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A COMPARATIVE STUDY OF THE EFFECT OF AMPICILLIN AND TETRACYCLIN ON BACTERIAL CULTURE BY MEASURING THE ZONE OF INHIBITION

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Abstract: Agar dilution susceptibility testing is regarded as the golden standard for all other susceptibility testing methods. The minimum inhibitory concentration (MIC) represents the concentration of antimicrobial at which there is complete inhibition of growth of organism. Agar plate dilution test was used to determine the MIC of the antibiotics (Ampicilin and Tetracycline). Agar plates with two fold dilutions of antibiotics were inoculated with bacterial cultures such as *E.coli* and *B.subtilis*; and were incubated. On the following day the MIC was recorded as the lowest concentration of antimicrobial growth with no visible growth. The MIC provides information regarding the degree of resistance. The agar diffusion methods are influenced by factors such as agar depth, diffusion rate of the antimicrobial agent and growth rate.

Keywords: Zone of Inhibition, Ampicillin, Tetracyclin, Pour Plate Technique, Spread Plate Technique, Minimum Inhibitory Concentration..

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INTRODUCTION

Antibiotics :

Antibiotics are substances that are produced by molds or bacteria and kill or inhibit the growth of other microorganisms. Antibiotics are among the most frequently prescribed medications in modern medicine. Antibiotics cure disease by killing or injuring bacteria. The first antibiotic was Penicillin, discovered accidentally from a mold culture. Today over 100 different antibiotics are available to doctors to cure minor discomforts as well as life-threatening infections.

Although antibiotics are useful in a wide variety of infections, it is important to realize that antibiotics only treat bacterial infections. Antibiotics are useless against viral infections (for example the common cold) and fungal infections (such as ringworm). Our doctor can best determine if an antibiotic is right for our condition.

How do they work :

Antibiotics specifically attack bacteria without harming cells belonging to the organism that produced them. Antibiotics such as penicillin kill bacteria by inhibiting them from making cell walls that are needed for their survival. Without their cell wall the contents of the cells leak out and the cell is destroyed. Human and animal cells don't require a cell wall in order to survive, thus these antibiotics do not damage them.

EXPERIMENTAL PROCEDURE

Materials Required :

Medium - 500 ml

Peptone - 2.5 gm

Agar - 8 gm

Yeast Extract - 1.5 gm

Beef Extract - 1.5 gm

Distill Water - 500 ml

Sterile Pipettes, Sterile Petri-Dishes, Cork Borer

Liquid Culture of Bacillus subtilis (24 hours old)

Liquid Culture of E.coli (24 hours old)

Antibiotic used – Ampicillin, Tetracyclin

Media Preparation :

Preparation of an agar medium of 500 ml by using peptone, Peptone – 2.5 gm

Yeast Extract – 1.5 gm , Beef Extract – 1.5 gm , Distill water – 500 ml

100 ml of Nutrient Broth is prepared (using *E.coli* and *B.subtilis*)

The media is autoclaved before use.

Dilution Process of Antibiotics :

Concentration for 100 mg / 10 ml

Antibiotic (Ampicillin or Tetracyclin) – 100 mg

Ethyl Alcohol – 1 ml

Distill Water – 9 ml

D_1 Solution : 1 ml of above solution + 9 ml sterile Distill water.

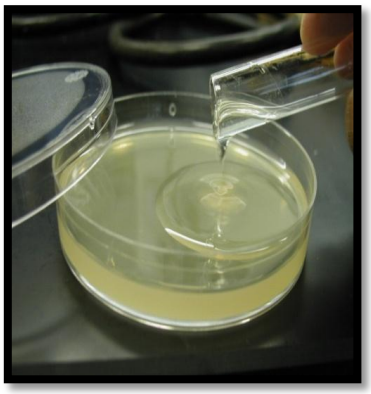
D_2 Solution : 1 ml of D_1 + 9 ml Sterile Distill water.

METHODS AND RESULTS

METHODS

POUR PLATE TECHNIQUE

Effect of Ampicillin and on E.coli and B.subtilis :



1. 10 ml of molten agar having a temperature of nearly 45^0 C (to avoid cell death due to high temperature) was inoculated with 0.1 ml of 24 hour old liquid culture (organism).
2. The agar and the inoculums were mixed well by boiling the test-tubes. Inoculated molten agar was poured.

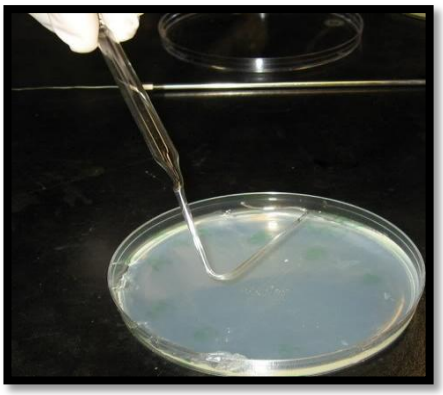
3. The sterilized petri dish was allowed to solidify.

4. Each petri dish was divided into three equal zones and three wells were dug with sterile cork borer at the middle of each zone.

5. Each well was marked as D_1, D_2 and C (dilution 1, dilution 2 and control).

6. 0.1 ml antibiotic of each concentration was poured in the well marked as D_1 and D_2 while sterile distill water was poured into C and allowed to stand without any disturbance.

7. The plates were kept under cold condition (Refrigerator) for 30 minutes before placing them in the incubator at 37^0 C in inverted position (to allow diffusion) for 24 hours.



8. After completion of the incubation period the plates were removed from the incubator and were observed to obtain the area of zone of inhibition created by the Ampicillin used.

SPREAD PLATE TECHNIQUE

Effect of Ampicillin on E.coli and B.subtilis :

1. 10 ml of molten agar was taken and poured into sterile petri dish.

2. The agar was allowed to solidify.

3. 0.1 ml of 24 hours old liquid culture (organism) was taken and poured at the middle of the petri dish.

4. Sterile glass spreader was taken and moved under the solid agar in circular motion to allow equal distribution of the liquid culture.

5. Plate was left undisturbed for few minutes.

6. Wells were dug in three equally divided zones and were marked as D_1, D_2 and C and were filled with the respective concentration of antibiotic.

7. The plates were left undisturbed for few minutes and were placed under cold condition.

8. The plates were placed in the incubator at 37^0 C in inverted position for 24 hours.

9. After completion of the incubation period the plates were removed from the incubator and were observed to obtain the area of zone of inhibition created by the Ampicillin used.

RESULTS

Using E.coli

Results of Ampicillin (Antibiotic) :

SL. No.	Concentration of Ampicillin (100 mg/10 ml)	Diameters of the Zone of Inhibition (cm)	Mean Zone Diameter (cm)
01.	D_1	3.5 cm , 3.4 cm	3.45 cm
02.	D_2	2.3 cm , 2.1 cm	2.2 cm

Using B.subtilis

Results of Ampicillin (Antibiotic) :

SL. No.	Concentration of Ampicillin (100 mg/10 ml)	Diameters of the Zone of Inhibition (cm)	Mean Zone Diameter (cm)
01.	D_1	4.4 cm , 4.1 cm	4.25 cm
02.	D_2	3.5 cm , 3.6 cm	3.55 cm

POUR PLATE TECHNIQUE

Effect of Tetracyclin on E.coli and B.subtilis :

1. 10 ml of molten agar having a temperature of nearly 45^0 C (to avoid cell death due to high temperature) was inoculated with 0.1 ml of 24 hour old liquid culture (organism).
2. The agar and the inoculums were mixed well by boiling the test-tubes. Inoculated molten agar was poured.
3. The sterilized petri dish was allowed to solidify.
4. Each petri dish was divided into three equal zones and three wells were dug with sterile cork borer at the middle of each zone.
5. Each well was marked as D_1 , D_2 and C (dilution 1, dilution 2 and control).
6. 0.1 ml antibiotic of each concentration was poured in the well marked as D_1 and D_2 while sterile distill water was poured into C and allowed to stand without any disturbance.
7. The plates were kept under cold condition (Refrigerator) for 30 minutes before placing them in the incubator at 37^0 C in inverted position (to allow diffusion) for 24 hours.
8. After completion of the incubation period the plates were removed from the incubator and were observed to obtain the area of zone of inhibition created by the Tetracyclin used.

SPREAD PLATE TECHNIQUE

Effect of Tetracyclin on E.coli and B.subtilis :

1. 10 ml of molten agar was taken and poured into sterile petri dish.
2. The agar was allowed to solidify.
3. 0.1 ml of 24 hours old liquid culture (organism) was taken and poured at the middle of the petri dish.
4. Sterile glass spreader was taken and moved under the solid agar in circular motion to allow equal distribution of the liquid culture.
5. Plate was left undisturbed for few minutes.

6. Wells were dug in three equally divided zones and were marked as D_1, D_2 and C and were filled with the respective concentration of antibiotic.

7. The plates were left undisturbed for few minutes and were placed under cold condition.

8. The plates were placed in the incubator at 37^0 C in inverted position for 24 hours.

9. After completion of the incubation period the plates were removed from the incubator and were observed to obtain the area of zone of inhibition created by the Tetracyclin used.

RESULTS

Using E.coli

Results of Tetracyclin (Antibiotic) :

SL. No.	Concentration of Ampicillin (100 mg/10 ml)	Diameters of the Zone of Inhibition (cm)	Mean Zone Diameter (cm)
01.	D_1	3.2 cm , 3.5 cm	3.35 cm
02.	D_2	2.2 cm , 2.4 cm	2.3 cm

Using B.subtilis

Results of Tetracyclin (Antibiotic) :

SL. No.	Concentration of Ampicillin (100 mg/10 ml)	Diameters of the Zone of Inhibition (cm)	Mean Zone Diameter (cm)
01.	D_1	3.8 cm , 3.5 cm	3.65 cm
02.	D_2	2.4 cm , 2.6 cm	2.5 cm

CONCLUSION

Zone of inhibition obtained in case of B.subtilis was found to be larger in diameter than that of E.coli suggesting E.coli is inhibited to a lesser degree by this chemotherapeutic agent while

B.subtilis is more susceptible between the two.

Ampicillin inhibits formation of cell wall thus growing cell were taken for the test and were found to be inhibited closer to the point where the antibiotic was placed (i.e. well containing Ampicillin). Moving away from the well or disc give the organisms less expose to antibiotic and allow them to grow.

No growth was observed within the zone of inhibition in both the cases suggesting there were no Ampicillin resistant strain or the concentration of antibiotic used did not allow the Ampicillin resistant strain to grow within the zone of inhibition.

Zone of inhibition obtained in case of B.subtilis and E.coli were found to have almost the same diameter by applying the antibiotic Tetracyclin.

No growth was observed within the zone of inhibition in both the cases suggesting there were no Tetracyclin resistant strain or the concentration of antibiotic used did not allow the Tetracyclin resistant strain to grow within the zone of inhibition.

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