

# What Concentration of Ampicillin is Required to Inhibit the Growth of E.Coli?



# Introduction

E.coli is a gram-negative bacterium where some strains are harmless, and other strains can be pathogenic. Of the six types of pathogenic E.Coli, Shiga-toxin producing E.coli is most commonly associated with food poisoning. The pathogenic strains can cause illness, either diarrhea or illness outside of the intestinal tract. (2). This type of E coli comes from contaminated food or water and is contagious between humans and animals.

To inhibit the growth of this rod-shaped bacteria, antibiotics are used to slow/stop the growth. Ampicillin is effective against both gram-positive and gram-negative bacteria, hence it is effective against E.coli. However, bacterial response to antibiotics is concentration dependent. Hence the practical investigates the concentration of ampicillin required to inhibit the growth of E.coli. It was expected that the higher the concentration of ampicillin, the slower the growth of E.coli would be; where the highest dose exposed to the bacteria in the experiment is 2.0 mg/mL.

E. coli's natural habitat is in any mammalian animal's digestive tract. Hence, if a higher dosage of ampicillin is given to a patient infected with E.coli, there will be greater bacterial growth inhibition.

# Methodology

## Material;

-Ampicillin stock solution      -Eight nutrient agar plates      -E.Coli broth  
8 swabs      -Incubator      -Ruler

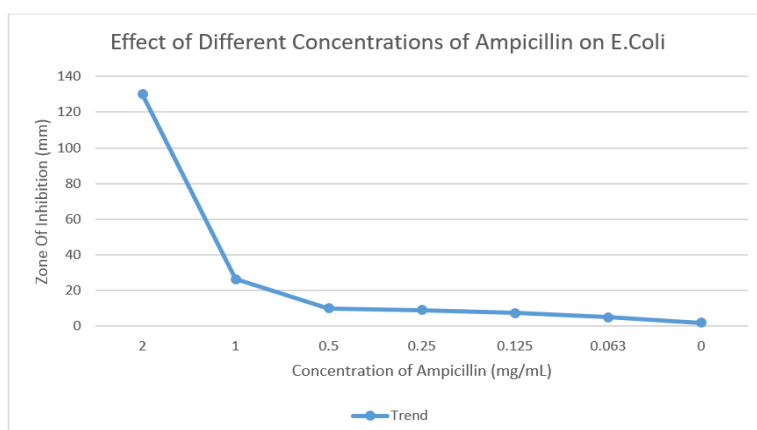
1. Put on safety equipment (i.e. lab coat and safety glasses and latex gloves). Make sure to be wearing gloves constantly when handling bacteria to minimize risk of infection.
2. Collect materials and equipment
3. Take one swab from the E.Coli broth and spread it uniformly over the surface of the agar jelly. Do this for eight samples. (when placing the swab on the nutrient plate, ensure to cover the whole jelly)
4. As a control, one agar plate does not receive any ampicillin. The other seven plates each receive varying amounts of ampicillin (independent variable) ranging from 2.0 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, and 0.063 mg/mL.
5. The starting amount of E. coli should be the same, as should their incubation temperature. Also, amount of light each colony is receiving as well as the same type and amount of nutrients in the agar jellies. The pH and oxygen : carbon dioxide ratio are also controlled.
6. Incubate sample at 30-37 degrees Celsius for 2-3 days.
7. After 2-3 days take samples from the incubator and measure the zone of inhibition (ZOI) (where bacteria hasn't colonized) –(dependent variable)
8. Record results. If ZOI is less than 6 mm, then the E.coli is resistant to that concentration, and if it is 6mm or more, its is sensitive to that concentration.

9. To reduce errors, the same experimenter must measure the ZOI of each sample.
10. Repeat the experiment twice over and then collate the average of the three trials.

## Results

Concentration of Ampicillin (mg/mL)	Zone of Inhibition (mm)
2.0	130
1.0	26.5
0.5	10
0.25	9
0.125	7.5
0.063	5
0	2

**Figure 1.0:** Table showing the effect of varying concentration of Ampicillin on the bacterial growth inhibition of E.Coli.



**Graph 1.0:** Graph showing the trend between the ampicillin concentration and zone of inhibition (where the bacteria didn't colonise).

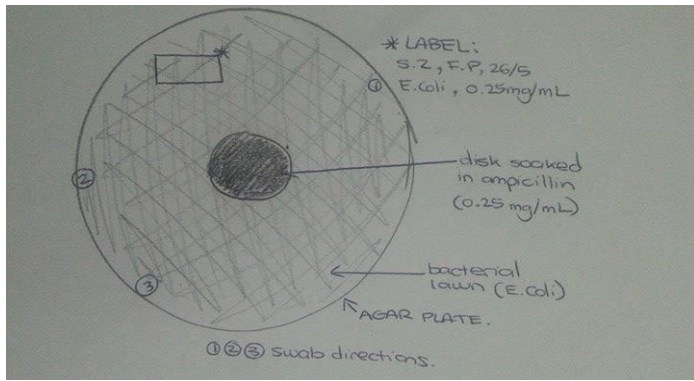


Figure 1.1: Drawing outlining the setup of the sample.

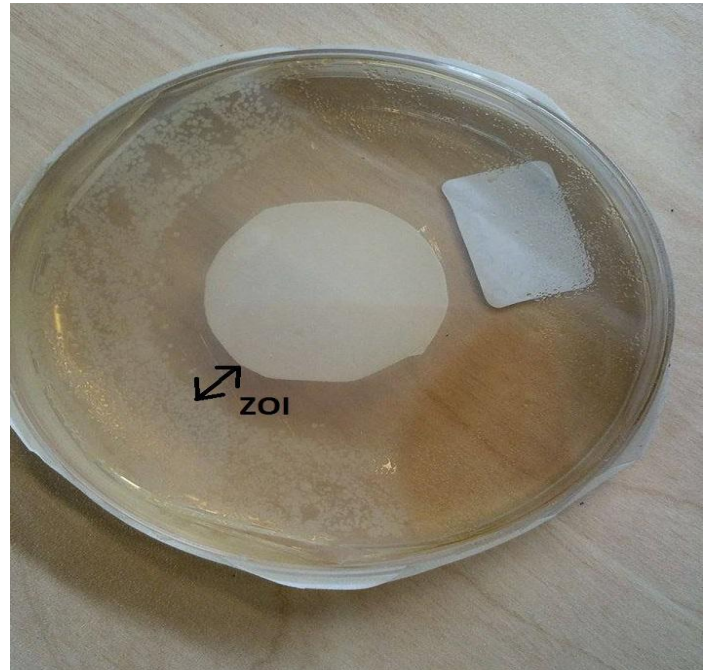


Figure 1.2: Image of bacterial growth and inhibition around the disk saturated with 0.25mg/mL concentration ampicillin. (ZOI = zone of inhibition).

## Discussion

*E. coli* is a gram-negative bacilli bacteria that inhabits the intestines of humans and animals, and has the ability to cause intestinal infection through its colonisation (6). Although most *strains of E. coli* are harmless, others can make an individual sick causing symptoms such as diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses (4).

Ampicillin contains an amino group that penetrates the lipopolysaccharide outer-membrane of the gram-negative *E. coli*, hence inhibiting its colonisation (3). It interferes with the enzyme, transpeptidase, needed for the formation of a rigid cell wall; causing it to become weak. This allows extracellular fluid to flow in and rupture the bacterial cell (2).

Through analysing the results from graph 1.0; it was found that the higher the concentration of ampicillin, the more efficient the inhibition of bacterial growth was. The 2.0mg/mL concentration effectively inhibited the growth of bacterial colonisation creating a ZOI of 130mm from the edge of the disc (disc-saturated with ampicillin). Whilst at a 0.25mg/mL concentration the zone of inhibition was 9mm, and decreased to 5mm at 0.063mg/mL concentration. Hence the efficiency steadily decreased as the concentration became lower.

If these results were to be generalised to have the same effect in humans, the suitable dose for treating patients with the *E. coli* pathogen would be 0.125mg/mL concentration. (Taking into consideration the criterion for a concentration being sensitive if the ZOI is larger than 6mm). As a 0.125mg/mL concentration effectively inhibited the growth of the bacteria at 7.5mm, it is an effective dosage to treat a patient with *E. coli* infection. The minimum inhibitory concentration (MIC) should be taken to reduce the issue of antibiotic resistance (7).

However, generalizing these results to humans would be inaccurate as a small effect of an antibiotic in an agar plate may not translate to fighting infection in the body. To create more applicable

results this experiment could be altered to test the effect of different concentrations in an environment that is more comparable to humans.

The negative control of no ampicillin exposed to the bacterial lawn in sample 7 gave unexpected results. It was observed that a 2mm zone of inhibition was created. This could be due to systematic parallax error. Hence to increase validity and reliability of the results, the experiment should be conducted at a minimum of 5 times, to reduce the skewing of results from an abnormal sample group. This would prevent any errors, including parallax error which could cause potential bias in the results whilst measuring the zone of inhibition.

The size of the disc should be strictly specified, as different sized discs could create biased results between the samples. The different sized discs correspond with the amount of ampicillin absorbed. If one disk is smaller than the other, it would be saturated with a different amount of ampicillin. Hence the bacterial growth response would be not valid. Additionally, the actual discs within the sample were not accurately cut, where the edges were uneven. The discs should be cut into accurate circles, with the diameter being the same through the whole circle to have control. To increase validity, the size and shape of the disc must be controlled.

## Conclusion

The study allowed the identification of the concentration of ampicillin required to inhibit the growth of E.Coli. The results indicated that E.coli was susceptible to antibiotics, with the most efficient concentration being 0.125mg/mL, taking into consideration MIC (to reduce antibiotics resistance)(7).

The hypothesis, stating that the higher the concentration of ampicillin, the more efficient bacterial growth inhibition would be, was supported. The concentration of 2.0mg/mL had the highest zone of inhibition, 130mm, showing that it was the most effective in making E.coli susceptible. Whilst the least effective was the ampicillin with the lowest concentration; 0.063mg/mL with a ZOI of 5mm.

The methods validity could be increased by the diameter of the disc array being specified, which could prevent potential bias in the results. Future work should replicate the methodology but with increased sample size, as well as a set standard size for the discs saturated with different concentrations of ampicillin. Furthermore, the experiment could be advanced to have more applicable results by ensuring that the results could be more comparable to the conditions of humans.

# References

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