KIRBY-BAUER TEST

BCH462- Practical

Antibiotic:

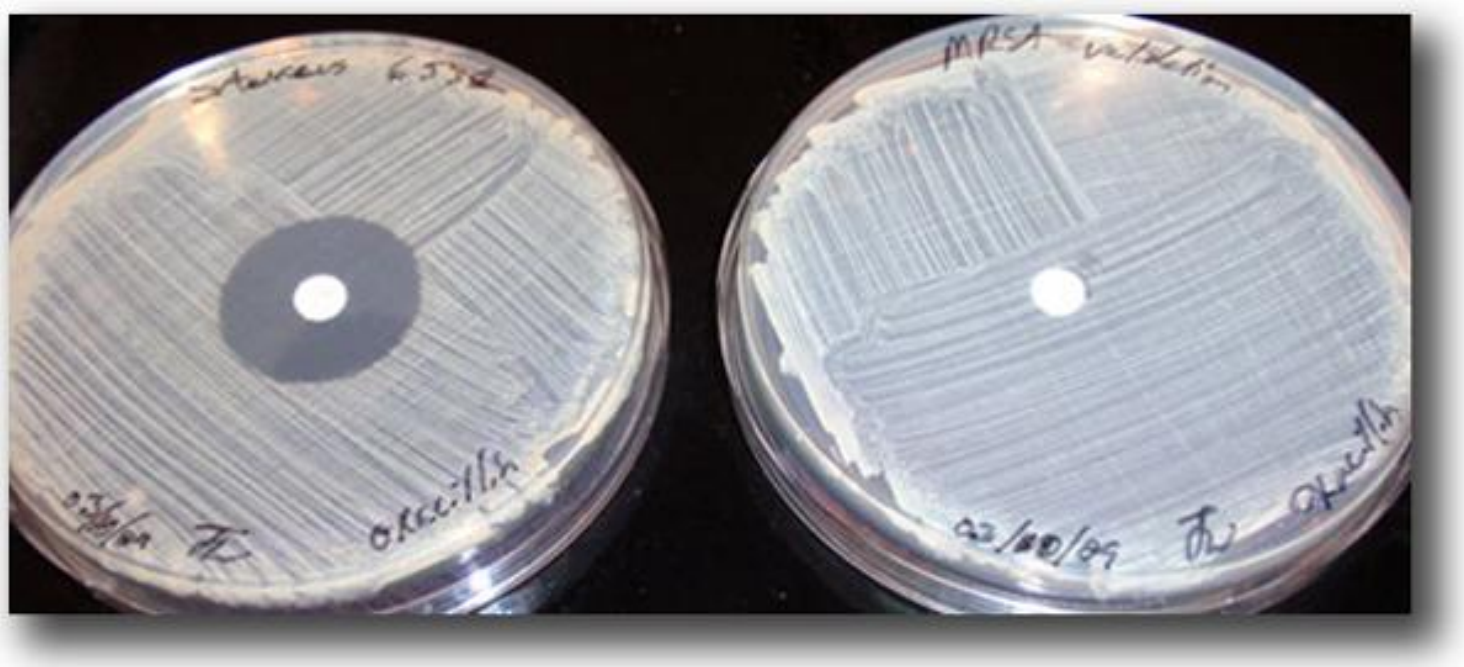
- A true antibiotic is an antimicrobial chemical produced by microorganisms against other microorganisms.
- In addition, many drugs are now completely synthetic or the natural drug is manipulated to change its structure somewhat, the latter called semi-synthetics.
- Bacteria respond in different ways to antibiotics. WHY?

Antimicrobial susceptibility testing :

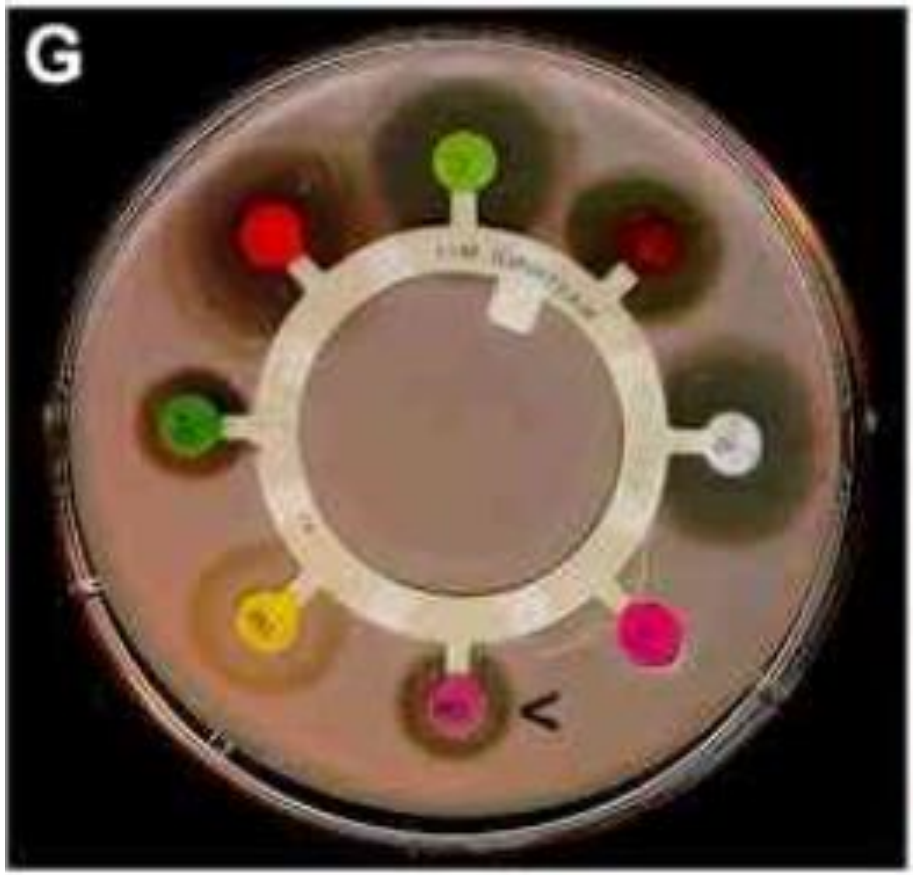
- Important task. WHY?
- Goal ?
- Automated and manual methods.

Kirby-Bauer test:

- Also called the **disc diffusion test** or **zone of inhibition 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.
- It tests the **ability of antimicrobial agents to inhibit the growth of microorganisms** over an 18-24 hour period of contact.
- The presence or absence of an inhibitory area (zone of inhibition) around the disc identifies the bacterial sensitivity to the drug.
- Advantages ?
- Disadvantages ?



A zone of Inhibition is evident around the oxacillin disk for S. aureus, left, but not for Methicillin-resistant S. aureus (MRSA), right.



Tests for sensitivity and resistance to antibiotics. The size of the zones of inhibition of microbial growth surrounding the antibiotic disks on the plate are an indication of microbial susceptibility to the antibiotic.

PRACTICAL PART

Aims:

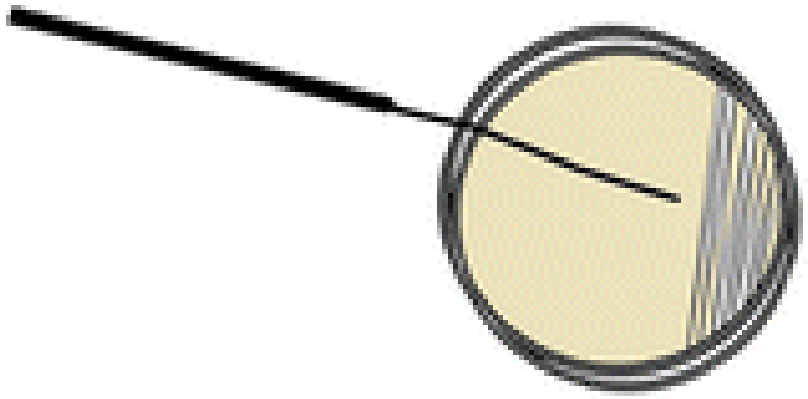
- To test the ability of different antimicrobial agents to inhibit the growth of Enterococcus faecalis using Kirby-Bauer test method.

Principle:

- The activity of the antimicrobial drug is evaluated by the ability of the antibiotic disks with certain concentration in inhibition of 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 potent**).

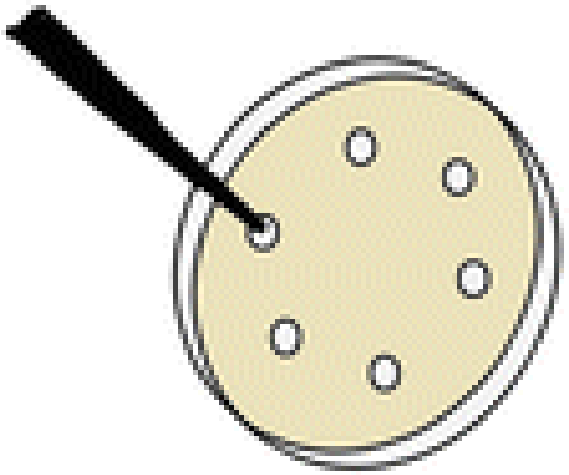
Performing steps:

1. The test is performed by applying a bacterial inoculum of approximately 1–2×108 CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate.



Performing steps cont':

2. Up to 12 commercially-prepared, fixed concentration, paper antibiotic disks are placed on the inoculated agar surface (Figure 1).



Performing steps cont':

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 millimetre

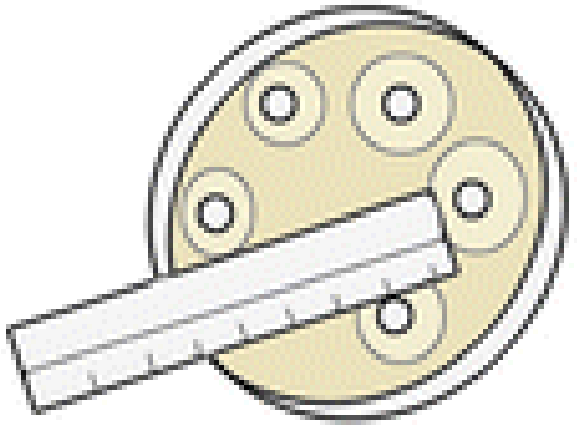


Plate design: