What is the minimum concentration of Ampicillin required to inhibit the growth of the bacteria E.coli?

Introduction

Escherichia coli also known as E.coli is a Gram-negative, rod-shaped bacterium that normally inhabits the intestines of humans and animals.[3] While not all strains of E.coli are pathogenic, some are, and these particular strains are considered to be the main causes of foodborne illnesses.[3] Therefore it would be useful to know if this bacteria is able to be treated and killed through the use of an antibiotic, such as Ampicillin. Ampicillin is a beta-lactam antibiotic that targets Gram-positive bacteria, and some Gram negative bacteria. It is the amino group in Ampicillin that allows it to attack some Gram-negative bacteria as it enables the antibiotic to penetrate the outer membrane of the bacteria. It then acts as an inhibitor of transpeptidase, which is an enzyme needed for the formation of the bacteria's cell wall. By interfering with formation of the bacteria's cell wall while it is growing, Ampicillin weakens the cell wall, rupturing it and causing the bacteria to die through lysis.[4]

The aim of this study and experiment, was to investigate the effects of Ampicillin on the growth of the bacteria E.coli, and more specifically, the minimum concentration of ampicillin required to inhibit the growth of E.coli.

Prior to the conduction of the experiment it was expected that the ampicillin would affect the growth of E.coli by inhibiting it. It was predicted that the higher the concentration of ampicillin, the more E.coli that would be inhibited, however the minimum inhibitory concentration was predicted to be 0.125 mg/mL.

Nutrient agar plates are the media chosen to grow the E.coli bacteria on. The effectiveness of the antibiotic ampicillin is to be measured using sterile blank discs that would display a Zone of Inhibition (ZOI) after the 48 hours that the bacteria was left to grow. The ZOI is a clear region that forms around the paper disc that is saturated in the given ampicillin concentration. This region indicates the area in which the antibiotic has diffused into the agar and where the bacteria would be unable to grow. A ZOI of <6mm indicates that the antibiotic is ineffective, and a ZOI 6mm or greater indicates that the antibiotic was effective in targeting the bacteria. Therefore by measuring the size of the ZOI in this experiment, the effectiveness of ampicillin on E.coli and the minimum amount needed to inhibit its growth can be determined.

Methodology

1. Set up a Bunsen burner station. Perform all bacterial work within about 30 cm of the Bunsen burner. The updraft created by the flame creates a working zone that reduces the chance of contaminants falling onto your agar plate.
2. Label the bottom of a nutrient agar plate with your initials, date, bacterial species and assigned concentration of ampicillin.
3. Spread a bacterial lawn (refer to diagram 1.0). Dip the sterile swab into the bacterial broth and spread it uniformly over the surface of the agar, rotate the plate as you spread and go to the edges of the agar surface.
4. Dip the forceps into a small volume of ethanol and pass its tips through the Bunsen burner flame to sterilise it.
5. Using forceps carefully pick up a single sterile paper disc and dip it into your assigned concentration of ampicillin.
6. Place disc onto centre of agar plate
7. Repeat for another agar plate
8. Tape lid on plate.
9. Place in a 30-37C incubator, overnight

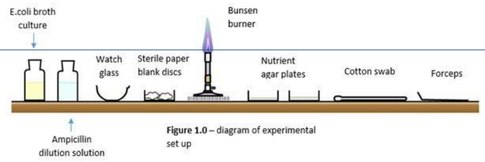

Figure 1.0 – diagram of experimental set up

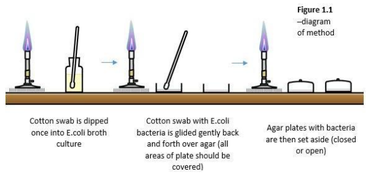

Figure 1.1 –diagram of method

Cotton swab is dipped once into E.coli broth culture

Cotton swab with E.coli bacteria is glided gently back and forth over agar (all areas of plate should be covered)

Agar plates with bacteria are then set aside (closed or open)

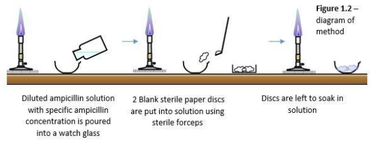

Figure 1.2 – diagram of method

Diluted ampicillin solution with specific ampicillin concentration is poured into a watch glass

2 Blank sterile paper discs are put into solution using sterile forceps

Discs are left to soak in solution

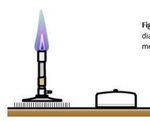

Figure 1.3 – diagram of method

Once soaked each disc is placed into one agar nutrient plate- plates are then placed in an incubator

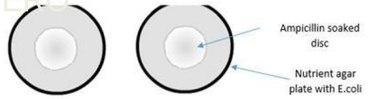

Figure 1.4 – diagram of top view of nutrient agar plates with ampicillin soaked discs

Results

- By observing the results in graph 1.0 and table 1.0, it is clear that there is a positive linear trend in the results obtained. As the concentration of ampicillin increases, the size of the Zone of Inhibition also increases, indicating that more E.coli bacteria growth is being inhibited. For example zero mg/mL ampicillin concentration shows to have the smallest ZOI of 1mm. In contrast the highest ampicillin concentration of 2.0 mg/mL shows to have the largest ZOI of 25.75mm. The concentrations in between these two figures increase from 0.063 mg/mL to 1.0 mg/mL, and show increasing ZOI's.
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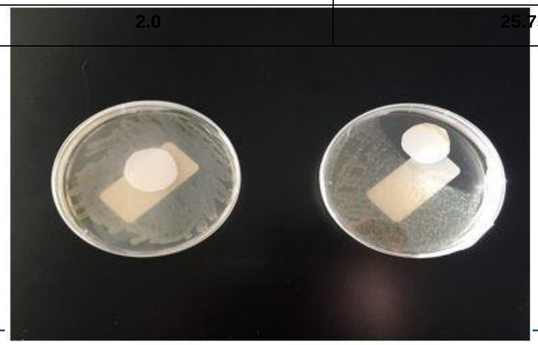
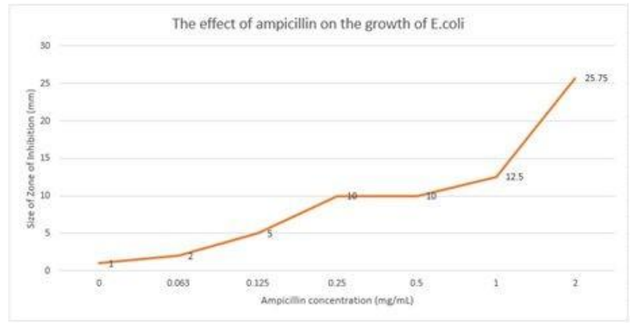
Results table 1.0 – Effect of ampicillin on the growth of E.coli bacteria

CONCENTRATION OF AMPICILLIN (mg/mL)	SIZE OF ZONE OF INHIBITION (mm)
0	1
0.063	2
0.125	5
0.25	10
0.5	10
1.0	12.5
2.0	25.75



1.5 - Image of agar plate results 48 hours after the experiment



Graph 1.0 - Results of experiment showing effect of ampicillin on growth of E.coli

Discussion

Independent variable: concentration of Ampicillin antibiotic (mg/mL)
Dependent variable: growth of E.coli (measured using Zone of Inhibition)

Describe trend in the data: By observing the results in graph 1.0 and the results table 1.0, it is clear that there is a positive linear trend in the results obtained. As the concentration of ampicillin increases, the size of the Zone of Inhibition also increases, indicating that more E.coli bacteria growth is being inhibited

Describe the effects of ampicillin as the concentration increased: By observing the class results both in graph 1.0 and table 1.0, the greater the ampicillin concentration, the larger than Zone of Inhibition (ZOI) was. The ZOI is the clear area around that disc that shows where the antibiotic has diffused into the agar and where the bacteria cannot grow. Therefore the results indicate that as the ampicillin concentration increases, so does the amount of E.coli growth that is inhibited. For example zero mg/mL ampicillin concentration shows to have the smallest ZOI of 1mm. In contrast the highest ampicillin concentration of 2.0 mg/mL shows to have the largest ZOI of 25.75mm. The concentrations in between these two figures increase from 0.063 mg/mL to 1.0 mg/mL, and show increasing ZOI's.

Minimum inhibitory concentration of ampicillin: The antibiotic ampicillin is shown to be effective only if the ZOI is 6mm or more. Therefore the minimum inhibitory concentration shown in this experiment was 0.25 mg/mL

Explain whether the negative control showed the expected result? The control in this experiment was that agar nutrient plate/s with zero concentration of the ampicillin antibiotic. The control was shown to have the smallest ZOI (1mm as seen in results table 1.0) which was expected. However the fact that the control had any ZOI at all was not expected as it was thought that the ZOI would be zero due to no antibiotic being present.

Is the methodology valid? Yes the methodology is valid. This method achieves its validity by having a control group (agar plates with zero concentration of ampicillin). This control serves as a baseline measure for comparison which contributes to validity because it shows that any changes in the zone of inhibition/growth of E.coli bacteria, are due to the ampicillin and no other factors. In addition the controlled variables of this experiment, such as the same size agar plates, same incubation temperature and same sample of E.coli broth culture, also add to validity for the similar reason that it ensures the experiment is a fair test and that only one variable is being tested at the one time.

Discuss the reliability of the experimental design. What are some possible sources of error?
The experiment was fairly reliable, as each concentration of ampicillin was tested on two agar plates rather than one. However the reliability could have been improved by having an even larger sample size, perhaps 4 agar plates per concentration to further ensure consistency of results and to prevent the possibility of an abnormal sample group from skewing the results. Reliability could have also been improved by repeating the experiment. There are many possible sources of errors that may have occurred during this experiment. One may include reading errors when measuring the zone of inhibition. This may have provided inaccurate results that indicate that a particular concentration of ampicillin worked more or less effectively than it actually did. Other sources of error may include that some bacteria in the atmosphere or on equipment may not have been killed by the heat of the Bunsen burner. Therefore it is possible that other bacteria may have gone onto the equipment and into the agar plates. This could have potentially altered the effectiveness of the ampicillin and decreased the reliability of the results. Other sources of error could include the inability to control the amount of E.coli broth culture absorbed by the cotton swab each time it was dipped into the bacteria mixture. Therefore the agar plates are likely to vary in the amount of E.coli that is present on them, meaning that this variable was unable to be controlled. This could alter the results as more or less bacteria on the agar plates may impact the effectiveness of the different ampicillin concentrations. Another error that may have altered the results was the slight differences in the diameters of the sterile paper disks. As some disks were therefore bigger or smaller than others, the results could potentially be affected as larger disks would be able to absorb more of the ampicillin solution.
Therefore these size differences may result in inconsistencies between disks that contain the same ampicillin concentration, as a larger disk may inhibit the growth of more E.coli than the smaller disk. Overall improvements to counter the occurrence of these errors could be to have more than one bunsen burner set up in order to futher reduce the chance of bacteria in the atmosphere from contaminating the agar plates and equipment, and measuring the diamters of the paper discs to ensure consistency in their size

Conclusion

In conclusion, the aim to investigate the minimum concentration of ampicillin needed to inhibit the growth of the bacteria E.coli, was successfully achieved. As seen in the results displayed in Table 1.0 and Graph 1.0, the general trend shown in the data is that as ampicillin concentration increases, so does the size of the ZOI, meaning the amount of E.coli growth inhibited is also increased. As discussed earlier, an antibiotic is only deemed as effective in inhibiting the growth of bacteria if it produces a ZOI of 6mm or more. Therefore the data obtained shows that 0.25 mg/mL of ampicillin is the minimum inhibitory concentration of ampicillin on the growth of E.coli, as it had a ZOI of 10mm. Hence the hypothesis is refuted. While the experiment can be considered valid, uncertainties lie in the reliability of the experiment as it lacked a large sample size and was not repeated. Similarly errors in the conduction of the investigation can be identified such as reading errors, ineffectiveness of the Bunsen burner heat, the inconsistency of the volume of E.coli on each plate and the different sizes of the paper discs, which were mentioned previously. These errors serve as imitations to the experiment and along with the reliability could have therefore potentially impacted the results.

References

1. Chemix - Draw Lab Diagrams. Simply.. 2017. *Chemix - Draw Lab Diagrams. Simply.*. [ONLINE] Available at: http://chemix.org/. [Accessed 29 May 2017].
2. Drugs.com. 2017. *Ampicillin solution: Indications, Side Effects, Warnings - Drugs.com*. [ONLINE] Available at: https://www.drugs.com/cdi/ampicillin-solution.html. [Accessed 29 May 2017].
3. CDC.gov. 2017. *E.coli (Escherichia coli) | E.coli | CDC*. [ONLINE]

Available at: https://www.cdc.gov/ecoli/. [Accessed 28 May 2017].
4. Netdoctor. 2017. *Penbritin (ampicillin)*. [ONLINE] Available at: http://www.netdoctor.co.uk/medicines/infections/a7323/penbritin-ampicillin/. [Accessed 29 May 2017].

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