A NEW METHOD FOR THE DEVELOPMENT OF ESCHERICHIA COLI RESISITANT AGAINST ANTIBIOTIC AMPICILLIN

Richa Thakre*, Shahbaz Eqbal, Abhishek Dwivedi and Shilpa Jain[1]

NRI Institute of Pharmaceutical Science, Bhopal, M.P-India

1, Sagar Institute of Research and Technolog, Pharmacy. Bhopal M.P-India

*Corresponding Author

ABSTRACT

Bacteria are like living paint, covering nearly every surface imaginable and living within a variety of living and nonliving things. An antibiotic is any substance produced by a microorganism, which harms or kills another microorganism. However, antibiotics do not harm viruses. A majority of antibiotic substances are natural products that certain bacteria and fungi produce as a natural defense to send outside their cells. Ampicillin is a prescription drug, introduced in 1961, used to treat infections caused by certain bacteria, including the specific bacteria family Enterobacteriaceae. Ampicillin is an antibiotic (drug produced by microbes). It is a semi-synthetic form of penicillin, belonging to the penicillin group of drugs. The present work was conceived to determine if Escherichia coli has the ability to become resistant to the antibiotic ampicillin and if yes, conclude the amount of time involved and how effective the mutant ability is.

Key-words: E. coli, Ampicillin, Antibiotics, Resistant

INTRODUCTION

E. coli, the most significant species in the genus Escherichia, is recognized as an important potential pathogen in humans. Being a gram-negative bacillus, it is a common isolate from the colon flora. E. coli are found in every human being's intestines. E. coli are essential for producing the particular vitamins K and B-complex. Our bodies are dependent on E. coli for the production of these vitamins, our only source. Although we are dependent on these helpful strains of E. coli there are some harmful strains of E. coli (like the O157:H7 strain of E. coli) that have been associated with a wide variety of diseases and infections, including meningeal (predominantly in the newborn), gastrointestinal, urinary tract, wound, and bacteremic infections in all age groups.. The prescription antibiotic ampicillin is a semi-synthetic form of penicillin. Ampicillin is also proven effective against the bacteria family Enterobacteriaceae. On most

occasions it may become visible as a non-lactose-fermenter or as a mucoid colony, E. coli usually produces a dry, pink (lactose positive) colony with a surrounding pink area of precipitated bile salts on Mac Conkey agar. The E. coli organism also possesses O, H, and K antigens. E. coli, first described by Theodore Esherich in 1885 was considered a non-harmful member of the colon flora. Since then, E. coli has been associated with a wide variety of diseases and infections, including meningeal (predominantly in the newborn), gastrointestinal, urinary tract, wound, and bacteremic infections in all age groups. E. coli may cause numerous types of diarrheal illnesses. There are five major categories of diarrheogenic E. coli. These include Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enterohemorrhagic (EHEC serotype O157:H7) and Enteroadherent (EAEC). [1-4]

About 90% of antibiotics used today come from bacteria. Although these are all natural, some antibiotics are completely synthetic (made in a laboratory) and some are semi-synthetic (only altered). To determine which antibiotic works best against a specific bacterium, tests are done in laboratories, such as a susceptibility test. On an agar plate a bacterium suspension is spread over the plate. Then small, circular, sterile disks, saturated with an antibiotic are placed on the bacterium covered agar plate. The plate is then incubated. After incubation the zone sizes around each disk are recorded for resistance or not. Where no growth occurred around disk, the bacterium is sensitive. Where growth around the disk occurs, the bacterium is resistant to that certain antibiotic. One of the major problems we face today is that many of the disease-causing bacteria have become resistant to the effects of different antibiotics. This occurs when a (need only be one cell) bacteria population genetically acquires the ability to destroy the antibiotic. This one resistant cell will divide and produce a population that is now no longer harmed by that particular antibiotic. This is a great concern, because certain strains of disease-causing bacteria now have only one antibiotic remaining which will kill them. Due to this concern there are tremendous efforts in finding new natural sources of antibiotics, and or make completely synthetic ones in the laboratory. One of the reasons for the cause of this problem is because of prior, indiscriminate use of antibiotics in human and domestic animal health (usually cattle and pigs).

Today synthetic antibiotics are in great demands by structural modification. Such thing has taken place to the antibiotic penicillin, and its semi-synthetic form called ampicillin. A particular kind of natural fungus (Penicillium – a kind of fungus that can grow on bread)

produces penicillin. However, penicillin is very sensitive to stomach acid, and will be broken-down before it can do any good fighting against the given infection. This is the reason that penicillin is given with a shot. The semi-synthetic derivative, called ampicillin has been made, because it is resistant to stomach acid. This is the reason why ampicillin can be taken in tablet form (orally). The reason for the production of ampicillin is, ampicillin unlike penicillin, can kill some bacteria that that are not effectively killed by penicillin G, such as Salmonella bacteria, which causes a form of food poisoning and it is also resistant to the stomach acids found in humans. Penicillin is sensitive to these stomach acids and is taken in the form of a shot unlike ampicillin which can be taken in pill form. There are different side effects when ampicillin is taken orally instead of in the form of a shot. People who choose to take the shot may experience minor side effects such as insignificant rashes. When taken orally (pill form) side effects may include diarrhea. An alternative to ampicillin is the semi-synthetic penicillin called amoxicillin, which in turn produces fewer side effects involving the stomach and intestines. [4-9]

OJECTIVE OF PRESENT WORK

The purpose of this experiment is to determine if Escherichia coli has the ability to become resistant to the antibiotic ampicillin and if yes, conclude the amount of time involved and how effective the mutant ability is. The information gained from this experiment could be found useful to those who work in the field of microbiology, those in the medical field, those having contact with antibiotics, including all antibiotic using citizens, and people who wish to know what they can do to prevent further growth of antibiotic resistance in bacteria.

HYPOTHESIS

It was based on verbal interviews with a qualified supervisor in the field of microbiology and medical technology; and information collected about general bacteria, the specific bacteria genes E. coli, antibiotic resistance, and the antibiotic ampicillin. Some bacteria have become resistant to the effects of different antibiotics, by slowly being exposed to the particular antibiotic. The resistance occurs when a minimum of one bacteria cell genetically acquires the ability to destroy the antibiotic. That single cell of bacteria then divides (possibly at a rate of every 20 minutes) and produces a population that is no longer affected by that specific antibiotic. For the most part E. coli is a harmless bacterium that can be found within the

intestines of all humans. E. coli has also been know to have the ability to exchange genetic information with other organisms gaining some of that organism's characteristics. The E. coli strain 0157:H7 is an example of this action. E. coli strain 0157:H7 was infected with a bacterial virus and that particular virus had the ability to insert its own DNA into the bacteria's chromosome without harming the bacterium. Ampicillin is a semi-synthetic from of penicillin that has a special feature that penicillin does not; ampicillin has a resistance to stomach acid, which penicillin is highly sensitive to. The bacterium E. coli has also been found to be sensitive to ampicillin.

The constants in this study were:

- The bacterial suspensions kept at 80% light transmission.
- The temperature at which the agar plates and their contents where incubated at.
- The amount of time at which the agar plates where incubated for.
- The type of antibiotic (ampicillin).
- The size of the test tubes used (12mmx75mm).
- The type of saline used (0.9%/normal).
- The type of incubator used (35C).
- The method used to dispose of the experimental material (autoclave).
- The type of growth medium (Mueller Hinton agar).
- The incubation atmosphere (35 ^{0}C; air).
- The Antimicrobial concentrations tested.

EXPERIMENTAL DESIGN

The manipulated variable was the amount of time ampicillin was exposed each week to the eleven strains of E. coli. All eleven strains of E. coli will be exposed to the same dose of ampicillin for that same week.

The responding variable will be the zone sizes around each ampicillin disk located on the agar plates. To measure the responding variable I record the diameter of the zone size around the ampicillin disk using calipers. The smaller the zone size around the ampicillin disk the more resistant the bacteria has become to the antibiotic. Meaning the ampicillin disk did not kill the bacterium that was around the antibiotic.

METHODOLOGY

The following procedures were adopted during the course of present experiment during five week of study. [10-12]

I. Week one

A. General procedure

1. Wash hands thoroughly as well as frequently (before and after handling experiment equipment.
2. Latex glove should be used at all times.
3. Fluid impermeable lab coat worn at times.
4. Sterilize all working surfaces per laboratory procedures.
5. Conduct work in a lab area not accessible to the public.
6. Use accepted aseptic procedures when transferring bacteria.

B. Making suspension strains of E. coli comply with testing needs

1. Gently dab E. coli strain # 1 with sterile cotton swab.
2. Smear E. coli strain #1 cotton swab on the inside of 12mmx75mm test tube.
3. Add saline to test tube at was just swabbed with E. coli strain #1 and tightly secure test tube screw cap.
4. Shake test tube at a reasonable rate.
5. Place test tube in colorimeter.
6. Test the light transmission of test tube contents.
7. For the proper amount of E. coli to saline, the colorimeter should read in the higher red zone (80% light transmission).
   a. If colorimeter reads higher than 80% light transmission add more E. coli, gradually lowering the light transmission to 80%.
   b. If colorimeter reads lower than 80% light transmission add more saline, gradually diluting the E. coli strain and increasing light transmission to 80%.
8. Once the test tube has met the 80% light requirement repeat steps B. 1-7 to all eleven E. coli strains.

C. Preparation of Mueller Hinton agar plates

1. Take E. coli suspension strain #1 and swab a coat of suspension on agar plate.

2. Using the same suspension swab an overlapping suspension coat over the first coat after the agar plate has been turned 90 degrees.
3. Once again using the same bacterial suspension swab another overlapping coat over the previous two, after the agar plate had been turned another 90 degrees.
4. The agar plate is finally finished when swabbed three times in three different directions.
5. Now place the ampicillin disks on the agar plate at equal distances apart.
6. Repeat steps C. 1-5 to all eleven E. coli suspensions.

D. Making trypitc soy broth (TSB) solution with suspension and ampicillin dilution

1. To empty test tube add 2.1mL TSB using pipette.
2. Add 200mg of ampicillin dilution (1mg to 1mL) to TSB.
3. Add 200mg of E. coli suspension to TSB+ ampicillin solution.
4. Repeat steps E. 1-3 to all eleven E. coli strains.
5. Incubate all eleven E. coli solutions for given time period.

E. Incubating

1. Incubate all eleven E. coli suspensions in Mueller Hinton agar plates for a minimum of 24 hours or longer.
2. After incubation time period measure the inhibition zones around all ampicillin disks for all eleven strains of E. coli.
3. Record inhibition zone diameters using calipers.

II. Week two

Partial reconfiguration of previous week

1. Repeat steps I.A.1-6, I.B.1-8 (with the exception of making bacterial suspensions from previous weeks TSB solutions), I.C.1-6, I.D.1-4, and I.E. 1-3.

III. Week three

Reconfiguration of previous week

1. Repeat steps I.A.1-6, I.B.1-8 (with the exception of making bacterial suspensions from previous weeks TSB solutions), I.C.1-6, I.D.1-4, and I.E. 1-3.

IV. Week four

Reconfiguration of previous week

1. Repeat steps I.A.1-6, I.B.1-8 (with the exception of making bacterial suspensions from previous weeks TSB solutions), I.C.1-6, I.D.1-4, and I.E. 1-3.

V. Week five

A. Reconfiguration of previous week

1. Repeat steps I.A.1-6, I.B.1-8 (with the exception of making bacterial suspensions from previous weeks TSB solutions), I.C.1-6, I.D.1-4, and I.E. 1-3.
2. After recording all information autoclave and dispose all materials used in experiment.

RESULTS

Having exposed the E. coli strains to increasing doses of ampicillin for five weeks will give the E. coli the ability to develop full or close to full resistance to the antibiotic, ampicillin. The bacterial zone size around the ampicillin saturated disk, when tested at five weeks for susceptibility, should be or close to a less than 13 mm zone size (using the NCCLS interpretive standards, an E. coli isolate that produces an ampicillin inhibition zone diameter of a less than 13 mm is classified as resistant), therefore showing resistance to the test antibiotic. During the course of present investigation it as concluded that produce ampicillin in zonediameter LT 13mm, classiy tested strains resistant.Different process involved are shown in the figure given below.

Fig. 1

Fig. 2

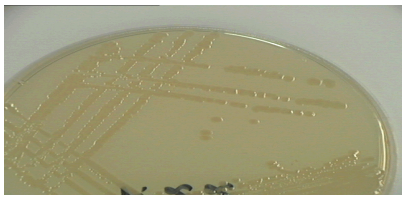

Fig 3

Fig. 4

Fig. 5

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