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Antibiotic susceptibility analysis of Escherichia coli *as* the cause of urinary tract infection

Melda Yunita*, Hasna Vuspita Latuconsina, Eka Astuty, Alessandra Flowrence Saija

Department of Medical Education, Faculty of Medicine, Universitas Pattimura, Indonesia.

Corresponding author: meldayunita22@gmail.com

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ABSTRACT

The incidence of urinary tract infection (UTI) in Indonesia is still quite high. The UTIs are generally treated with antibiotic. However, the use of antibiotics has recently increased tremendously thus triggering bacterial resistance. This study was to analyze the susceptibility of the *Escherichia coli* as the cause of the UTI to 15 antibiotics at the UPTD Health Laboratory Center of Maluku Province. The study was an experimental laboratory study using the disk diffusion method. The antibiotic disc was placed on the media and the inhibition zone against *E. coli* was measured. The results showed that the *E. coli* was resistant (26.67%) to 4 types of antibiotics, namely amoxilin, ampicillin, sulfametazole dan penicillin. Meanwhile, *E. coli* was sensitive (46.66%) to 7 types of antibiotics, namely kanamycin, gentamicin, subactam ampicillin, chloramphenicol, amikacin, ceffazedine and azithromycin. Furthermore, *E. coli* was intermediate (26.67%) against 4 types of antibiotics, namely ciprofloxacin, ceptriaxone, cefoxitin and colistin. This study concluded that of the 15 antibiotics tested, gentamicin, amikacin, and kanamycin were the most effective antibiotics in inhibiting the growth of *E. coli* that causes UTI. Nevertheless, the appropriate choice of antibiotic needs to be adjusted only by physicians based on local, regional and national patterns of susceptibility.

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INTRODUCTION

Urinary tract infection (UTI) is one of the crucial public health problems and often occurs in both developed and developing countries. A total of 150 million people suffer from UTIs worldwide each year. This infection is caused by pathogenic microorganisms that multiply beyond normal limits in the urinary tract, thereby damaging the walls of the urinary tract (Hermiyanty, 2012). Various studies have reported that urinary tract infections are generally caused by

pathogenic bacteria which are dominated by *Escherichia coli,* followed by *Klebsiella pneumoniae* ,and *Proteus mirabilis* (Rachman et al., 2015). In Indonesia, the incidence of UTI is still quite high. The Ministry of Health of the Republic of Indonesia reports that the incidence rate of UTI reaches 90-100 cases per 100,000 population (Kemenkes RI., 2015). Furthermore, the Maluku Provincial Health Laboratory Center records that more than 10 people per month are infected with pathogenic bacteria in the urinary tract with a total of more than 100 people per year (Medical Record data from the Maluku Provincial Health Laboratory Center, 2021).

Generally, urinary tract infections are treated with antibiotic therapy (Yunita et al., 2021a). However, various studies report that the use of antibiotics has recently increased tremendously. In Indonesia, about 40-62% of antibiotics are used irrationally for diseases that do not actually require antibiotics, thus further causing crucial problems such as the emergence and development of antibiotic-resistant bacteria which in turn can thwart treatment therapy for UTI infections (Rahman et al. al. 2015). The results of the Antimicrobial Resistance in Indonesia (AMRIN study) showed that of the 2494 samples investigated, 43% of *Escherichia coli* were known to be resistant to various types of antibiotics, including ampicillin, cotrimoxazole, and chloramphenicol (Kemenkes RI., 2015). In addition, the results of a study by the Indonesian Ministry of Health also reported that of 781 patients hospitalized, 81% of *E. coli* were also resistant to several types of antibiotics, namely ampicillin, cotrimoxazole (56%), chloramphenicol (43%), ciprofloxacin (22%), and gentamicin (18%) (Kemenkes RI., 2011).

Considering the seriousness of the risk posed by the problem of bacterial resistance, particularly *E. coli,* which is the most common cause of UTI, various efforts have been made to overcome this problem. Information about bacterial resistance to certain types of antibiotics is important in an effort to avoid the risk or impact of bacterial resistance. Bacterial resistance can be identified through a susceptibility analysis. Through susceptibility analysis, information about the appropriate antibiotics for the treatment of UTI caused by *E. coli* can also be obtained. In addition, there are not many study results that can be accessed on the susceptibility test of *E. coli* to various antibiotics in Maluku province. Therefore, the objective of this study was to analyze the susceptibility of *Escherichia coli* as the cause of urinary tract infections to various antibiotics at the UPTD Maluku Provincial Health Laboratory Center so that it can be used as a basis for knowing the appropriate type of antibiotic to treat urinary tract infections caused by *E. coli.*

RESEARCH METHODS

Research Design

The study was conducted in March-May 2021 at the UPTD Maluku Province Health Laboratory and Microbiology Laboratory, Faculty of Medicine, University of Pattimura. This study is a descriptive-observative laboratory research with a true experimental approach using the Disk Paper Diffusion Method of Kirby Bauer. A total of 15 types of antibiotics were tested for *Escherichia coli* to determine whether the bacterial response was sensitive, intermediate or resistant to these antibiotics.

Tools and Materials

The tools used in this research were incubator, ose needle, tube rack, bunsen, petri disk, marker, tweezers and bio safety cabinet. While the bacterial isolate used was *Escherichia coli* obtained from the UPTD Maluku Provincial Health Laboratory, Sodium Agar, Muller Hinton Agar, 70% alcohol, spirit, and 15 types of antibiotic discs, namely: Amoxilin, Ampicillin, Sulfametazole, Kanamycin, Gentamicin, Ampecilin-Subactam, Penicillin, Chloramphenicol, Ciprofloxacin, Amikacin, Ceptriaxone, Cefazedine, Azithromycin, Cefoxitin, and Colistin.

Procedures

Nutrient Agar Media Preparation

A total of 9 grams of Nutrient Agar (Merck) was dissolved in 500 ml of distilled water, then sterilized using an autoclave. The sterile medium was then poured into a sterile petri dish for one night at 37 ^{0}C.

Reculturing Bacterial Isolate

Escherichia coli isolated from urine samples of patients with urinary tract infections were obtained from the UPTD of the Health Laboratory of Maluku Province and re-grown on new Nutrient Agar media by streaking the isolate on NA media and incubated overnight at 37 ^{0}C.

Preparation of Tested Isolate

Escherichia coli that had been previously recultured were taken using a needle ose and then put in Mac Concey media and the suspension was measured until the MacFarland Densitometer showed the number 5.0 or turbidity standard was made from 0.5 ml BaCl22 H_2O, 1.175% added with 99.5 ml sulfuric Acid 1 % into a standardized turbidity bacterial suspension. Furthermore, sterile cotton buds were dipped in bacterial suspension and left for a while so that the suspension was absorbed by cotton and then swabbed on the surface of Mueller Hinton Agar (MHA) media. Swabbing was performed on the agar surface until it is full. The MHA media was allowed for 5-15 minutes so that the suspension was absorbed into the agar medium. Subsequently, antibiotics were prepared for bacterial susceptibility analysis including Amoxilin, Ampicillin, Sulfametazole, Kanamycin, Gentamicin, Ampicillin-Subactam, Penicillin, Chloramphenicol, Ciprofloxacin, Amikacin, Ceptriaxone, Ceffazedine, Azithromycin, Cefoxitin, and Colistin.

Susceptibility Analysis

The susceptibility analysis of *Escherichia coli* to 15 antibiotics was carried out using the Kirby Bauer disc diffusion method (Davis & Stout, 1971). The principle of this method is to observe and measure the inhibition of antibiotics against *E. coli* which is represented by a clear zone around the antibiotic disc used. Antibiotic discs were placed on MHA media that had been swabbed with *E. coli* with equal distances. As a negative control, paper discs were immersed in aquadest solution and placed in a petri dish in the same way as antibiotic discs. The clear zone that appeared around the antibiotic disc was measured using a caliper to determine its inhibition. The inhibition is determined by the formula:

Inhibition = Overall inhibition zone diameter – antibiotic disc diameter

The response of *E. coli* to the various antibiotics tested in this study was determined according to the Clinical and Laboratory Standards Institute (CLSI) (2019). The determination of the susceptibility of *E. coli* refers to the CLSI guidelines to determine the response of the *E. coli* tested whether it is sensitive, intermediate, or resistant (Table 1).

Table 1. Determination of bacterial response to antibiotics

No.	Type of Antibiotic	Determination		
		Sensitive	Intermediet	Resisten
1	Amoxicillin	>18	14-17	<12
2	Ampicillin	>18	14-17	<12
3	Sulfamethoxazole	>16	14-15	<12
4	Kanamycin	>21	16-20	<15
5	Gentamicin	>15	13-14	<12

6	Ampicillin-Subactam	>15	12-14	<11
7	Penicillin	>15	13-14	<12
8	Chloramphenicol	>18	15-17	<14
9	Ciprofloxacin	>18	12-17	<11
10	Amikacin	>20	17-19	<16
11	Ceptriaxone	>16	10-15	<9
12	Ceffazedine	>18	15-17	<14
13	Azithromycin	>18	16-17	<15
14	Cefoxitin	>18	12-17	<11
15	Colistin	>16	11-15	<10

Data Analysis

Data were presented by tabulation, graph and figure. While data were analyzed descriptively-qualitatively by observing the diameter of the inhibition zone formed by the 15 antibiotics against *E. coli.*

RESULTS

Analysis of the susceptibility of *E. coli* to 15 types of antibiotics showed varying results which were reflected in the clear zone, then the diameter of the clear zone was compared with the Zone Diameter Interpretive Standards from Kirby-Bauer to determine the category and level of susceptibility (Table 2).

Table 2. Results of antibiotic susceptibility analysis on the growth of *Escherichia coli*

No	Antibiotic Code	Type of antibiotic (20 µg)	Inhibition Zone Diameter (mm)	Inhibition Category	Determination
1	A	Amoxicillin	0	-	Resistance
2	B	Ampicillin	0	-	Resistance
3	C	Sulfamethoxazole	0	-	Resistance
4	D	Kanamycin	21	Strong	Sensitive
5	E	Gentamicin	18	Strong	Sensitive
6	F	Ampicillin-Subactam	15.1	Strong	Sensitive
7	G	Penicillin	0	-	Resistance
8	H	Chloramphenicol	20.2	Strong	Sensitive
9	I	Ciprofloxacin	12	Strong	Intermediates
10	J	Amikacin	20.2	Strong	Sensitive
11	K	Ceptriaxone	12	Strong	Intermediates
12	L	Ceffazedine	18	Strong	Sensitive
13	M	Azithromycin	19	Strong	Sensitive
14	N	Cefoxitin	12	Strong	Intermediates
15	O	Colistin	11	Strong	Intermediates
16	P	K-	0	-	-

The results of antibiotic susceptibility analysis against *Escherichia coli* showed that amoxicillin, ampicillin, sulfametazol, and penicillin did not show any inhibition against *E. coli* which was indicated by the absence of a clear zone around the disc (Figure 1). In contrast, kanamycin, ampicillin-subactam, gentamicin, chloramphenicol, ciprofloxacin, amikacin,

ceptriaxone, cefazedine, azithromycin, cefoxitin and colistin have inhibitory activities that are in the strong category against the growth of *E. coli.*

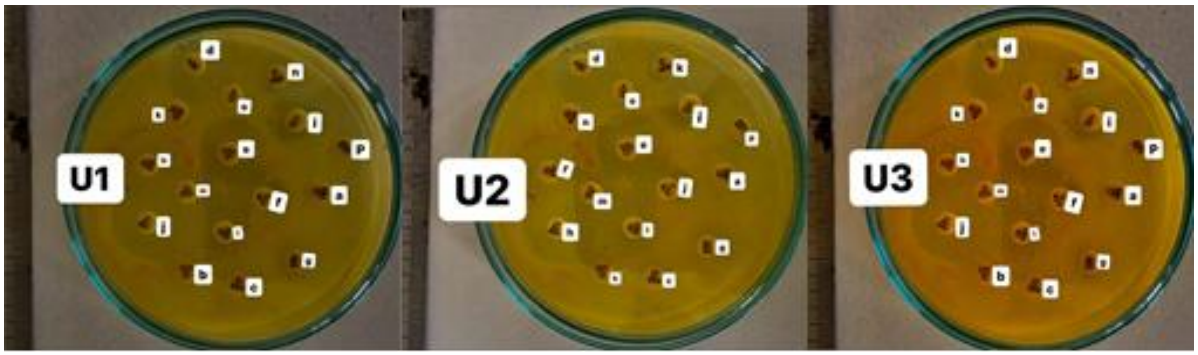

Figure 1. The results of the antibiotic susceptibility analysis on the growth of *E. coli* with 3 repetitions. a: amoxicillin; b: ampicillin; c: sulfametazole; d: kanamycin; e: gentamicin; f: ampicillin-subactam; g: penicillin; h: chloramphenicol; i: ciprofloxacin; A: amikacin; k: ceptriaxone; l: ceffazedine; m: azithromycin; n: cefoxitin; o: colistin; p: K (-)

According to the clinical and laboratory standards institute (CLSI) guidebook (2019), the results showed that *E. coli* was resistant (26.67%) to 4 types of antibiotics, namely amoxicillin, ampicillin, sulfametazole and penicillin. In contrast, *E. coli* was sensitive (46.66%) to 7 types of antibiotics, namely kanamycin, gentamicin, ampicillin subactam, chloramphenicol, amikacin, ceffazedine and azithromycin. Meanwhile, *E. coli* is intermediate (26.67%) to 4 types of antibiotics, namely ciprofloxacin, ceptriaxone, cefoxitin and colistin.

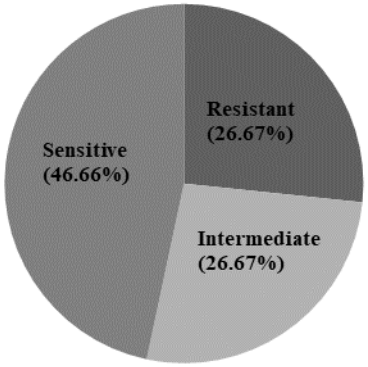

Figure 2. Percentage of antibiotic susceptibility analysis on the growth of *E. coli*

DISCUSSION

Escherichia coli is the predominant facultative flora in the gastrointestinal tract of humans and animals. Some strains of *E. coli* cause disease of the gastrointestinal, urinary, and central nervous systems. Prolonged exposure of *E. coli* to antibiotics contributes to the development of antibiotic resistance. Antibiotic-resistant bacteria, including *E. coli*, can serve as important reservoirs for colonization and infection in humans. Previous study has shown that drug-resistant *E. coli* can be transmitted to humans from the environment through direct or indirect contact, such as from ingestion of contaminated food and water (Sahoo et al., 2012).

Urinary tract infections (UTIs) are currently ranked among the most common infectious diseases worldwide, with chronic and recurrent infections being a major problem. UTIs are often treated with broad-spectrum antibiotics, and treatment is initiated empirically without performing culture and sensitivity. This inappropriate and unwise use of antibiotics has resulted in the worldwide development of antibiotic resistance in bacteria, leading to the emergence of multi drug

resistant strains of pathogenic bacteria. The infection gradually becomes difficult to treat, and can lead to a treatment stalemate (Ahmed et al., 2019).

The results of the susceptibility analysis showed that *E. coli* was sensitive (46.66%) to kanamycin, ampicillin-subactam, gentamicin, chloramphenicol, amikacin, cefazedine, and azithromycin which are generally included in the aminoglycoside group. This indicates that these antibiotics have the ability to inhibit the growth of *E. coli.* Generally, aminoglycoside antibiotics inhibit protein synthesis by binding to the 30S ribosomal subunit. Aminoglycosides diffuse through the water channel formed by the porin protein on the outer membrane of gram-negative bacteria into the periplasmic space. Once inside the cell, aminoglycosides bind to the 30S ribosome and inhibit protein synthesis. The binding of aminoglycosides to the ribosomes accelerates the transport of aminoglycosides into the cell, followed by destruction of the cytoplasmic membrane, and followed by cell lysis. This occurs due to a miss reading of the mRNA genetic code which results in disruption of protein (Cox & Wright, 2013). Interestingly, similar results but in higher numbers were also reported by Kebira et al., (2009) where 75% of *E. coli* isolated from urine of UTI patients were sensitive to amikacin, and 80% of isolates were sensitive to gentamicin.

Furthermore, *E. coli* was intermediate (26.67%) against ciprofloxacin, ceptriaxone, cefoxitin and colistin with an inhibitory zone that was included in the strong category. Intermediate is a condition where there is a shift from a sensitive state to a state that is resistant but not completely resistant (Peterson & Kaur, 2018). The large zone of inhibition found in this study was due to the antibiotic used to treat urinary tract infections, uncomplicated acute cystitis in women, chronic bacterial prostatitis, lower respiratory tract infections, acute sinusitis, skin infections, bone and joint infections, nosocomial pneumonia. Intermediate results generally occur during bacterial reproduction where the double helix of bacterial DNA is separated into 2 single strands of DNA. The separation of DNA strands can be overcome by the enzyme DNA gyrase produced by bacteria where this enzyme functions to reconnect the separated bonds (Marston et al., 2016). These results are supported by the findings reported by Vicky (2016) that ciprofloxacin, norfloxacin and cefoxitin have moderate sensitivity (intermediate) of 40%.

Furthermore, the *E. coli* analyzed in this study were resistant (26.67%) to amoxicillin, ampicillin, sulfametazole, and penicillin. Amoxicillin and ampicillin are antibiotics belonging to the penicillin group which are bacteriolytic (moderate spectrum) and are usually the first choice in their class because of their better absorption. Amoxicillin together with ampicillin belongs to the semisynthetic β-lactam group which functions to inhibit the synthesis of peptidoglycan and muerein which are the main components of cell walls. However, these antibiotics can be degraded by the β-lactamase enzyme produced by *E. coli,* resulting in bacterial resistance to these antibiotics. The results of our study are supported by a study conducted by Edityandari (2017) which showed that *E. coli* was resistant to amoxicillin, ampicillin, and sulfamethzole with a percentage of 100%. Furthermore, similar results were also reported by Sotto et al., (2001) that *E. coli* was resistant to amoxicillin (48.1%), ticarcillin (46.9%), penicillin (40.6%) and amoxicillin-clavulanic acid (20.3%). Furthermore, Kebira et al., (2009) reported that 75% of *E. coli* strains isolated from urine of UTI patients were resistant to sulfometazole.

Various studies reported that *E. coli* was indeed resistant to the same antibiotics in this study. Ny et al., (2019) in their study found that the non-susceptibility of the 775 strains of *E. coli* analyzed, nitrofurantoin and fosfomycin appeared as the most active drug tested with susceptibility of 1.2% and 1.3%, respectively, followed by by cefoxitin (3.1%) and mecillinam (4.1%). No resistance was detected to meropenem among the *E. coli* isolates collected. The highest resistance rates were found for ampicillin (39.6%), trimethoprim (23.8%), trimethoprim/sulfamethoxazole (22.4%) and amoxicillin-clavulanic acid (16.7%). More than 15% of all strains of *E. coli* showed resistance to ciprofloxacin.

In general, the emergence of bacterial resistance to an antibiotic can occur through several mechanisms, including: bacteria synthesize an enzyme inactivating or destroying antibiotics, bacteria change their permeability to drugs, bacteria develop a change in the target structure for drugs, bacteria develop changes in metabolic pathways that are directly inhibited by the drug. drugs, and bacteria develop enzyme changes that can still carry out their metabolic functions (Marston *et* al., 2016).

Escherichia coli expresses multi-drug resistance. Patterns of antibiotic resistance have shown extensive interregional differentiation. Appropriate antibiotic choice needs to be adjusted based on local susceptibility patterns. Generally, empiric antimicrobial treatment is initiated in almost all cases of UTI before urine culture laboratory results are obtained and, thus, antibiotic resistance may increase in uropathogens due to repeated inappropriate antibiotic selection (Prasada et al., 2019).

CONCLUSION

Based on the research results that of the 15 antibiotics tested, it was found that *E. coli* was resistant to 4 types of antibiotics, namely amoxilin, ampicillin, sulfametazole, and penicillin; sensitive to 7 types of antibiotics namely kanamycin; gentamicin; ampicillin subactam; chloramphenicol; amikacin; ceffazedine; and azithromycin, and is intermediate to 4 types of antibiotics, namely ciprofloxacin; ceptriaxone; cefoxitin; and colistin. It can be concluded that gentamicin, amikacin, and kanamycin are the most effective antibiotics in inhibiting the growth of *E. coli* that causes urinary tract infection in this study. Therefore, considering the relatively high rates of drug resistance observed in this study, the appropriate choice of antibiotic needs to be adjusted only by physicians based on local, regional and national patterns of susceptibility.

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