Department of Medical Laboratories Techniques
Practical Microbiology
Lab.9: Antibiotic Sensitivity Test
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Antibiotic sensitivity: Is a term used to describe the susceptibility of bacteria to antibiotics, to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*.

Different methods have been employed in laboratories to determine the susceptibility of microorganisms to antimicrobial agents. Two main methods for the *in vitro* determination of the susceptibility of microorganisms against antimicrobials are the **disk agar diffusion test**, wherein antibiotic-impregnated disks are used with an agar medium, and the **dilution techniques**, wherein the test microorganism is exposed to increasing concentration of an antibiotic either in broth or agar.

Minimum inhibitory concentration (MIC)

The MIC is the minimum (lowest) concentration of an antibiotic that will inhibit the growth of a bacterial strain.

Minimum bactericidal concentration (MBC)

The MBC is the lowest concentration of the antibiotic that will kill a bacterial strain.

The disk agar diffusion method

Is the most widely used laboratory technique for antimicrobial susceptibility test. This method is simple, requires a short duration, and easy to perform. Bacterial isolation can be done using a general medium, wherein various bacteria can grow, and selective media that allows growth of specific genera.

The disk diffusion method is performed using Mueller-Hinton Agar (MHA), which is the best medium for routine susceptibility tests because it has good reproducibility, and gives satisfactory growth of most bacterial pathogens.

Disk sensitivity tests

1. Prepare MHA from the dehydrated medium according to the manufacturer's instructions. Media should be prepared using distilled water or deionized water.
2. From a pure bacterial colonies, Inoculate the agar by streaking with the swab containing the inoculum.
3. place antibiotic disk on the surface of the inoculated and dried plate. Immediately press it down lightly with the instrument to ensure complete contact between the disk and the agar surface.
4. Position disks such that the minimum center - center distance is 24 mm and no closer than 10 to 15 mm from the edge of the petri dish. A maximum of six disks may be placed in a 9-cm petri dish and 12 disks on a 150 mm plate. Reduce the number of disks applied per plate if overlapping zones of inhibition are encountered.
5. Incubate plates in an inverted position at 30°C or at an optimum growth temperature.
6. Observe for the zone of inhibition after 16 to 18 hours. Slow growing organisms may require longer incubation period.
7. Read and record the diameter of the **zones of inhibition** using a ruler graduated.
8. Compare the diameter of the zone of inhibition of the test isolates with those in the chart of interpretative standard for veterinary pathogens.
9. Report result as Resistant (R), Intermediate (I) or Susceptible (S).

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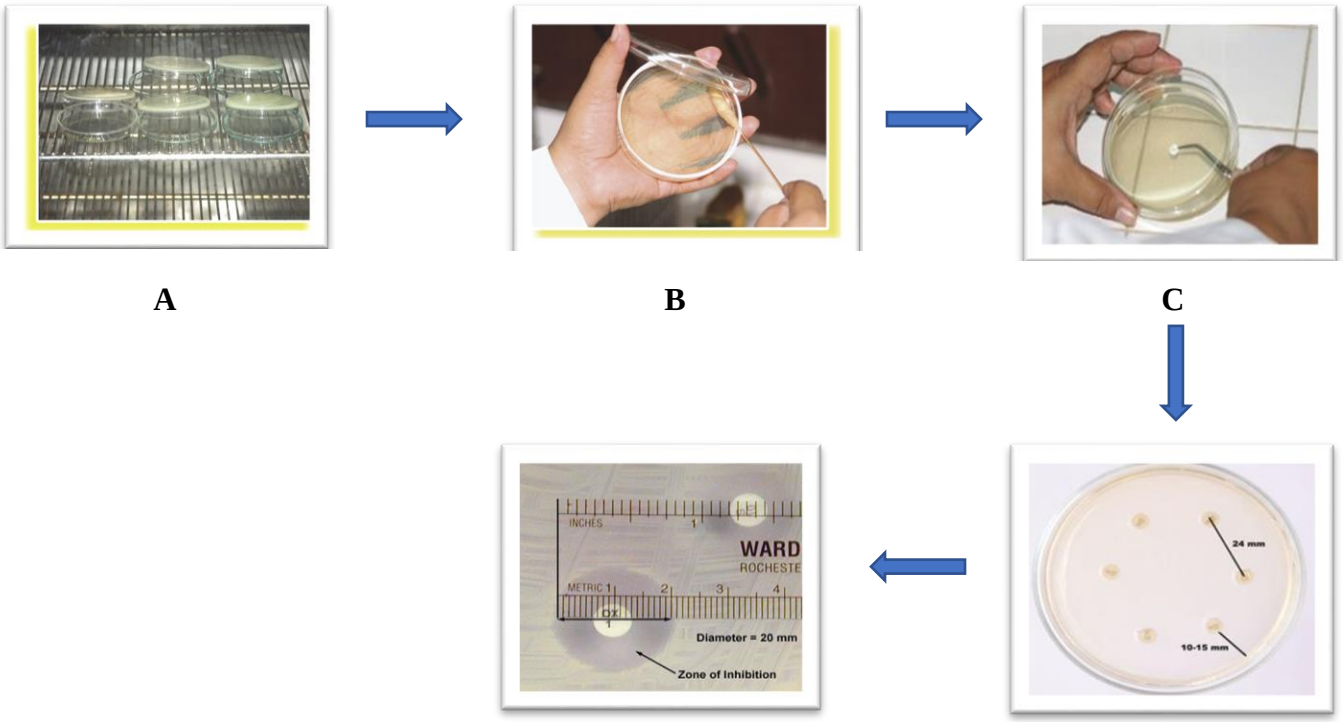

TABLE 1
Details of the antibiotics that were used in the study to test for antibiotic resistance

Group	Antibiotic	Abbreviation	Generally accepted antibiotic disc concentrations (μg)	Inhibition zone (mm)		
				Resistant	Intermediate resistant	Susceptible
Aminoglycosides	Streptomycin	S	10	≤11	12 – 14	≥15
Macrolides	Erythromycin	E	15	≤13	14 – 22	≥23
Tetracyclines	Oxytetracycline	OT	30	≤14	15 – 18	≥19
Beta-lactams	Ampicillin	AP	10	≤11	12 – 14	≥15
	Penicillin G	PG	10	≤20	21 – 28	≥29
	Methicillin	MT	5	≤9	10 – 13	≥14
Glycopeptides	Vancomycin	V	30	≤9	10 – 11	≥12
	Nitrofurantoin	NI	300	≤14	15 – 18	≥19
Sulphonamides	Sulphamethoxazole	Smx	300	≤10	11 – 15	≥16

Source: The concentration used as well as the inhibition zone measurements were according to the National Committee on Clinical Laboratory Standards[23]
Note: The abbreviations are as they appeared on the antibiotic discs.