Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY LABORATORY

EXPERIMENT # 8

AIM

To study the anti-microbial sensitivity of antibiotics.

BACKGROUND

Different antibiotics are produced by different microorganisms (moulds and Streptomyces). Their mode of action on the microorganisms are different. Some antibiotics interfere with protein synthesis eg. Chloromycetin, kanamycin, tetracycline, etc. Others like penicillin and Ampicillin stop cell wall synthesis. Some affect DNA synthesis, eg. Novobiocin. Clinically different antibiotics are selected for different causative microorganisms of different diseases. Their selection depends upon antimicrobial sensitivity of antibiotics and their side effects.

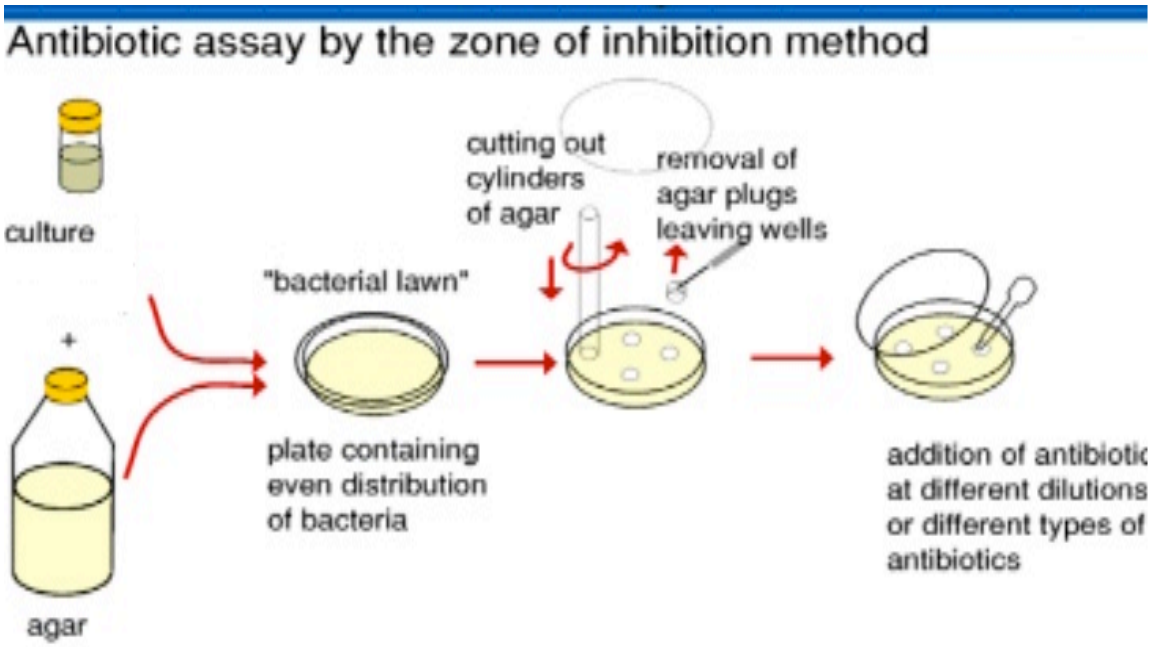
After the causative organism is isolated, a broth culture is grown in a medium like Nutrient Broth and flooded on a suitable agar plate. After the surface is dry, the antibiotic discs are placed carefully and the plates are incubated for 18-20 hours. The zones of inhibition can be measured and the result is written as sensitive, moderately sensitive or resistant.

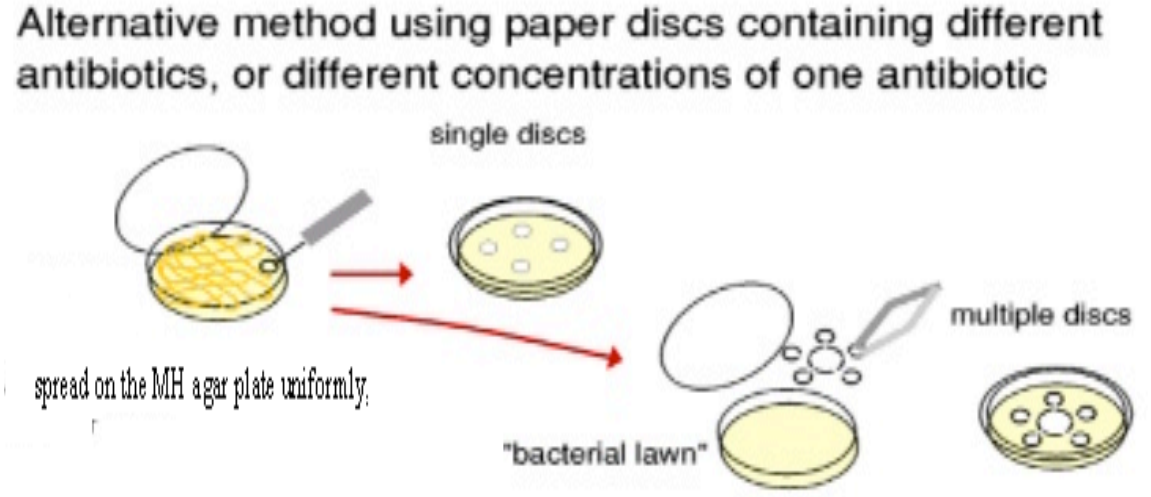
PROCEDURE

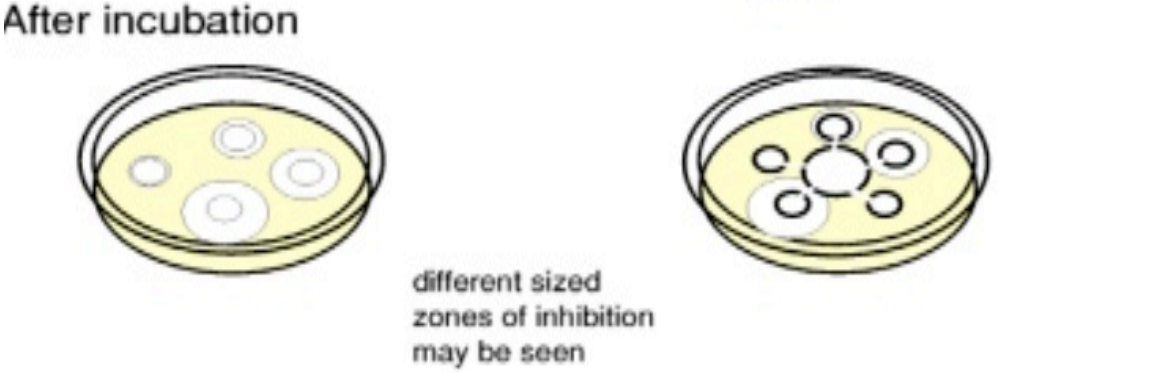
There are various methods for doing antimicrobial sensitivity, but the disc diffusion method is widely used

1) Take 5-10 similar clones of causative organisms/test culture and transfer to about 2 ml of Nutrient Broth in a test tube and incubate in a shaker at 30-37°C for 4-6 hours, until a very fine turbidity appears.
2) Take 100 µl(SECOND METHOD) of this culture and spread on the MH agar plate uniformly, using a glass rod and turntable and allow the surface to dry in Laminar Flow Hood for sometime (care must be taken that not a single drop of culture falls on the laminar flow bench).
3) Aseptically place the antibiotic disc, with a pair of forceps, onto the agar surface. The firm contact of these discs on the agar surface is necessary for uniform diffusion of antibiotic in the surrounding medium(Fig-1).
4) Write the codes of the antibiotics on the other side of the plate. Refer to Table 1 for the antibiotic codes.
5) Incubate the plates upside down in an incubator at 30-37°C for 18-20 hours.
6) Measure the zones of inhibition (clear zones) of each antibiotic with the help of a scale and express the results as sensitive, moderately sensitive or resistant(Pictures 1 and 2).

Antimicrobial sensitivity testing by different methods







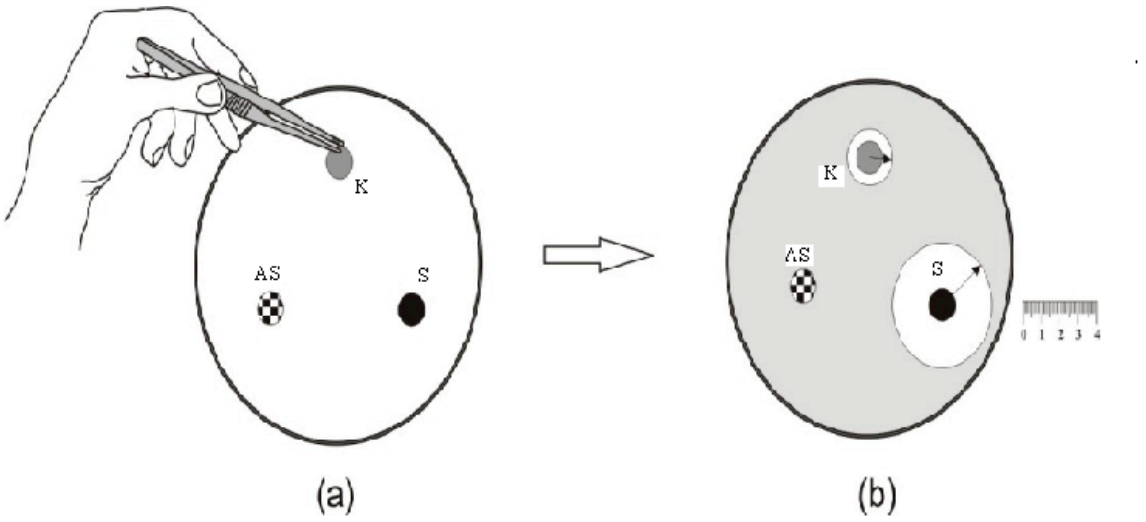
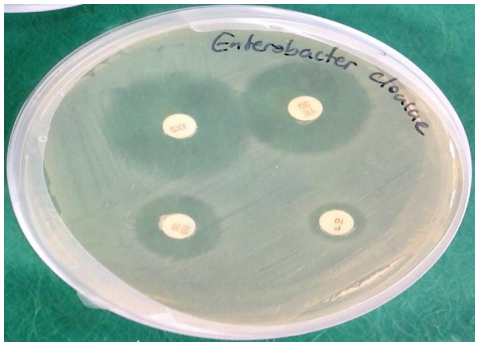


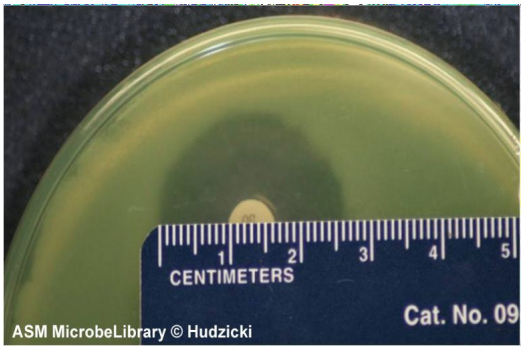
Fig-1

Table 1: Nomeclature for antibiotic discs to be used in diffusion sensitivity tests

Antibiotic	Symbol
Ampicillin	AS
Kanamycin	K
Streptomycin	S



1



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PRECAUTIONS

1. Do not over incubate your test plates

2. Prepare bacterial lawn of appropriate dilution