ANTIBIOTICS SENSITIVITY TEST ON *ESCHERICHIA COLI* AND *SHIGELLA SONNEI* USING DISK WITH DIFFUSION AND DILUTION METHODS

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ABSTRACT

An antibiotic sensitivity test is a method to test the response of bacteria to antibiotics. This study aims to determine the effectiveness of an antibiotic against microbial activity. The sensitivity test was conducted with two methods, namely, the diffusion method and the dilution method. The diffusion method was performed using paper discs (Kirby-Bauer) against *Escherichia coli* and *Shigella sonnei* bacteria with amoxicillin, neomycin, and sulfonamide antibiotics. The data required is the diameter of the inhibition zone. Results showed that *Escherichia coli* was sensitive to amoxicillin but resistant to sulfonamide and neomycin. Meanwhile, *Shigella sonnei* was resistant to amoxicillin, neomycin, and sulphanilamide. Furthermore, the dilution method was performed to test the potency of amoxicillin against *Escherichia coli* bacteria using the liquid dilution method. The data required were test tubes with liquid media that showed no turbidity. The results showed that the minimal inhibitory concentration of amoxicillin against *Escherichia coli* was 0.25%. Based on the results of the antibiotic sensitivity test using the diffusion and the dilution methods, it can be concluded that amoxicillin has high effectiveness against *Escherichia coli bacteria* with a minimum inhibitory concentration of 0.25%, while *Shigella sonnei* is resistant to the antibiotics tested.

Keywords: Antibiotic; Diffusion; Dilution; *Escherichia coli*; *Shigella sonnei.*

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INTRODUCTION

This research aims to train students in developing crucial skills in the field of microbiology. Upon completion of this experiment, it is anticipated that students will acquire proficiency in antibacterial sensitivity testing techniques, including disc diffusion (Kirby Bauer) and liquid dilution, as well as the ability to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of antibacterial compounds.

The urgency of this research lies in the need for a profound understanding of microbial responses to antibacterial compounds. With the escalating resistance of bacteria to conventional antibiotics, this study plays a crucial role in providing new insights into the potential of antibacterial compounds. This urgency is further reinforced by global challenges related to increasingly complex and difficult-to-treat bacterial infections.

The novelty of this research is found in its focus not only on conventional antibacterial sensitivity testing methods but also on the

assessment of MIC and MBC for antibacterial compounds. This approach makes a significant contribution to a deeper understanding of the effectiveness of these compounds in inhibiting and even killing bacterial growth. By combining these two aspects, this research opens the door to new discoveries in the field of antimicrobial agent development.

Antibacterials represent substances with the capability to inhibit or even eradicate bacterial growth, posing 20risks of infection, diseases, and spoilage of food materials. These antibacterial compounds fall under the category of antimicrobials, playing a crucial role in arresting bacterial growth (Ayu, Angga, & Masyitah, 2020). Antibacterial compounds can be either bactericidal, causing bacterial death, or bacteriostatic, merely inhibiting bacterial growth. Antibiotics such as aminoglycosides, β-lactams, tetracyclines, and quinolones exhibit diverse mechanisms, including inhibiting cell wall synthesis, altering cell wall permeability, affecting protein synthesis, interfering with nucleic acid synthesis, and disrupting microbial cell metabolism (Jawetz et al., 2008).

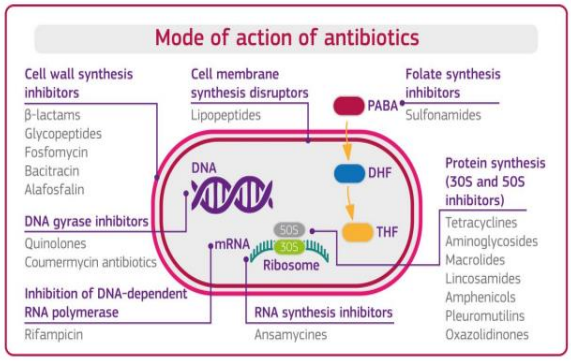


Figure 1. Mechanisms of antibiotics (Sanseverino, 2018)

Sulfonamide antibiotics, for instance, hinder folate metabolism crucial for purine and pyrimidine biosynthesis, influencing the synthesis of nucleic acids vital for bacterial survival and replication (Sanseverino, 2018). Antibiotics can also impact bacterial growth by targeting cell walls, cell membranes, nucleic acid synthesis, and protein synthesis. Some act as antimetabolites by inhibiting folate metabolism, while others inhibit enzymes like DNA gyrase involved in replication and transcription (Sanseverino, 2018).

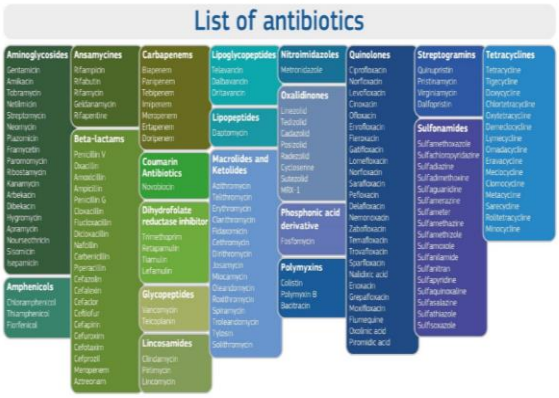


Figure 2. List of antibiotics (Sanseverino, 2018)

Based on the purpose of this research we have 2 different methods: Diffusion method and dilution method. Both of them have their specific intentions with different ways of procedure. The fundamental principles of diffusion and dilution methods provide valuable information about microbial responses to antibacterial compounds, aiding in understanding the antimicrobial activity of a substance and determining its effective concentration against test microorganisms.

Diffusion methods, encompassing cylinder, paper disc, and well methods, are frequently employed to analyze antibacterial activity. These techniques measure the diameter of clear zones as an indicator of the response to inhibiting bacterial growth by antibacterial compounds. Diffusion methods provide insights into microbial sensitivity to antimicrobial agents (Pratiwi, 2008). There are 3 different methods of diffusion, Cylinder method, Paper disc method, and well method. The cylinder method compares the growth inhibition zones of microorganisms exposed to varying doses of the tested antibiotic with zones of a standard antibiotic on agar plates (Martin et al., 1993). The paper disc method employs filter paper discs saturated with antimicrobial substances to assess antibacterial activity (Jawetz & Adelberg, 2005). The well method involves creating

wells on agar media inoculated with microorganisms, with the tested antibacterial agent placed in these wells (Pratiwi, 2008). The disk diffusion test measures the diameter of the clear zone, indicating the inhibition of bacterial growth by antibacterial compounds. The size of the clear zone reflects the antibacterial capacity of the compound (Hermawan et al., 2007).

Dilution have 2 different type of method. Dilution methods are employed to evaluate the potential of a compound against microbial activity by identifying the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (Lennette et al., 1991). Broth dilution method and Solid dissolution method. The broth dilution method measures MIC and MBC by progressively diluting the antimicrobial substance in liquid media containing the test bacteria (Pratiwi, 2008). The solid dilution method is used to determine MBC and involves diluting a series of test samples in solid media, such as agar, with microorganisms inoculated on the surface (Pratiwi, 2008).

MATERIALS AND METHODS

a. Materials

The tools used in this research are autoclave, blue tips, petri dish, measuring cup, Hockey Stick, incubator, vernier calipers, ose needle, paper disk, Flask, Laminar Air Flow, bunsen, micropipette, test tube rack, refrigerator, test tube, analytical balance, and vortex. Materials used in this research are paper disk, Amoxicillin disk (Oxoid), antibiotics, Etanol 70%, NaCl 0.9%, Sterile water, Muller Hinton Agar (Himedia), and Nutrient broth(Himedia). Bacterial used in this research are ATCC *Escherichia coli* (25922) and *Shigella* sp.

b. Method

Firstly, all tools and materials used are washed clean, dried, wrapped in paper and sterilized in an oven at 160-180°C for 2 hours and the materials to be used are sterilized in an autoclave at 121°C for 10-20 minutes.

Second, 28 grams of NA media were made for making Nutrient Agar (NA) and dissolved in an Erlenmeyer flask with distilled water until it reached a volume of 1 L, then heated until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes. Pour about 25 mL of the media into a petri dish and leave it until it solidifies.

Making Nutrient Broth (NB) media: 8 g of NB was weighed and dissolved in an Erlenmeyer flask with distilled water until it reached a volume of 1 L, then heated until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes. Pour about 25 mL of the media into a petri dish and leave it until it solidifies. Making MHA media Making Mueller Hinton Agar (MHA) media begins by weighing 19 grams of MHA and dissolving it in an Erlenmeyer flask with distilled water until it reaches a volume of 500 mL, then heating until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes. Pour about 25 mL of the media into a petri dish and leave it until it solidifies.

Third, a suspension of *Escherichia coli* and *Staphylococcus aureus* test colonies was made by taking one cycle of colonies from solid NA media into a test tube containing 5 mL of physiological NaCl. Turbidity in the test colony suspension was standardized to the 0.5 McFarland standard (approximately 1.5 x 108 CFU/mL). The suspension should be used as an inoculum within 15 minutes.

Fourth, a sterile cotton swab is dipped in the bacterial suspension and then gently pressed against the tube wall until the cotton is not too wet. A sterile cotton swab containing the test bacterial suspension is inoculated on the surface of the media using a streak plate so that it is evenly distributed. The disc paper containing antibiotics is then placed on the surface of the media aseptically. Each disc of paper is made at a certain distance regularly, so that there is no overlapping of the inhibition zones formed. The petri dish was incubated for 24 hours at 37°C and the clear zone around the paper disc was observed. The test was replicated 3x.

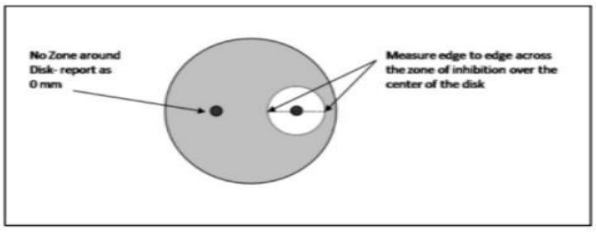


Figure 3. How to determine the zone of inhibition of bacterial growth
Sumber: Hudzicki, 2016

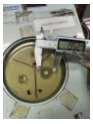
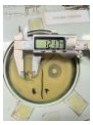
Fifth, making the control solution. The control solution is made in a test tube consisting of: Bacterial control (K1): 2 ml microbial suspension. Solvent control (K2): 1 ml liquid media + 1 ml sterile distilled water. Media control (K3): 2 ml liquid media. Next, make a sample solution, namely test tube number 1 is filled with 2 ml of 2% antibiotic solution. Test tubes number 2, 3 and 4 are filled with 1 ml of sterile distilled water A total of 1 ml of antibiotic solution from tube number 1 was transferred into tube number 2 and then homogenized with a vortex to obtain a 1% antibiotic solution. A total of 1 ml of antibiotic solution from tube number 2 was transferred into tube number 3 and then homogenized using a vortex to obtain a 0.5% antibiotic solution. A total of 1 ml of antibiotic solution from tube number 3 was transferred into tube number 4 and then homogenized using a vortex to obtain a 0.25% antibiotic solution. A total of 1 ml of antibiotic solution from tube number 4 was discarded. Lastly, Antibacterial testing of liquid and solid dilution methods.

Test tubes containing antibiotic solutions with concentrations of 2%, 1%, 0.5%, 0.25% w/v were added with 1 ml of microbial suspension and homogenized, the tubes were then incubated for 24 hours at 37°C. MIC is determined by observing the turbidity/clarity in each tube. KBM was determined by etching each liquid in the tube onto solid media and incubating for 24 hours at 37°C. After incubation, the scratch results were observed to see whether colony growth occurred on the solid media. The test was replicated 3x.

RESULT

Table.1 Diffusion Observation

Bacterial Growth Inhibition Zone (mm)
Escherichia coli

Sample	R1	R2	R3	Mean ± SD
Control (blank disc)	0 mm	-	-	0 mm
Amoxicillin	 20,84 mm	 13,52 mm	 22,71 mm	19,02 mm ± 4.86 mm
Neomisin	 13,72 mm	 6,17 mm	 18,36 mm	12,75 mm ± 6,15 mm
Sulphanilamide	0 mm	0 mm		3,24 mm ± 5,6 mm

Bacterial Growth Inhibition Zone (mm)
Shigella sp

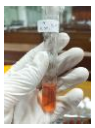
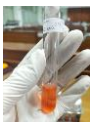
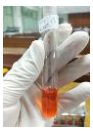
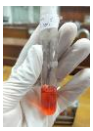
Sample	R1	R2	R3	Mean ± SD
Control (blank disc)	0 mm	-	-	
Amoxicillin	 8,94 mm	 7,55 mm	 13,71 mm	10,06 mm ± 3,23 mm
Neomisin	 4,11 mm	 2,54 mm	0 mm	2,21 mm ± 2,074 mm
Sulphanilamide	0 mm	0 mm	0 mm	0 mm ± 0 mm

Descriptions :
R1 : Petri dish 1
R2 : Petri dish 2
R3 : Petri dish 3

Interpretation: *Escherichia coli* bacteria are sensitive to the antibiotic Amoxicillin, Intermediate to neomycin, and resistant to sulfonamides. Meanwhile, *Shigella* sp bacteria are resistant to Amoxicillin, Neomycin and Sulphanilamide.

Table 2. Dilution Observation

Dilution Observation
Escherichia coli

Sample	Turbidity/Clarity			
bacterial control				
Solvent control				
Media control				
Concentration				
Amoxicillin	0,25 %	0,5%	1%	2%
	 Clear	 Clear	 Clear	 Clear

Interpretation: The MIC (Minimum Inhibitory Concentration) of Amoxicillin against *Escherichia coli* bacteria is 0,25%

DISCUSSION

The antibiotic sensitivity test employs the paper disc diffusion method. The paper disc or Kirby-Bauer method is a frequently utilized approach to assess the antimicrobial activity of an antibiotic against pathogenic microbes causing diseases. In this method, the inhibitory zone is observed as a clear area surrounding the paper disc immersed in the antibiotic solution (Kaesang et al., 2016). The sensitivity of microbes to antibiotics is discerned based on the size of the clear zone formed. The larger the diameter of the inhibitory zone, the lower the minimum inhibitory concentration value of the compound (Cappucino & Sherman, 2001).

The clear zone signifies the unobstructed or inhibited growth of microbes due to the antimicrobial substance excreted by their competitors (Atlas & Bartha, 1998). The paper disc method is frequently employed in both research and laboratory testing due to its numerous advantages over other methods. Its simple and efficient mechanism, along with relatively affordable costs, renders this method more economical compared to other sensitivity testing methods requiring specialized equipment. Additionally, the paper disc method has the capability to test a large number of microbes and provides significant results based on the inhibitory zones formed around the paper disc, offering visual information regarding the sensitivity of bacteria to antibiotics, facilitating interpretation by the examiner (Mounyr et al., 2015).

The McFarland standard is utilized as a reference to regulate the turbidity of bacterial suspensions, ensuring that the bacterial quantity falls within a specific range for microbial testing standards. The McFarland standard employs a scale of 1-10 representing bacterial concentrations/mL.

Cat No.	McFarland Standard	1% $BaCl_2$ (mL)	1% H_2SO_4 (mL)	Approximate Bacterial Suspension / mL
TM50	0.5	0.05	9.95	1.5×10^8
TM51	1.0	0.10	9.90	3.0×10^8
TM52	2.0	0.20	9.80	6.0×10^8
TM53	3.0	0.3	9.7	9.0×10^8
TM54	4.0	0.4	9.6	1.2×10^9
TM55	5.0	0.5	9.5	1.5×10^9
TM56	6.0	0.6	9.4	1.8×10^9
TM57	7.0	0.7	9.3	2.1×10^9
TM58	8.0	0.8	9.2	2.4×10^9
TM59	9.0	0.9	9.1	2.7×10^9
TM60	10.0	1.0	9.0	3.0×10^9

Figure 4. McFarland Standard Scale Value

Sensitivity testing necessitates the use of standardized inoculum. McFarland Standard 0,5 is recommended for preparing the inoculum to conduct antimicrobial disc diffusion sensitivity tests (Gayathiri & Ekambaram, 2018). McFarland Standard 0.5 represents $1,5 \times 10^8$ CFU/mL. Based on the McFarland standard scale table, McFarland Standard 0,5 has the least bacterial colony count, simplifying observations.

In the observation results of Escherichia coli bacterial diffusion, the negative control or blank did not exhibit any inhibitory effect (0 mm). Firstly, with Amoxicillin antibiotic, inhibitory zones were sequentially observed as 20,84 mm, 13,52 mm, and 22,71 mm in three repetitions, yielding an average or mean of 19,02 mm and a standard deviation of 4,86 mm. Secondly, with Neomycin antibiotic, inhibitory zones were sequentially observed as 13,72 mm, 6,17 mm, and 18,36 mm in three repetitions, yielding an average or mean of 12,75 mm and a standard deviation of 6,15 mm. Thirdly, with sulphanilamide antibiotic, inhibitory zones were sequentially observed as 0 mm, 0 mm, and 9,7 mm in three repetitions, yielding an average or mean of 3,24 mm and a standard deviation of 56 mm. According to the experimental data, Escherichia coli bacteria showed resistance to sulphanilamide, resulting in no inhibitory effect in tests R1 and R3. However, inhibitory effects were observed with Neomycin and Amoxicillin antibiotics. If the standard deviation is smaller than the mean, the performance can be considered good (Ghozali, 2016). The standard deviations for Amoxicillin and Neomycin are smaller than their respective means, indicating good performance. Meanwhile, the standard deviation for sulphanilamide is larger than its mean, suggesting poor performance.

In the observation results of *Shigella* sp bacterial diffusion, the negative control or blank exhibited no inhibitory effect (0 mm). With Amoxicillin antibiotic, inhibitory zones were sequentially observed as 8,94 mm, 7,55 mm, and 13,71 mm in three repetitions, yielding an average or mean of 10,06 mm and a standard deviation of 3,23 mm. With Neomycin antibiotic, inhibitory zones were sequentially observed as 4,11 mm, 2,54 mm, and 0 mm in three repetitions, yielding an average or mean of 2,21 mm and a standard deviation of 2,074 mm. With sulphanilamide antibiotic, inhibitory zones were sequentially observed as 0 mm in all three repetitions, yielding an average or mean of 0 mm and a standard deviation of 0 mm. According to the experimental data, *Shigella* sp bacteria exhibited resistance to sulphanilamide, resulting in no inhibitory effect in tests R1, R2, and R3. However, inhibitory effects were observed with Neomycin and Amoxicillin antibiotics. If the standard deviation is smaller than the mean, the performance can be considered good (Ghozali, 2016). The standard deviations for Amoxicillin and Neomycin are smaller than their respective means, indicating good performance. When comparing the standard deviation to the mean, Amoxicillin exhibits better performance than Neomycin. Meanwhile, *Shigella* sp exhibited resistance to sulphanilamide.

In the diffusion experiment, a blank disc, which lacks antimicrobial content, was used to determine the inhibitory zone of bacteria when not exposed to antimicrobials. The results obtained from the observation of the blank will serve as the standard for comparing observations against other antibacterial inhibitory zones (Rizka & Pandapotan, 2022).

The Clinical and Laboratory Standards Institute (CLSI) has established different classes of antimicrobial susceptibility based on the results of

antimicrobial susceptibility testing. Those categories include susceptible, intermediate, and resistant. A bacteria is categorised as susceptible if it shows significant response towards antimicrobial activity. An intermediate class of bacteria shows an intermediate level of susceptibility to the antimicrobial agent. Whereas resistant bacteria show no response and growth are not inhibited by antimicrobial agents (CLSI, 2020). *Escherichia coli* and *Shigella sp* belong to a group of bacteria known as *Enterobacteriales* which are susceptible to amoxicillin, neomycin, and sulphanilamide.

In the case of *Escherichia coli.* The administration of amoxicillin disc to *Escherichia coli* culture resulted in an inhibitory zone of 19,02 mm ± 4,86 mm and is categorised as susceptible according to CLSI which needs to be >18 mm. In other hand, administration of neomycin and sulphanilamide resulted in an inhibitory zones of 12,75 mm ± 6,15 mm and 3,24 mm ± 5,6 mm which is categorised as resistant due to it being <12 mm (CLSI, 2020).

In *Shigella sp,* the results of the inhibitory zone of amoxicillin, neomycin, and sulphanilamide are <12 mm which makes *Shigella sp* a resistant class of bacteria (CLSI, 2020).

The antibiotics that were used in this practice had different mechanisms of action towards *Escherichia coli* and *Shigella sp.* Amoxicillin is a β-lactam antibiotic. Amoxicillin works by inhibiting bacterial cell wall synthesis by binding to one or more penicillin-binding proteins (PBPs), which are responsible for the cross-linking process in cell wall synthesis, a process called transpeptidation. This binding leads to the lysis of bacterial cell wall and eventually the death of the bacteria. Gram negative bacteria like *Escherichia coli* are more resistant to amoxicillin due to its thick layer of lipopolysaccharide cell wall. In gram-positive bacteria such as *Shigella sp,* lysozyme is an enzyme that can damage the cell wall. Meanwhile, in Gram-negative bacteria, the lysozyme enzyme is not found in the membrane layer so it can prevent damage to bacterial cells (Kurniawan, 2019).

sulphanilamide is another type of antibiotic that works by inhibiting the synthesis of folic acid. Folic acid is an essential compound for bacterial growth and reproduction. sulphanilamide acts as a competitive antagonist to para-aminobenzoic acid (PABA) and inhibit the enzyme dihydropteroate synthase which is responsible for synthesizing the necessary folic acid in bacteria. Without folic acid, bacteria cannot synthesize DNA, RNA, and proteins, leading to their death (Hassanein, 2019). In the bacteria used, Escherichia coli, dihydropteroate synthase (DHPS) activity was resistant to sulfonamides. Some strains have enzymes that makes it resistant to sulfonilamide. In *Shigella sp,* resistance to sulfonilamide can be caused by sul1 sul2 and sul3 gene. One study stated that the sulfonamide 2 gene is one of three genes responsible for sulfonamide resistance. This gene is usually located on a plasmid, a type of genetic material, which can be transferred between bacteria (Reza & Abbas, 2019).

Neomycin is an antibiotic belonging to the aminoglycoside group which works by inhibiting bacterial protein synthesis. Aminoglycosides have excellent activity against aerobic gram-negative bacteria because they can pass through the outer membrane of bacteria. The positively charged nature of aminoglycosides allows them to bind to the negatively charged outer membrane to form holes and penetrate the bacterial cytoplasmic membrane to the ribosome. This antimicrobial carries out an active transport mechanism that relies on energy that requires oxygen and active proton power. For this reason, aminoglycosides work poorly in anaerobic and acidic environments. Each aminoglycoside works by binding to the 30S subunit of the bacterial ribosome, which causes a mismatch between the mRNA codon and the aminoacyl-tRNA and ultimately protein translation errors (Anggita, Dwi., et al. 2022). This causes the formation of non-functional proteins. Aminoglycoside antibiotics can also cause rupture of the

polysome structure so that protein is not synthesized. This antibiotic also has bactericidal properties and is often used in Gram-negative bacterial infections, especially Escherichia coli, Enterobacter sp., Proteus mirabilis and Pseudomonas aeruginosa (Sulaeman, L.P., 2015). Antibiotics work by stopping the growth of bacteria, either by targeting the cell wall or cell membrane, or through their effect on nucleic acid and protein synthesis. The final process occurs in ribosomes, nucleoprotein complexes that have small and large subunits. Apart from that, antibiotics can also act as antimetabolites by inhibiting folate metabolism, which in turn affects DNA synthesis through a pathway involving para-aminobenzoic acid (PABA), dihydrofolic acid (DHF), and tetrahydrofolic acid (Anggita, et al. 2022). The relationship between the diffusion speed of antibacterial compounds and the size of the clear zone in inhibiting bacterial growth shows a positive correlation. When the diffusion rate of antibacterial compounds increases, the size of the clear zone also tends to increase. The reason is because the faster the antibacterial compound diffuses, the faster it spreads around the antibacterial disc in the media (Hermawan, 2007). In the tests carried out, different inhibition zones were obtained between the antibiotics amoxicillin, neomycin and sulphanilamide on the three plates containing *Escherichia coli* and *Shigella* bacteria. However, the results show that there is an inhibition zone, which means that the antibiotic sample is in accordance with the theory of being effective in killing bacteria.

In testing using the dilution method, a different type of media is used to the diffusion method, namely using a type of liquid media, namely Nutrient Broth (NB) media. The dilution method is also one of the methods used to determine the effectiveness of a compound on the activity of a microorganism. The parameters used are the Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (KBM). A number of antimicrobial substances are included in solid or liquid bacteriological media. Usually a twofold dilution of the antimicrobial agent is used. The medium is finally inoculated with the tested bacteria and incubated.

The ultimate goal of the dilution method is to find out how much antimicrobial substance is needed to inhibit the growth or kill the bacteria being tested. Dilution susceptibility testing is quite time consuming, and its usefulness is limited in certain circumstances. The broth dilution test is cumbersome and of little use if dilutions have to be made in test tubes, but the advent of a series of broth dilution preparations for different drugs in microdilution plates has improved and simplified the method. The advantage of the dilution test is that it allows for quantitative results, indicating the amount of a particular drug required to inhibit (or kill) the microorganism being tested. (Mulyadi et al, 2013).

The observation results obtained from the liquid dilution method were clear or no turbidity in all test solutions which had antimicrobial amoxicillin concentrations of 2%, 1%, 0.5% and 0,25%. This shows that no bacteria grow and develop due to good antimicrobial activity. Then the MIC value of amoxicillin against *Escherichia coli* bacteria can be determined by looking at the minimum concentration that can inhibit bacterial growth, namely 0,25%. The MIC value itself or minimum inhibitory concentration is a value used to quantify the strength or effectiveness of an antimicrobial. In this practicum, it was not possible to determine the KBM value or minimum kill concentration because the test suspension was not inoculated again on the agar medium to determine whether there were live bacteria or not.

In dilution experiments, the control solution is very important in maintaining the accuracy and validity of the results of microbial sensitivity tests to antimicrobial compounds. The control solution consists of a bacterial control solution, solvent control, and media control. The bacterial control solution consists of nutrient broth media and bacterial suspension to see the bacterial inhibition zone when there is no antimicrobial activity. A

control solvent solution was used to ensure that the antimicrobial effects detected did not originate from the solvent itself. Meanwhile, media control is carried out by incubating nutrient broth to ensure that the media does not affect the resulting inhibition zone. (Christina et al, 2014).

In the test results obtained, the liquid dilution test showed that the clearest, or most effective, sample was at a concentration of 0,25%. This means that at this concentration amoxicillin is effective in inhibiting the growth of *Escherichia coli* bacteria in accordance with MIC theory, namely the lowest concentration that is still able to inhibit bacterial growth. So, in this case, it can be seen that the MIC of amoxicillin against *Escherichia coli* bacteria is at a concentration of 0,25%. The results of the amoxicillin MIC experiment on *Escherichia coli* bacteria showed that the minimum concentration required to inhibit bacterial growth was 0,25%.

Based on the practical results of the dilution test which was carried out where the tube containing the media was incubated for 24 hours, the antimicrobial concentration of amoxicillin was obtained at 2%, 1%, 0,5% and 0,25%. From these results, it was observed that the dilution was clear in colour and there was no turbidity, which means that no bacteria were growing. So it can be concluded that antibiotics work effectively in killing and stopping the growth of bacteria.

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CONCLUSION

The diffusion method can determine the sensitivity or sensitivity of bacteria to antimicrobial compounds by measuring the inhibition zone. Based on the results of the practicum, *Escherichia coli* bacteria are categorized as sensitive to amoxicillin antimicrobials and resistant to sulfonamide and neomycin. While *Shigella* sp. bacteria are categorized as resistant to amoxicillin, sulphanilamide, and neomycin. In the dilution test amoxicillin has a KHM value of 0,25%. This shows that amoxicillin at a concentration of 0,25% can effectively inhibit the growth and development of *Escherichia coli* bacteria.

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