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Review Article

ANTIMICROBIAL SCREENING

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ABSTRACT

To measure effectiveness of different antibiotics by measuring zones of inhibition on bacterial culture plates by diffusion method. Objective: The goal of research project is to measure the effectiveness of different antimicrobial agents by measuring zones of inhibition on bacterial culture plates. Antibiotics activity of these compounds has been assayed against two different of bacteria (one gram-positive and one gram-negative) by agar diffusion method.

KEY WORDS: antibacterial activity, antifungal activity, minimum inhibitory concentration, minimum bactericidal concentration.

INTRODUCTION

Antimicrobial agents are chemicals that are used against bacteria. There are many such agents available. Because there are many different situations where bacterial control is important, no antimicrobial agent is effective in all situations[1]. There are many additional factors that have to consider in order choosing an appropriate antimicrobial agent for a given situation. For example, the chemical properties of the agent (e.g., Ph and solubility). To know whether the compound is toxic-to humans, other animals, plants, or beneficial bacteria. Finally, definitely want to know that the compound is effective against the organism(s) trying to eliminate[2].

This shows one method of measuring the effectiveness of an antimicrobial agent against bacteria grown in culture. This is called the Kirby-Bauer disk-diffusion method, and here is how it works. The bacteria of interest are swabbed uniformly across a culture plate. Then a filter-paper disk, impregnated with the

compound to be tested, is placed on the surface of the agar. The compound diffuses out from the filter paper into the agar[2,.3]. The concentration of the compound will be higher next to the disk, and will decrease gradually as distance from the disk increases[4]. If the compound is effective against bacteria at a certain concentration, no colonies will grow wherever the concentration in the agar is greater than or equal to that effective concentration. This region is called the "zone of inhibition." Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound[5]

MATERIALS AND METHODS[6,7]

Antibiotics activity of these compounds has been assayed against two different of bacteria (one gram-positive and one gram-negative) by agar diffusion method. Generally, the antibiotics activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar. The bacterial inhibition can be measured by two methods:

1. Serial dilution method
2. Diffusion method

The serial dilution method is very much useful for the determination of the antibiotics activity. But it is not much useful for the qualitative detection tests and also for the evaluation of large number of compounds. The agar diffusion method consists of

1. Cup-plate method: - In this method with the help of a sterile borer, cup or disc is formed in the nutrient agar. The test solution or standard solution is added in this cup around which we get the zone of inhibition after incubation.
2. Filter paper strip method: - In this method the test solution that has to be screened is adsorbed on the. Filter paper which is then placed sterile plate inoculated with' the organism.

The method adopted in this investigation was cup-plate method. In this method, cups or discs of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums. The test compounds are introduced into the disc and the diameter of the zone of inhibition was measured. All the test compounds were evaluated for antibiotics activity against *Staphylococcus aureus* (gram-positive) and Escherichia coli (gram negative).

Diffusion test

Here the drug is allowed to diffuse through a solid medium so that a gradient is established, the concentration being highest near the site of application of the drug and decreasing with distance. The test bacterium is seeded on the medium and its sensitivity to the drug determined from the inhibition of its growth. Several methods have been used for the application of the drug. It may be added to ditches or holes cut in the medium or to hollow cylinders placed on it. The method most commonly employed is to use filter paper discs, impregnated with antibiotics[8].

Ditches or hole is 6 mm in diameter and charged with appropriate concentrations of the drugs. The discs are stored dry in the cold. They may be prepared in the laboratory or purchased commercially A suitable dilution of a broth culture or a broth suspension of the test bacterium is flooded on the surface of a solid medium (Mueller—Hinton agar or nutrient agar). The plate is tilted to ensure uniform spreading and the excess broth pipette off. Inoculation may also be performed by spreading with swabs. After drying the plate (37 °C for 30 mins), antibiotic discs (four or five per 10 cm plate) are applied with sterile forceps. After overnight incubation, tile degree of sensitivity is determined by measuring the zones of inhibition of growth around the disc.

Dilution Test

The tube dilution method of performing antibiotic sensitivity tests is commonly employed as an accurate method for defining the minimal inhibitory concentration in

relation to pathogenic organisms. It is also used as a reference for comparing minimal inhibitory concentrations with the size of the zone of inhibition in the agar diffusion test. Although surveys have shown that there is no standardized method and technique of performing the tube dilution test, it is generally assumed that all of the diversified methods will yield the same results and interpretations. With the assistance of five experts, seven different tube dilution methods were compared; 16 antibiotics, and three organisms for each antibiotic, were used. The conclusions drawn are that, although the accuracy of a single method within its own confines is acknowledged, the minimal inhibitory concentrations and interpretations cannot be interpolated from one laboratory to another where a different technique is employed. The results are frequently discrepant. It is suggested that a uniform method be developed and promulgated for general use[9,10].

Materials and Equipment

Its require the following materials and equipment:

- at least 6 nutrient agar plates:
  - 3 plates will serve as controls, with no disinfectants,
  - 3 plates will serve as test plates, with disinfectant disks.
- Live *E. coli* culture & *Stapyloccus aureas* culture
- sterile swabs,
- sterile tube with 10 ml sterile water,
- filter paper,
- hole punch,
- forceps,
- Permanent marker.
- Autoclave
- Incubator
- Pipttes
- Burners

Safe Disposal of Plates

At the conclusion of the experiment, all plates should be disinfected for safe disposal.

1. The best way to dispose of bacterial cultures is to pressure-sterilize (autoclave)
2. Them in a heat-stable biohazard bag.
3. If autoclaves or pressure cookers are not available, an alternative is to bleach the plates.

a. Wear proper safety equipment (gloves, lab coat, eye protection) when working with the bleach solution; it is corrosive.
b. Saturate the plates with a 20% household bleach solution (in other words, one part bleach and four parts water).
c. Allow the plates to soak overnight in the bleach solution before disposing of them.
d. Please note that the bleach solution is corrosive and needs to be thoroughly rinsed afterwards.

CULTURE MEDIUM

Nutrient broth was used for the preparation of inoculums of the bacteria and nutrient agar was used for screening method.

Composition of the medium, Nutrient Agar

Peptone	5.0 gm
Sodium Chloride	5.0 gm
Beef Extract	1 .5 gm
Yeast extract	1.5 gm
Agar	1.5gm
Distilled water upto	1000 ml
pH	7.4 ± 0.2

The test organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1°C for 24 hours. They were stored in refrigerator. The stock cultures were maintained. Bacterial inoculums was prepared by transferring a loop full of stock culture to nutrient broth (100 nil) in conical flasks (250 ml) the flasks were incubated at 37±l °C for 48 hours before experimentation.

Solution of the test compounds. Solutions of test compounds were prepared by dissolving 1mg each in alcohol (10 ml; annular

grade). A reference standard for gram positive and gram negative bacteria were made from accurately weighed quantities of ceftazidime in alcohol. The nutrient medium was sterilized by autoclaving at 121 °C (151b/sq.inch) for 15 minutes. The Petri plates, tubes and flasks plugged with cotton were sterilized in hot air oven at 160°C for an hour. Into each sterilized Petri plate (10 cm diameter), about 30 ml of molten nutrient agar medium inoculated with the respective strains of the bacteria was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, one disc of 6 mm diameter was made with a sterile borer. The test solution at the concentration 10µg/ml was added to each disc aseptically and labeled accordingly. The plates were kept undisturbed for at least two hours at room temperature to allow diffusion of the solution properly in the nutrient agar medium. After incubation of the plates at 37±1°C for 24 hours, the diameter of zone of inhibition surrounding each of the discs was measured with the antibiotic zone reader.

This shows one method of measuring the effectiveness of an antimicrobial agent against bacteria grown in culture. This is called the Kirby-Bauer disk-diffusion method, and here is how it works. The bacteria of interest are swabbed uniformly across a culture plate. Then a filter-paper disk, impregnated with the compound to be tested, is placed on the surface of the agar. The compound diffuses out from the filter paper into the agar. The concentration of the compound will be higher next to the disk, and will decrease gradually as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow wherever the concentration in the agar is greater than or equal to that effective concentration. This region is called the "zone of inhibition." Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound.

Observation Table

The Compound showed antibiotics activity at the concentration of 10 □g/ml. Results of the activity are represented in table.

Sr. No.	Name of Compound	*Staph. Aureus*	*E.coli*
1.	Amoxicilin	18.0 mm.	23.0 mm.
2.	Ampicillin	15.0 mm.	18.0 mm.
3.	Cefixime	25.0 mm.	27.0 mm
5.	Standard Cefotaxime	32.0 mm.	28.0 mm

RESULT AND DISCUSSION

In the present research work two strains of microorganisms are selected. *Staphylococcus aureus is gram* positive strain& *E. coli* is as a negative strain for antibacterial screening. Ampicillin & Amoxicillin are two derivatives of Penicillines and Cefixime is a third generation Cephalosporin antibiotic.

According to antibiotics screening results zone of inhibition for gram negative microorganism *E.coli* shows less activity to Ampicillin (15 mm) and for Amoxycillin (18 mm) and shows more activity for Cefixime(25mm).

Zone of inhibition values for gram positive cocci i.e. *Staphylococcus aureus* for penicillins i.e. Ampicillin and Amoxycillin are 18 mm and 23 mm respectively.

Third generation Cephalosporines shows broad spectrum of activity against gram positive and gram negative microorganisms. Cefixime shows excellent activity aganist *Staphylococcus aureus* (27 mm).

From present research work it is observed that beta lactam antibiotics shows activity against gram positive and gram negative microorganism.

CONCLUSION

1) Amoxycillin shows more activity to *E. coli* and *S. aureus* as compared to Ampicillin.
2) In case of Amoxycillin, due to hydroxyl benzyl group attached to Penicillin nucleus, it shows more activity as compared to Ampicillin. Activity of Ampicillin is less due to amino benzyl group attached penicillin nucleus.
3) Cefixime is third generation Cephalosporin antibiotic so it shows enhanced spectrum of activity to gram positive *Staphylococcus aureus* and gram negative *E. coli* microorganism.

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