Antimicrobial Activity

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome .In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Several bioassays can provide rapid results of the antimicrobial agent's effects and a better understanding of their impact on the viability and cell damage inflicted to the tested microorganism.

After the revolution in the "golden era", when almost all groups of important antibiotics, were discovered, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance. Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health.

For this reason, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major sources of new drug molecules today. They are derived from prokaryotic bacteria, eukaryotic microorganisms, plants and various animal organisms. Microbial and plant products occupy the major part of the antimicrobial compounds discovered until now.

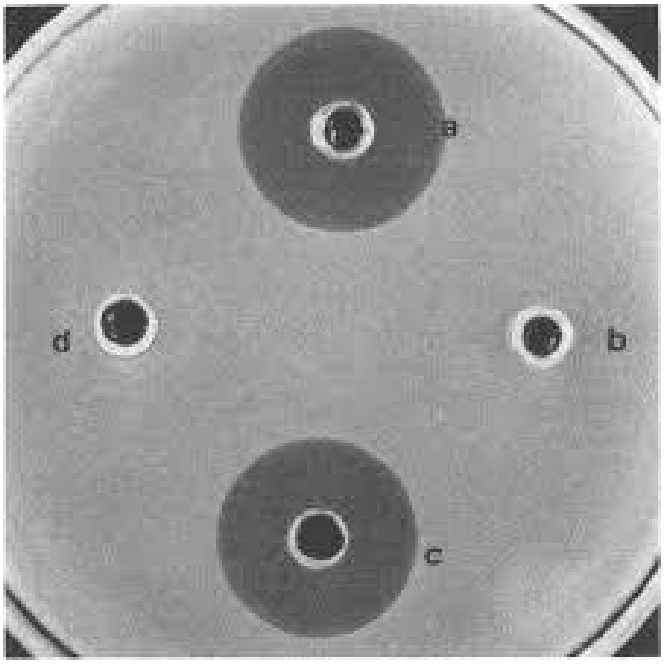
There are 4 *in vitro* antimicrobial susceptibility testing methods

A. The Well-Diffusion method

1. Inoculate 10 ml of *E. coli* broth with 0.2 ml of N.B.
2. Incubate at 37°C for 24 hours in aerobic conditions.
3. Spread 0.1 ml of bacteria (pathogenic bacteria) on the Muller-Hinton agar plates.
4. Cut 5 wells with 6mm sterile cork-borer, fill with 100 µl of the Antimicrobial material solution (e.g. plant extract).
5. Incubate the plates at 37°C for 24 hours.

6. Measure the inhibition zones and record the results.

Antimicrobial activity is detected by a zone of clearing around the supernatant well.



B. The Disk-Diffusion Method

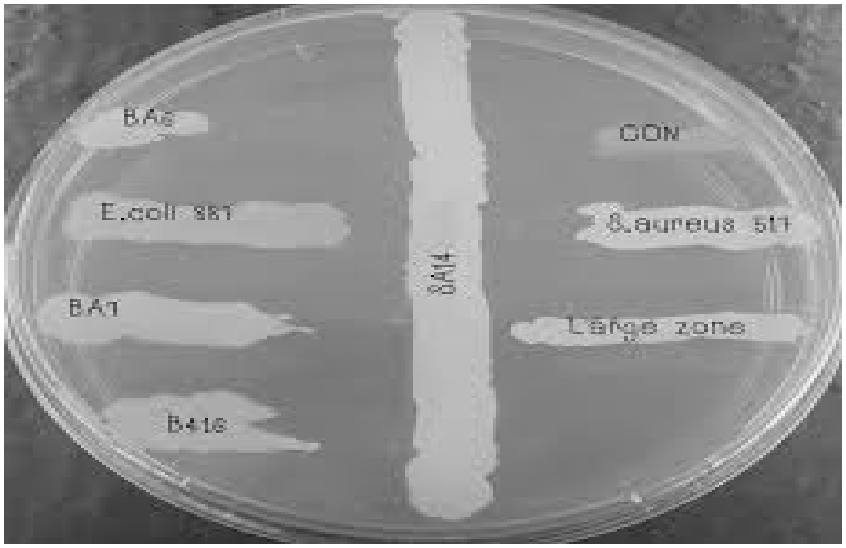
1. Inoculate 10 ml of *E. coli* broth with 0.2 ml of N.B.
2. Incubate at 37°C for 24 hours in aerobic conditions.
4. Spread 0.1 ml of indicator bacteria (pathogenic bacteria) on the Muller-Hinton agar plates.
5. Submerse 6mm sterile disks of filter paper in Antimicrobial material solution (e.g. plant extract).
6. By sterile forceps, place the submersed disks on the inoculated plates.
7. Incubate the plates at 37°C for 24 hours.
8. Measure the inhibition zones and record the results.

Antimicrobial activity is detected by a zone of clearing around the disk.

C. The Flip-Streak Method

1. Streak Antimicrobial material solution (e.g. plant extract) across the surface of Muller-Hinton agar plates
2. Flip the agar onto Petri dish cover with a sterile spatula.
3. Streak indicator bacteria (pathogenic bacteria) across the surface of the agar perpendicularly to the producer streaks.
4. Incubate the plates at 37°C for 24 hours.

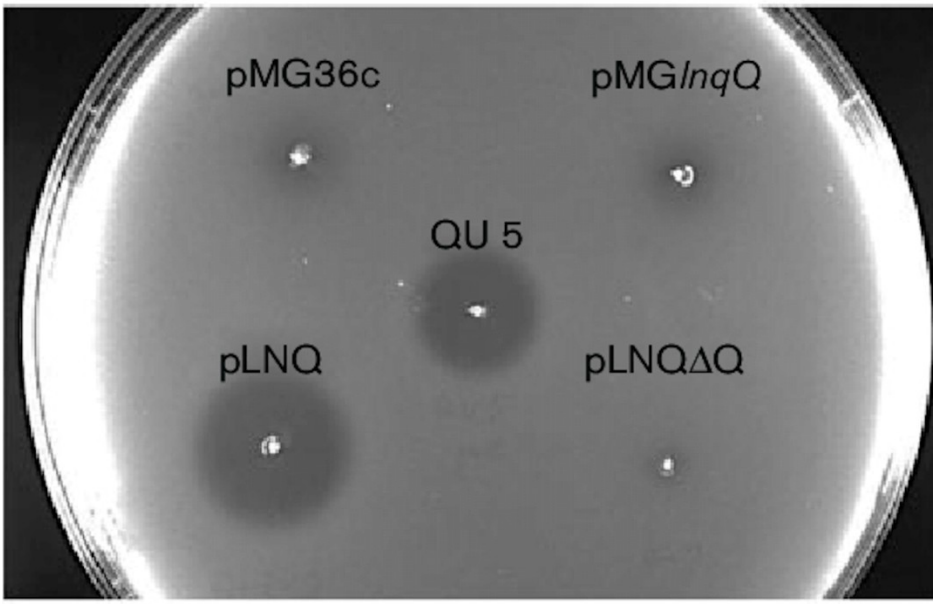
Antimicrobial activity is detected by a zone of inhibition at the intersection of the material and indicator isolate.



D. The Spot-on-the-Lawn Method

1. Spot Petri plates containing M.H agar with 2µl of the antimicrobial solution.
2. Melt NA agar (9 ml in each tube) to 45°C, inoculate with indicator bacteria (pathogenic bacteria) and mix well.
3. Pour the inoculated tubes on the spotted plates.
4. Incubate the plates at 37°C for 24 hours.
5. Measure the inhibition zones and record the results.

Antimicrobial activity is detected by a zone of clearing around the producer colony.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pMG*lnq*Q
QU 5
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