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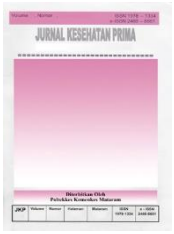

In Vitro Sensitivity Test of *Escherichia coli* ATCC 25922 to Various Antibiotics with Well Diffusion Method

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Abstract

Escherichia coli ATCC 25922 is an opportunistic normal flora bacteria that can cause diarrhea and other intestinal tract diseases. Acute diarrhea is the discharge of loose/liquid stools with a frequency of more than three times a day or more, lasting less than 14 days. Until now, acute diarrheal infections are caused by Escherichia coli, usually treated with antibiotics. However, there is resistance of Escherichia coli bacteria to several antibiotics such as ampicillin, tetracycline, sulfamethoxazole-trimethoprim, cotrimoxazole, chloramphenicol, ciprofloxacin, and gentamicin caused by controlled use of antibiotics. This study aims to analyze the sensitivity of various antibiotics to Escherichia coli ATCC 25922 bacteria in vitro. This research is an experimental study with a post-test only with a control group research design and uses various antibiotics as samples. The test bacteria used in this study was Escherichia coli ATCC 25922, with the agar diffusion method (well). The diameter of the inhibition zone formed was measured using a caliper. The results of the sensitivity test of various antibiotics against Escherichia coli found to be five resistants, namely sulfamethoxazole-trimethoprim 1.25µg (8,90mm), cefotaxime 30µg (12,26mm), cefixime 30µg (5,20mm), ceftriaxone 30µg (10,66mm), chloramphenicol 30µg (8,80mm), three intermediates namely ampicillin 10 µg (16,00mm), gentamicin 10 µg (13,55mm), erythromycin 15µg (17,53mm), and two sensitives, namely tetracycline 30µg (25,80mm), ciprofloxacin 5 µg (23,31mm).

Keywords: Antibiotics; Diarrhea; *Escherichia coli*; Resistance

INTRODUCTION

Acute diarrhea is the passage of loose or liquid stools with a frequency of more than three times a day or more often than usual that lasts less than 14 days (Setiati dkk., 2014). According to the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF), it is estimated that there are two billion cases of diarrheal disease worldwide each year. In the United States, an estimated 8,000,000 patients see a doctor and over 250,000 patients are hospitalized yearly (Farthing dkk., 2012). The incidence of acute diarrhea in developing countries, including Indonesia, is 2-3 times higher than in developed countries. One of the causes of acute diarrhea is *Escherichia coli* (Setiati dkk., 2014). Diarrhea caused by Escherichia coli can cause dehydration by various mechanisms depending on the type of pathotype, and the number of colonies in the intestine can affect the severity of diarrhea symptoms, including fatality (Halim dkk., 2017).

Based on the results of a study conducted by Bonkoungou dkk. (2013) on children under five years

old, it was found that *Escherichia coli* was the second rank cause of diarrhea (24%) after Rotavirus (30%), followed by *Salmonella* sp. (9%), *Shigella* sp. (6%), Adenovirus (5%), and *Campylobacter* (2%). Another study, Halim dkk. (2017) has reported that 50% of acute diarrhea in children was caused by *Escherichia coli* from 50 children with acute diarrhea, followed by *Klebsiella* sp., *Staphylococcus hemolytic*, *Yersinia pseudotuberculosis*, *Providencia better*, *Proteus stuartii*, and *Streptococcus* sp.

Escherichia coli is a normal flora found in the large intestine of humans. *Escherichia coli* belongs to the group of Gram-negative bacteria with coccobacillus form and is opportunistic. Several *Escherichia coli* strains are known to cause diarrhea and other intestinal tract diseases (Syahrurachman dkk., 2019). Most diseases caused by *Escherichia coli* infection can transmit through contaminated water or food, direct contact with animals or people, and usually occurs in places with poor sanitation and environment (Brooks dkk., 2018). From acute diarrhea, it was found that *Escherichia coli* ATCC 25922 is a reference strain of O6 ETEC (O6:H1) serotype that causes diarrhea (Kwon dkk., 2017).

Many strategies have been proposed for diarrhea management, including antibiotic and dehydration management. However, the handling of cases of infectious diseases is primarily relying on antibiotics. Various studies stated that 40-60% of antibiotics used are not as indicated, irrational, frequent, and have excessive usage, leading to bacterial resistance to various antibiotics (multidrug resistance). This results in ineffective treatment, increased health costs, and even impact death (Sjahriani & Pattiyah, 2019). Published research by Buntuan & Warouw (2012) has stated that antibiotic sensitivity and resistance tests against microbes causing diarrhea in children under five, such as sulfamethoxazole-trimethoprim (100%), cefazolin (100%), chloramphenicol (96,7%), doxycycline (83,3%), ampicillin (100%), and ceftriaxone (60%). In addition, the Antimicrobial Resistant in Indonesia study (AMRIN-Study) showed that from 2494 individuals in the community, 43% of *Escherichia coli* were found to be resistant to various types of antibiotics, such as ampicillin (34%), cotrimoxazole (29%), and chloramphenicol (25%). Moreover, the results of the study of 781 patients also showed that 81% of *Escherichia coli* were resistant to various types of antibiotics such as ampicillin (73%), cotrimoxazole (56%), chloramphenicol (43%), ciprofloxacin (22%), and gentamicin (18%) (Depkes RI, 2011).

Due to the high resistance of antibiotics to the growth of *Escherichia coli*, this study aimed to test the sensitivity of *Escherichia coli* ATCC 25922 to various types of in vitro antibiotics. In addition, this study will provide valuable information regarding the selection of antibiotics against diarrheal infections caused by *Escherichia coli*.

METHOD

Research participant and design

This research is an observational descriptive study to determine the sensitivity of *Escherichia coli* ATCC 25922 isolates to various antibiotics in vitro by healthy diffusion method.

Research timeline

This research was conducted at the Microbiology and Parasitology Laboratory of Abdurrab University between May - June 2021.

Research procedures

a. Making the concentration of antibiotics

Each antibiotic tablet is crush using a mortar. Then it was dissolved with distilled water until the desired antibiotic concentration was obtained

(sulfamethoxazole-trimethoprim 1,25µg, ampicillin 10 µg, cefotaxime 30µg, cefixime 30µg, ceftriaxone 30µg, tetracycline 30µg, chloramphenicol 30µg, gentamicin 10 µg, erythromycin 15µg, and ciprofloxacin 5 µg).

b. Making *Escherichia coli* ATCC 25922 0.5 Unit McFarland isolates

0,5 homogenized McFarland standard made by adding 1% H_2SO_4 and 1% $BaCl_2$ solution. The McFarland standard is equivalent to 10^8 CFU/mL.

c. Sensitivity test of *Escherichia coli* ATCC 25922 to various antibiotics by sound diffusion

The sensitivity test of several antibiotics for *Escherichia coli* ATCC 25922 using the well-diffusion method (Nurhayati dkk., 2020). *Escherichia coli* ATCC 25922 bacteria were dissolved in 0.9% NaCl adjusted with 0,5 McFarland standard solution, then swabbed into the MHA medium using a sterile cotton swab and left for 5-10 minutes. After that, holes were made in the MHA medium using a 6mm cork borer. Each antibiotic and negative control put as much as 25µl into the well using a micropipette, then incubated at 37°C for 24 hours. After incubation, the inhibition zone would observe and measured using a caliper. The determination of sensitive, intermediate, and resistant categories was determined using the Clinical and Laboratory Standards Institute standards.

Data analysis

The obtained data were analyzed descriptively. Data present in the form of tables and pictures. Following intervals of interpretation for the antibiotics used: Sulfamethoxazole-trimethoprim (Sensitive: ≥20mm to mm, resistance: ≤14mm, Intermediates: 15-19mm). Ampicillin (Sensitive: ≥17mm, resistance: ≤13mm, Intermediates: 14-16mm). Cefotaxime (Sensitive: ≥23mm to mm, Resistance: ≤14mm, Intermediates: 15-22mm). Cefixime (Sensitive: ≥20mm to mm, resistance to ≤14mm, intermediate to 15-19mm). (Sensitive: ≥20mm to mm, resistance: ≤14mm, Intermediates: 15-19mm). Tetracycline (Sensitive: ≥19mm to mm, resistance: ≤14mm, Intermediates: 15-18mm). Chloramphenicol (Sensitive: ≥18mm, resistance: ≤12mm, Intermediates: 13-17mm). Gentamicin (Sensitive: ≥17mm to mm, Resistance: ≤12mm, Intermediates: 13-14mm). Erythromycin (Sensitive: ≥20mm to mm, resistance: ≤14mm, Intermediates: 15-19mm). Ciprofloxacin (Sensitive: ≥21mm to mm, Resistance: ≤15mm, Intermediates: 16-20mm).

RESULT AND DISCUSSION

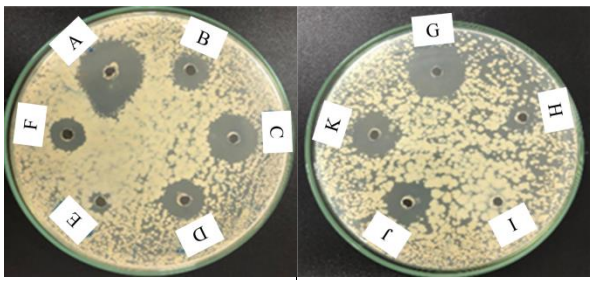
The results of the sensitivity test of *Escherichia coli* ATCC 25922 to various types of antibiotics present in table 1 and picture 1. The results show that five have developed resistance, namely sulfamethoxazole-trimethoprim 1,25µg, cefotaxime 30µg, cefixime 30µg, ceftriaxone 30µg, and chloramphenicol 30µg, three intermediates are ampicillin. 10µg, gentamicin 10µg, and erythromycin 15µg, 2 were sensitive namely tetracycline 30µg and ciprofloxacin 5µg.

The antibiotic sensitivity result showed that the antibiotics sulfamethoxazole-trimethoprim, cefotaxime, cefixime, ceftriaxone, and chloramphenicol classify as resistant to the growth of *Escherichia coli* ATCC 25922 with an average diameter of inhibition zone of 8,90mm, 12,26mm, 5,20mm, 10,66mm, and 8,80mm. This result follows research conducted by Onifade et al. (2015), which stated that *Escherichia coli* bacteria isolated from the feces of diarrhea patients were resistant to the antibiotic sulfamethoxazole-trimethoprim by 100%, cefixime by 96,63%, and chloramphenicol by 64,03%: another study, Ugwu dkk. (2017) stated that 78% of

Escherichia coli bacteria isolated from diarrhea patients resistant to the antibiotic ceftriaxone. The study of Cho dkk. (2011) stated that 70% of *Escherichia coli* bacteria were resistant to cefotaxime isolated from diarrhea patients.

Table 1. The results of the sensitivity test of various antibiotics against *Escherichia coli* ATCC 25922on MHA medium

Antibiotics	Mean (SD)	Sensitivity category
Sulfamethoxazole-trimethoprim	8,90 (0,35)	Resistance
Ampicillin	16,00 (0,85)	Intermediate
Cefotaxime	12,26 (0,15)	Resistance
Cefixime	5,20 (1,01)	Resistance
Ceftriaxone	10,66 (1,00)	Resistance
Tetracycline	25,80 (0,20)	Sensitive
Chloramphenicol	8,80 (0,70)	Resistance
Gentamicin	13,55 (1,08)	Intermediate
Erythromycin	17,53 (1,02)	Intermediate
Ciprofloxacin	23,31 (1,13)	Sensitive



Picture 1: The results of the sensitivity test of various antibiotics against *Escherichia coli* ATCC 25922 on MHA medium. A: tetracycline, B: sulfamethoxazole-trimethoprim, C: ampicillin, D: cefotaxime, E: cefixime, F: ceftriaxone, G: ciprofloxacin, H: chloramphenicol, I: negative control, J: gentamicin, K: erythromycin,

The results showed that ampicillin, gentamicin, and erythromycin are intermediates against *Escherichia coli* ATCC 25922. This research is contradictory to another study conducted by Pervin dkk. (2019) stated that *Escherichia coli* bacteria isolated from the feces of diarrhea patients experienced resistance to ampicillin by 96,08% and erythromycin by 90,02%. Research conducted by Sartika dkk. (2020) stated that the antibiotic gentamicin experienced Resistance to *Escherichia coli* bacteria isolated from the feces of hospitalized patients in pediatric wards aged <5 years by 75%.

The results obtained in tetracycline and ciprofloxacin antibiotics classified as sensitive to *Escherichia coli* ATCC 25922. These results indicate that the tetracycline and ciprofloxacin antibiotics are still quite effective against *Escherichia coli* ATCC 25922. Research conducted by Sumampouw (2018) states that tetracycline and ciprofloxacin antibiotics are still sensitive to *Escherichia coli* bacteria by the good diffusion method, which is characterized by the formation of inhibition zone diameters of 21,00mm and 35,00mm.

Another study conducted by Syafriana dkk. (2020) stated that tetracycline and ciprofloxacin antibiotics were still sensitive to *Escherichia coli* bacteria isolated from ISTN lake water by the paper disc diffusion method, which characterize by the formation of an inhibition zone diameter of 22,30mm on tetracycline antibiotics and ciprofloxacin of 25,31mm. Tetracyclines are antibiotics often used by the public and classified as broad-spectrum antibiotics. Tetracyclines inhibit the binding of aminoacyl-tRNA to the acceptor site in the mRNA-ribosomal complex through the reversibly binding to the 30S subunit of the bacterial ribosome, thereby. This prevents the addition of amino acids to the peptide being formed (Ogawara, 2019).

Ciprofloxacin is a fluoroquinolone group that has a mode of action affecting bacterial nucleic acid metabolism. Fluoroquinolones will inhibit topoisomerase II and IV in bacteria, each of which functions for DNA relaxation during transcription in the DNA replication process and for the separation of new DNA formed after the bacterial DNA replication process is complete (Brooks dkk., 2018).

The emergence of resistance to some antibiotics is because some bacteria have a natural ability to resist the effects of treatment, even though

they do not interact directly. This can happen because the bacteria have enzymes that can destroy the drug. The receptor on which the antimicrobial agent reacts can change both the affinity of the receptor for the antimicrobial and the response of the receptor, which can increase activity so that it can overcome the drug. Reduced drug accumulation by the presence of resistant cells occurs with a decrease in cell membrane permeability to antibiotics and variations in these metabolic pathways by antimicrobials. Drugs that inhibit the growth of competitive antagonists of normal metabolism can produce metabolic overload. As a result, the drug is no longer effective against bacteria (Brooks dkk., 2018).

In addition, previous researchers said that there are two mechanisms of adaptation of bacteria to antibiotics, namely 1) mutations in genes that are often associated with the mechanism of action of compounds and 2) acquisition of foreign DNA coding for determinants of resistance through horizontal gene transfer (HGT) (Munita & Arias, 2016). Furthermore, the most common cause of antibiotic resistance is the widespread and irrational use. For example, the use of inappropriate (irrational), i.e., use that is too short, in doses that are too low, the initial diagnosis is wrong, and in inadequate potency (Utami, 2012).

In this study, phenicol resistance in *Escherichia coli* ATCC 25922 was probably mediated by three main mechanisms, namely 1) enzymatic inactivation of no fluorinated phenicol by chloramphenicol acetyltransferases, 2) active efflux of no fluorinated chemicals (cmlA gene) or fluorinated and no fluorinated phenicol (floR gene) by major facilitatory superfamily proteins, and 3) methylation of target sites by rRNA methylase encoded by the multi-resistance gene CFR, which confer resistance to five classes of antimicrobial agents, including fluorinated and no fluorinated phenicol. Trimethoprim resistance detected in Enterobacteriaceae and other gram-negative bacteria is known to contain multiple dfr genes (dfrA and dfrB). In addition, the Resistance of *Escherichia coli* to cephalosporins may occur due to the presence of AmpC-type enzymes, thus providing a high level of resistance to these antimicrobial agents (Poirel dkk., 2018).

CONCLUSION

Tetracycline and ciprofloxacin classified as sensitive to the growth of *Escherichia coli* ATCC 25922. for further research, it is necessary to conduct sensitivity tests using other methods and try more antibiotics, as well as molecular detection of genes that cause *Escherichia coli* ATCC 25922 resistance to these antibiotics.

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