

Experiment (6): Kirby-Bauer test

Aim:

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

Introduction:

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 and chemosynthetic drugs, even within the same species. ⁽¹⁾

- ✚ PAUSE AND THINK → What could be the cause of the different response of different bacterial species or even bacteria from the same specie toward the antibiotic? How?

An important task is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. Different automated and manual methods have been developed in order to screen the antimicrobial susceptibility. ⁽²⁾

The Kirby-Bauer test (also called the disc diffusion test or zone of inhibition test) is a standard test for antibiotic susceptibility that has been used for years. First developed in the 1950s, it was refined and by W. Kirby and A. Bauer. It has been superseded in clinical labs by automated tests.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.

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. ⁽¹⁾

The advantages of the disk method are the test simplicity that does not require any special equipment, least costly of all susceptibility methods and flexibility in selection of disks for testing. The disadvantages of the disk test are the lack of mechanization or automation of the test. Although not all fastidious or slow growing bacteria can be accurately tested by this method, the disk test has been standardized for testing *streptococci*, *Haemophilus influenzae*, and *N. meningitidis* through use of specialized media, incubation conditions, and specific zone size interpretive criteria. ⁽³⁾

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).⁽⁴⁾

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 (Figure 1).
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 (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).

References:

1. [https://bio.libretexts.org/Demos%2C_Techniques%2C_and_Experiments/Microbiology_Labs_I/09%3A_Kirby-Bauer_\(Antibiotic_Sensitivity\)](https://bio.libretexts.org/Demos%2C_Techniques%2C_and_Experiments/Microbiology_Labs_I/09%3A_Kirby-Bauer_(Antibiotic_Sensitivity)).
2. Reller L, Weinstein M, Jorgensen J, Ferraro M. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clinical Infectious Diseases* 2009;49: 1749–55.
3. Clinical and Laboratory Standards Institute, Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A10, 2009 Wayne, PA Clinical and Laboratory Standards Institute
4. <https://microchemlab.com/test/zone-inhibition-test-antimicrobial-activity>