhibition** test.
- Is a standard test for antibiotic susceptibility.
- It has been superseded in clinical labs by automated tests.
- However, the K-B is still used in some labs or used with certain bacteria that automation does not work well with.





Remember !!

Resistance × **Sensitivity** = Susceptibility

Kirby-Bauer test

- This test is used to determine the resistance or sensitivity of aerobes or facultative anaerobes to specific chemicals, which can then be used by the clinician for treatment of patients with bacterial infections.
- **Resistance** is the ability of microbes to survive in the presence of antibiotic.
- **Antibiotic sensitivity** is the susceptibility of bacteria to antibiotics.
- It tests the ability of antimicrobial agents to inhibit the growth of microorganisms over an 18-24 hour period of contact.



Pause and Think Can K-B test used with anaerobic bacteria?

Kirby-Bauer test

Advantages:

1. Test simplicity
2. Does not require any special equipment
3. Least costly of all susceptibility methods
4. Flexibility in selection of disks for testing

Disadvantages:

1. Lack of mechanization or automation of the test.
2. Not all fastidious or slow growing bacteria can be accurately tested by this method.
3. Qualitative

Practical Part

Practical part

- **Aim:**

To test the ability of antimicrobial agents to inhibit the growth of microorganisms using Kirby-Bauer test method.

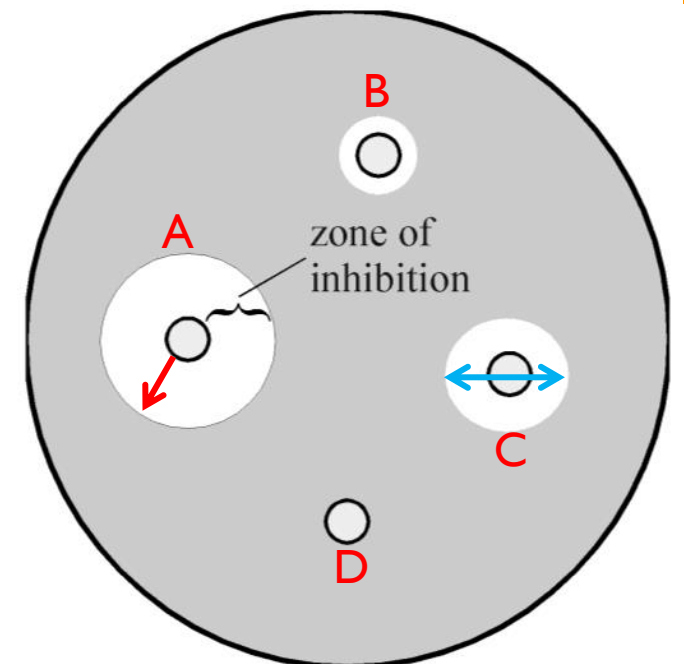
- **Principle:**

- The activity of the antimicrobial drug is evaluated by the ability of the antibiotic disks with certain concentration to inhibit the microbial growth.
- If substantial antimicrobial activity is present, then a zone of inhibition appears around the test product.
- The zone of inhibition is simply **the area on the agar plate that remains free from microbial growth.**
- The diameter of the zone of inhibition is usually related to the level of antimicrobial activity present in the sample or product.
 - (a larger zone of inhibition usually means that the antimicrobial is more effective).

Notes

- The **presence or absence** of an inhibitory area (zone of inhibition) around the disc identifies the bacterial sensitivity to the drug.
- A **larger zone** of inhibition usually means that the antimicrobial is **more potent.**
- The **concentration** of antibiotic that diffuses into the media **decreases** with **increasing distance** from the source.
- Therefore, the **more sensitive** the bacteria are to a given antibiotic, the **larger** the clear bacteria-free zone that forms around the disk containing that antibiotic.

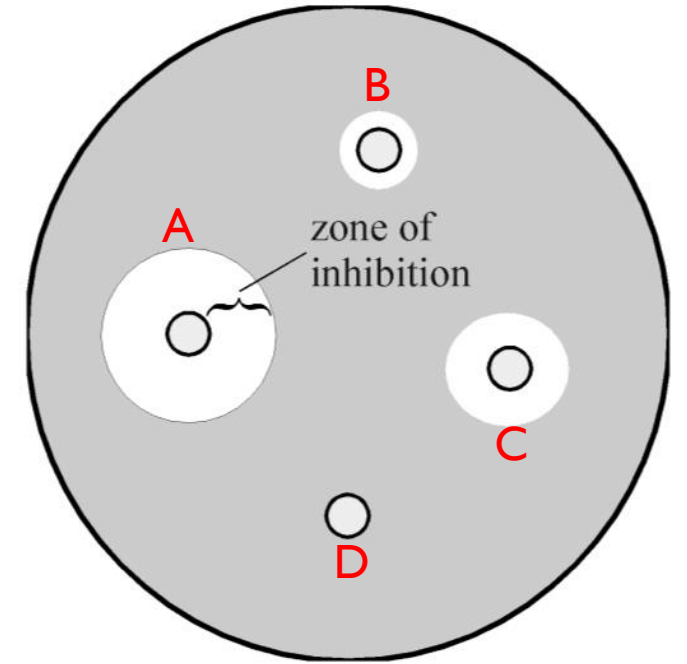
💡 **Pause and Think** What types of test is Kirby-Bauer? quantitative OR qualitative?



Choose the right answer regarding the figure:

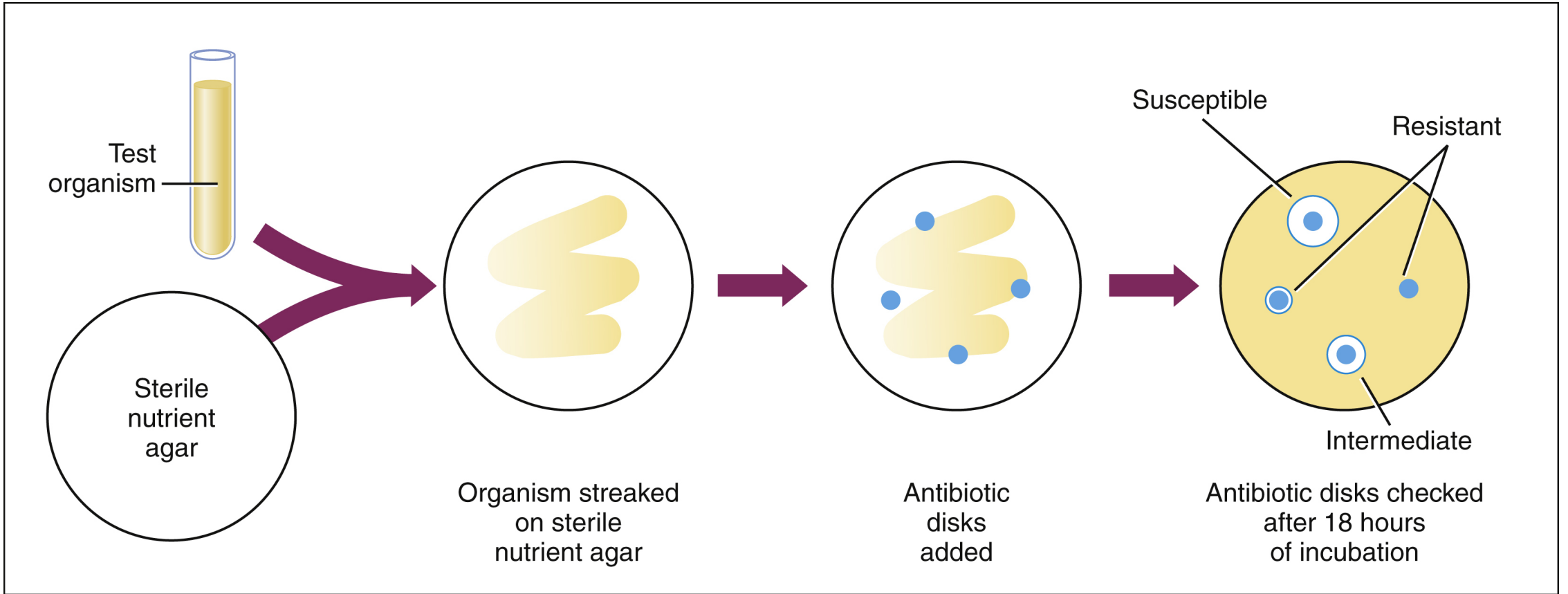
- 1- To which antibiotic bacteria was the **most resistance**?
- 2- To which antibiotic bacteria was the **most sensitives**?
- 3- which antibiotic was the **most potent**?

A , B , C ,D

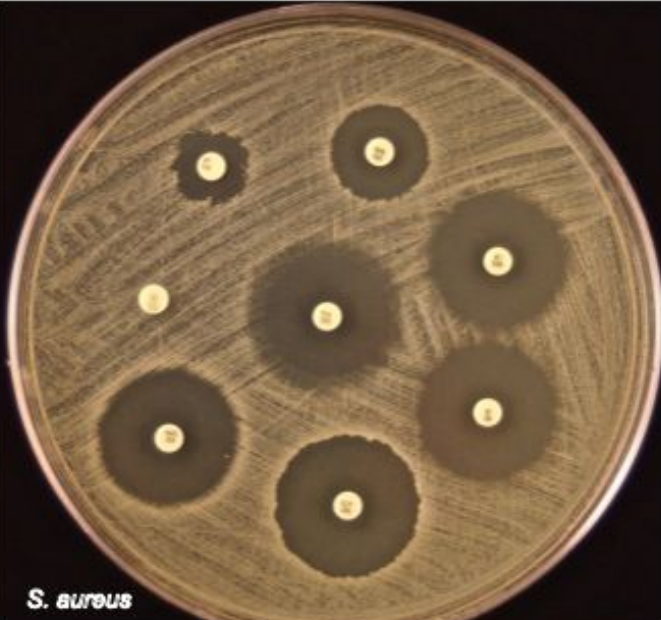


Performing steps

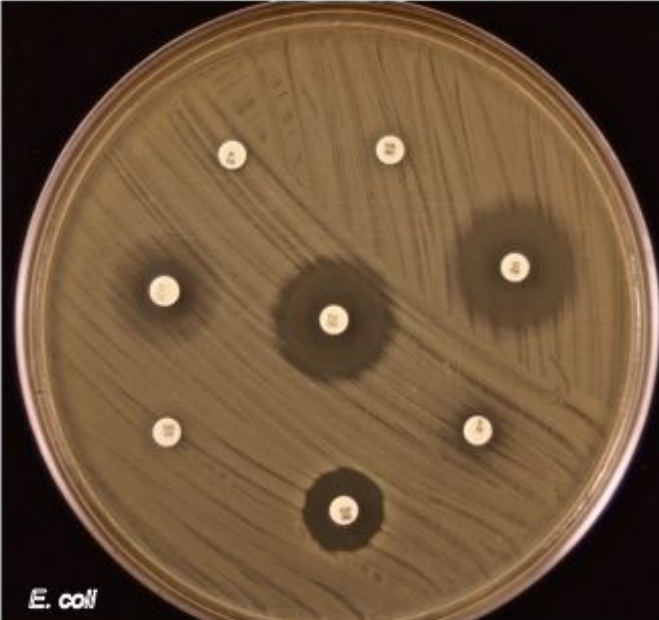
1. The test is performed by applying a bacterial inoculum of approximately $1-2 \times 10^8$ CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate.
2. Up to 12 commercially-prepared, fixed concentration, paper antibiotic disks are placed on the inoculated agar surface.
3. Plates are incubated for 16–24 h at 35°C prior to determination of results.
4. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter (The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium).



Overview of Kirby-Bauer test



S. aureus



E. coli

