Riwayat

Original Article

Resistance Testing of Escherichia Coli Bacteria Producing Extended Spectrum Beta-Lactamase (ESBL) in Urinary Tract Infection (UTI) Patients at Indramayu Regional General Hospital

Fitri Zakia[1]✉, Bambang Karsidin[2], Ade Hasan Basri[3]
[1,2,3]Universitas YPIB majalengka, Indonesia.
Correspondence Author: fz8880@gmail.com✉

Abstract:

Urinary Tract Infection (UTI) is an infection caused by the growth of microorganisms in the human urinary tract. The bacteria Escherichia coli can cause UTIs. Escherichia coli is a bacterium that can become resistant to antibiotics in UTIs because it produces the Extended-Spectrum β-lactamase (ESBL) enzyme. ESBL causes high morbidity and mortality because it can make it challenging to manage infectious diseases. Research on ESBL-producing bacteria has been carried out in several hospitals in Indonesia. The difference in place and time of research causes the pattern of resistance of UTI-causing bacteria to antibiotics to be different. The samples in this study were urine specimens from patients with UTI diagnoses who were examined for culture at the microbiology laboratory of Indramayu Hospital. The sampling technique in this study is the total sampling method. The research method used is Quantitative Descriptive. Bacteria identification and Resistance tests were carried out using the Vitec 2 Compact tool. The data obtained from the research results are then collected in tables and used as a reference in the preparation of data analysis. Escherechia coli is the most common type of bacterial species, at 41%. Ampicillin is 100% resistant to all isolates, and Meropenem and Ertapenem are 100% sensitive. Penicillin MIC value for Sensitive outcomes ≤ 4; Intermediate 16; and Resistant ≥ 32. Cephalosporin group MIC value for Sensitive results ≤ 4; Intermediates 8; and Resistant ≥ 64. Carbapenem group MIC value for Sensitive ≤ 0.5; Intermediates 8; and Resistant ≥ 64. Identification of ESBL Escherichia coli bacteria is 89% ESBL positive and 11% ESBL negative.

Keywords: ESBL, Escherichia coli, Resistance

https://jurnal.usk.ac.id/riwayat

Introduction

The number of infection cases continues to increase with the development of disease

Received: Maret 11, 2024 – Revised: Mei 12, 2024- Accepted: June 15, 2024 - Published online: July 30, 2024

one of which is Urinary Tract Infection (UTI). According to the World Health Organization (WHO) data from 2013, Urinary Tract Infection is the second most common infectious disease in the human body after Respiratory Tract Infection.According to research by (Terlizzi et al., 2017), it is estimated that around 150 million people worldwide suffer from UTIs each year. According to the Ministry of Health of the Republic of Indonesia, in 2014, the number of UTI cases in Indonesia was 90-100 per 100,000 people per year (Darsono et al., 2016). Urinary Tract Infection is a significant cause of morbidity and mortality if not treated quickly and appropriately.

Urinary Tract Infection is an infection caused by the growth of microorganisms in the human urinary tract. Bacteria, viruses, and fungi can cause UTIs. The type of bacteria that causes UTIs is Gram-negative anaerobic bacteria commonly found in the digestive tract (Enterobacteriaceae) (Terlizzi et al., 2017). Escherichia coli is one of the pathogenic opportunistic Enterobacteriaceae bacteria that ranks highest as the cause of UTI incidence (Prasetya et al., 2019). Escherichia coli can shift from normal flora in the intestines to pathogenic flora that grows and persists in the urinary tract. Escherichia coli has virulence factors that can infect and cause disease in the urinary tract. Strains of Escherichia coli consistently associated with uropathogenicity are known as UPEC (Uropathogenic Escherichia coli) (Shah et al., 2019).

Urinary Tract Infection is a significant cause of morbidity and mortality if not treated quickly and appropriately. A common problem is when UTIs become challenging to treat due to antibiotic resistance. Antibiotic resistance occurs when antibiotics cannot kill or inhibit the growth of bacteria. Escherichia coli is a bacterium that can become resistant to antibiotics in UTI cases. Escherichia coli can become resistant because it produces the enzyme Extended-Spectrum β-lactamase (ESBL), one of the highest ESBL-producing bacteria (Prasetya et al., 2019). Extended Spectrum Beta-Lactamase (ESBL) is a form of beta-lactamase enzyme with the ability to hydrolyze a broader range of beta-lactam antibiotics such as penicillins, first, second, and third-generation cephalosporins, and aztreonam (except cephamycins and carbapenems) compared to previous generations (Biutifasari, 2018). The ability to produce ESBL is due to improper antibiotic use, leading to genetic changes in Escherichia coli (Siregar et al., 2022).

According to a 2013 CDC (Centers for Disease Control and Prevention) survey, 26,000 infections are caused by ESBL-producing Enterobacteriaceae each year, with about 1,700 resulting in death. In 2007, the Study for Monitoring Antimicrobial Resistance Trends (SMART) conducted a survey in Asia, and the prevalence of ESBL-producing Escherichia coli from intra-abdominal infections was 42% (Fahirah Arsal, 2019).

The production of the ESBL enzyme by Escherichia coli bacteria leads to chronicity, persistence, and recurrence of infections, causing high morbidity and mortality. The occurrence and spread of resistance in ESBL-producing bacteria complicate the management of infectious diseases. Treatment options for infections caused by ESBL-producing bacteria are limited, creating a severe health crisis (Dumaru et al., 2019). Furthermore, this leads to increased costs, more extended hospital stays, and increased side effects from dual and high-dose medications (Lia Yunita et al., 2021).

Strategies to address the emergence and development of resistant microbes can be controlled through two main activities: implementing the prudent use of antibiotics and applying the principles of preventing the spread of resistant microbes through standard precautions (Kemenkes, 2015). Based on these strategies, one of the principles of preventing the spread of resistant microbes through standard precautions is the need

for valid data on microbial patterns and sensitivity. This data is crucial in determining the appropriate antibiotic selection for infections caused by bacteria, such as in UTI cases.

Research on ESBL-producing bacteria has been conducted in several hospitals in Indonesia. For example, the prevalence of ESBL production at Dr. Kariadi Central General Hospital in Semarang from 2004-2005 was 50.6%. However, a lower percentage was found regarding the prevalence of ESBL-producing Enterobacteriaceae infections in ICU patients at the central RSCM in 2011, which was 7.2%. In another study at Dr. M. Djamil Central General Hospital in Padang from 2011-2013, the percentage of ESBL-producing Gram-negative bacteria was 72%, showing that the prevalence was 61.3% for Klebsiella sp, 17.1% for Pseudomonas aeruginosa, 10.3% for Proteus sp, 9.7% for Escherichia coli, and 1.7% for Enterobacter sp. Research at H. Adam Malik Central Hospital in Medan showed that ESBL-producing bacteria were more prevalent in Klebsiella pneumonia (55.5%) than Escherichia coli (45.5%). A study conducted at Arifin Achmad Regional General Hospital in Pekan Baru in 2015 showed a prevalence of ESBL-positive Klebsiella pneumoniae at 73.5%, while Escherichia coli was 26.4%. Research results from several hospitals in Indonesia indicate varying high and low percentages of ESBL-producing prevalence in each hospital studied (Sari, 2019).

Differences in research location and time lead to changes in the antibiotic resistance patterns of UTI-causing bacteria. Therefore, it is essential to continuously monitor the resistance patterns of UTI-causing bacteria in each healthcare institution (Imaniah, 2015). Based on data from the Indramayu Regional General Hospital, there is no data on microbial patterns and sensitivity, especially in ESBL-producing Escherichia coli bacteria. Therefore, this study aims to conduct resistance testing of ESBL-producing Escherichia coli bacteria in UTI cases at Indramayu Regional General Hospital. This research aims to identify and describe the results of Escherichia coli bacteria identification in urine samples diagnosed with urinary tract infections (UTIs) at Indramayu Regional General Hospital. Additionally, this study aims to describe the results of resistance testing of Escherichia coli bacteria isolated from the urine of UTI patients, determine the Minimum Inhibitory Concentration (MIC) in beta-lactam antibiotic resistance testing against Escherichia coli, and determine the percentage of ESBL-positive Escherichia coli bacteria isolated from the urine of UTI patients at Indramayu Regional General Hospital.

Fitri Zakia, et al. (2024). https://doi.org/10.24815/jr.v7i3.40563

Methods

Conceptual Framework

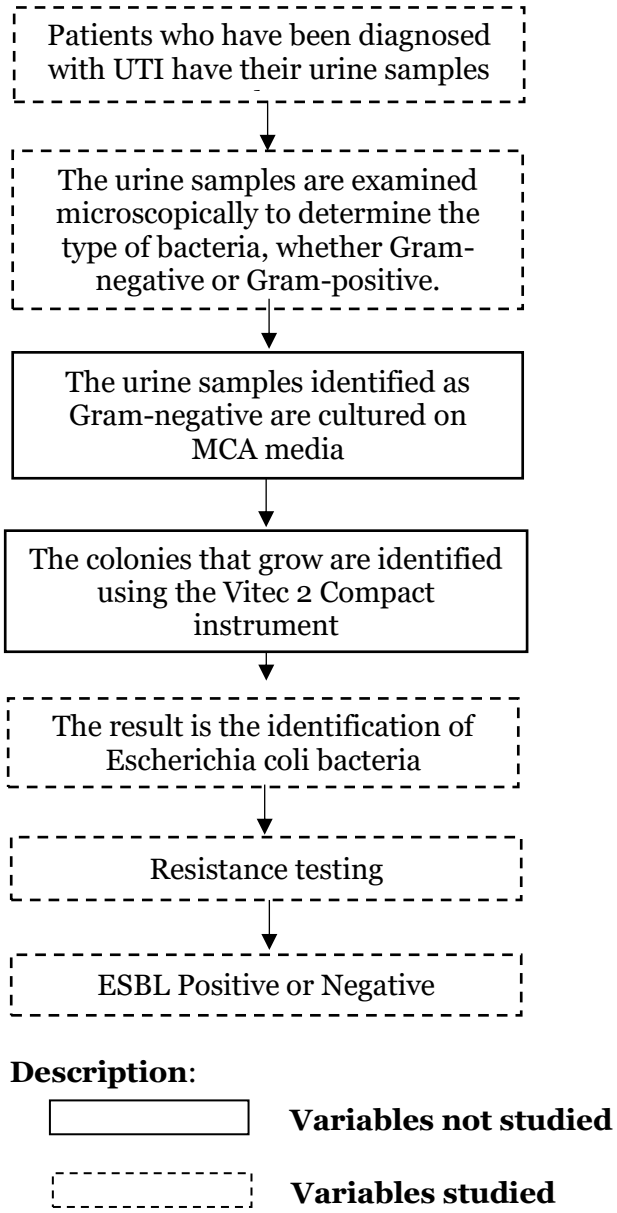

Figure 1. Conceptual Framework Chart

The research methodology used is Descriptive Quantitative, which systematically describes the facts and characteristics of the object or subject being studied accurately. This research involves the identification and resistance testing to provide an overview of ESBL-producing Escherichia coli bacteria in UTI patients at RSUD Indramayu.

Research Object The object of this research is Escherichia coli bacteria isolated from cultured urine samples of patients diagnosed with UTI.

Population. A population is a generalization area consisting of objects or subjects with certain quantities and characteristics determined by the researcher to be studied and then concluded (Sugiyono, 2019). The population used in this study includes all patients diagnosed with UTI who were treated at RSUD Indramayu. The number of patients diagnosed with UTI during the research period was 57.

Sample and Sampling Technique

A sample is a portion of the population's quantity and characteristics (Sugiyono, 2019). The sample in this study is urine specimens from patients diagnosed with UTI, which were cultured in the microbiology laboratory of RSUD Indramayu. The total number of urine specimens examined was 57. The urine specimens that met the inclusion criteria, such as being of good quality and containing Gram-negative bacteria, were 22 samples. Specimens of poor quality that did not contain bacteria or contained Gram-positive bacteria were excluded.

The sampling technique used in this study was Total Sampling. Total Sampling is a technique in which the sample size equals the population size. Because the population size was less than 100, the entire population was included as the research sample.

Tools and Materials

The tools used in this research include a Bio-safety cabinet, incubator (± 37°C), microscope, inoculation loop, object glass, Bunsen burner, micropipette, sterile tips, Densicheck plus standard, polypropylene plastic tubes, Vitek 2 Compact, autoclave, measuring glass, Erlenmeyer flask, digital scale, and magnetic stirrer. The materials used include Vitek ID Cards (GN), Antibiotic Susceptibility Testing Card (AST GN), MacConkey Agar Plate, urine specimens, Gram staining reagents, sterile Vitek saline (0.45% NaCl, pH 4.5 – 7.0), and urine containers.

Procedure Midstream urine collection is aimed at avoiding contamination from the genital area. The procedure differs for men and women: Women should clean the labia with sterile tissue before collecting the midstream urine, while men, especially those who are uncircumcised, should clean the urethra before collecting the urine. After the sample is obtained, bacterial identification is conducted through a microscopic examination of the centrifuged urine sediment. Gram staining is performed to determine the morphology and bacterial reaction. If Gram-negative bacilli are detected, the analysis proceeds to the next stage.

The bacterial culture process uses MacConkey Agar (MCA) media to grow bacterial colonies from urine samples, which are incubated for 24 hours at 37°C. The colonies that grow are identified using the Vitek 2 Compact instrument. This process involves preparing a bacterial colony suspension in sterile saline, measuring the turbidity of the suspension, and inputting patient data into the instrument. The instrument then provides results on bacterial species identification and antibiotic resistance testing, helping to determine which antibiotics are effective and which are not against the bacteria (Prihatini et al., 2018).

Results

This research was conducted by collecting primary data from the Microbiology Laboratory at the Indramayu Regional General Hospital from March to June 2024, and 57 urine specimens were obtained from patients diagnosed with UTI. The research began with the microscopic identification of bacterial content in the urine, followed by Gram staining. Then, the urine samples containing Gram-negative bacteria were cultured on MCA media, and the bacterial species of the resulting colonies were identified using the Vitek 2 Compact instrument. The isolates identified as Escherichia coli were then tested for antibiotic resistance and ESBL production using the Vitek 2 Compact instrument.

Microscopic Identification and Gram Staining Results

The results of microscopic identification and Gram staining are shown in Figures 2 and 3.

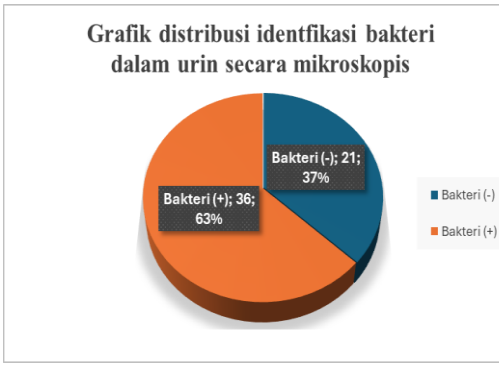

Figure 2. Distribution of Bacterial Identification in Urine Microscopically

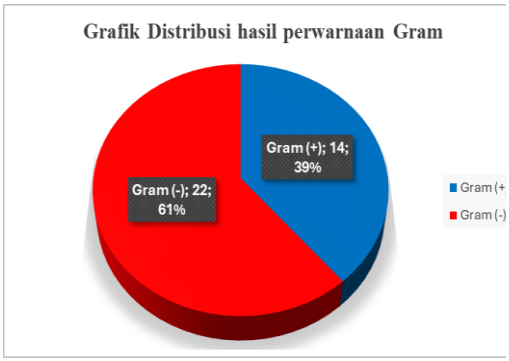

Figure 3. Distribution of Gram Staining Results

The first step in this research was the microscopic identification of bacteria in urine samples from patients diagnosed with UTIs, followed by Gram staining. This step was taken to facilitate the identification of Escherichia coli bacteria, which is the focus of this research. Based on the microscopic identification of bacteria in 57 urine samples from patients diagnosed with UTIs at Indramayu Regional General Hospital, it was found that 21 urine samples, or 37%, did not contain bacteria. In comparison, 36 urine samples, or 63%, contained bacteria. This indicates that more urine samples contained bacteria than those that did not. This result can be attributed to the fact that in UTI cases, bacteriuria occurs in 70% of cases before patients receive antibiotic therapy or experience antibiotic resistance (Flores-Mireles et al., 2015). This result is consistent with the study by Anggreini et al., 2023, which reported that 40 out of 57 samples in UTI cases tested positive for bacteria.

The next step in this research was Gram staining, performed on the urine samples that tested positive for bacteria. Based on the previous microscopic results, Gram staining was conducted on 36 urine samples identified as containing bacteria. The results showed that 14 samples, or 39%, contained Gram-positive bacteria, while 22 samples, or 61%, contained Gram-negative bacteria. This indicates that Gram-negative bacteria were more prevalent than Gram-positive bacteria. This is because the primary cause of UTI cases is Enterobacter bacteria, such as Escherichia coli and Klebsiella pneumoniae, which are Gram-negative anaerobes commonly found in the digestive tract (Enterobacteriaceae) (Terlizzi et al., 2017). These findings are also in line with the study by Sunaidi & Putri Maharani Mansyur, 2022, which explained that the most common pathogenic bacteria causing UTIs are Gram-negative bacteria such as Escherichia coli and Klebsiella pneumoniae.

Bacterial Identification

The results of bacterial identification are shown in Figure 4.

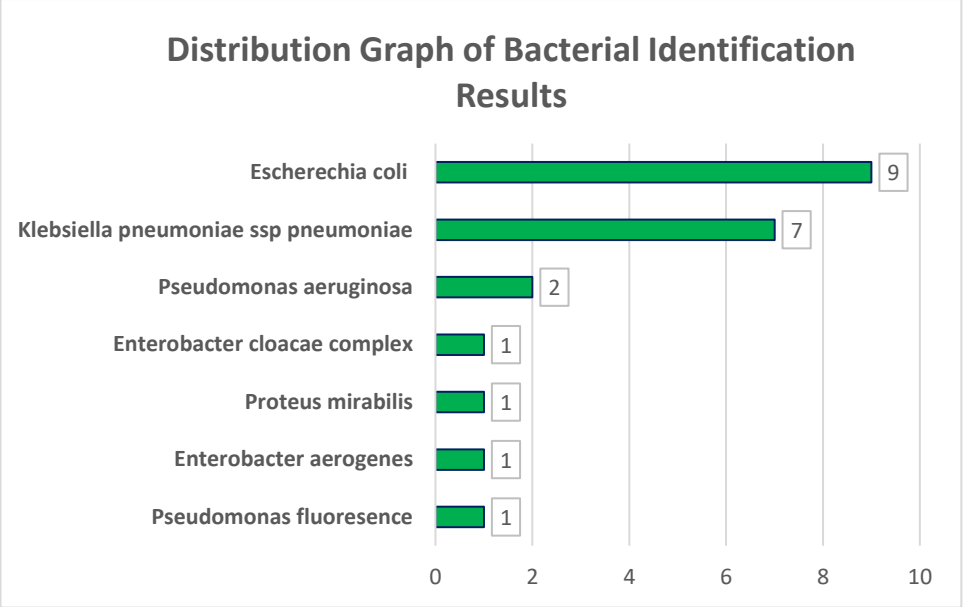

Figure 4. Distribution of Bacterial Identification

Based on the Gram staining results, 22 urine samples were identified as containing Gram-negative bacteria. Following this, bacterial identification was carried out to determine the bacterial species in the urine, facilitating the selection of isolates containing *Escherichia coli* bacteria. The first step in bacterial identification involved culturing the urine samples on MacConkey agar (MC). The culture results showed the growth of colonies on the MC medium. The next step was to isolate the colonies and prepare a 0.5 McFarland suspension for identification using the Vitec 2 Compact device. The bacterial identification results revealed seven bacterial species: *Escherichia coli* with 9 isolates, *Klebsiella pneumoniae ssp pneumoniae* with 7 isolates, *Pseudomonas fluorescens* with 1 isolate, *Pseudomonas aeruginosa* with 2 isolates, *Enterobacter aerogenes* with 1 isolate, *Proteus mirabilis* with 1 isolate, and *Enterobacter cloacae complex* with 1 isolate.

Based on these results, *Escherichia Coli was* the most frequently identified species, accounting for 9 isolates or 41% of the 22 isolates from urine samples diagnosed with urinary tract infection (UTI) at RSUD Indramayu. This occurrence is consistent with that urinary tract infections are most commonly caused by *Escherichia coli*, responsible for approximately 90% of cases (Erlinawati et al., 2024). *Escherichia coli* is an opportunistic pathogenic member of the Enterobacteriaceae family and is the leading cause of UTI incidence (Prasetya et al., 2019). *Escherichia coli* can transition from its status as normal intestinal flora to a pathogenic flora that grows and persists in the urinary tract. *Escherichia coli* possesses virulence factors that allow it to infect and cause disease in the urinary tract. Strains of *Escherichia coli* consistently associated with uropathogenicity are called UPEC (Uropathogenic *Escherichia coli*) (Shah et al., 2019). These findings align with research conducted by (Erlinawati et al., 2024), which reported that bacteria found in urine from suspected UTI cases include *Escherichia coli* (65%), *Enterobacter aerogenes* (18%), *Proteus vulgaris* (9%), *Pseudomonas aeruginosa*(4%), and *Citrobacter freundii* (4%).

Antibiotic Resistance Testing of *Escherichia coli* and ESBL Identification

The results of the antibiotic resistance testing and ESBL (Extended Spectrum

Beta-Lactamase) identification are presented in Table IV.4.

Table 1. Results of Escherichia coli Antibiotic Resistance Testing and ESBL Identification

No	Kode Sampel	ESBL (Positif/Negatif)	Ampicillin		Ampicillin/Sulbactam		Piperacillin/Tazobactam		Cefazolin		Ceftazidime		Ceftriaxone		Cefepime		Ertapenem		Meropenem	
			MIC	R/S/I	MIC	R/S/I	MIC	R/S/I	MIC	R/S/I	MIC	R/S/I	MIC	R/S/I	MIC	R/S/I	MIC	R/S/I	MIC	R/S/I
1	UR2	POSITIF	≥32	R	≥32	R	≤ 4	S	≥ 64	R	≥64	R	≥64	R	≥64	R	≤ 0,5	S	≤ 0,25	S
2	UR6	POSITIF	≥32	R	≥32	R	≤ 4	S	≥ 64	R	4	S	≥64	R	2	S	≤ 0,5	S	≤ 0,25	S
3	UR9	POSITIF	≥32	R	16	I	≤ 4	S	≥ 64	R	8	I	≥64	R	≥64	R	≤ 0,5	S	≤ 0,25	S
4	UR15	POSITIF	≥32	R	≤ 4	S	≤ 4	S	≥ 64	R	4	S	≥64	R	2	S	≤ 0,5	S	≤ 0,25	S
5	UR23	POSITIF	≥32	R	≥32	R	≤ 4	S	≥ 64	R	≥64	R	≥64	R	≥64	R	≤ 0,5	S	≤ 0,25	S
6	UR24	POSITIF	≥32	R	4	S	≤4	S	≥ 64	R	4	S	≥ 64	R	2	S	≤ 0,5	S	≤ 0,25	S
7	UR25	NEGATIF	≥32	R	≥ 32	R	≥ 128	R	4	S	≤ 1	S	≤ 1	S	≤ 1	S	≤ 0,5	S	≤ 0,25	S
8	UR31	POSITIF	≥ 32	R	≥ 32	R	≤ 4	S	≥ 64	R	≥ 64	R	≥ 64	R	≥ 64	R	≤ 0,5	S	≤ 0,25	S
9	UR44	POSITIF	≥ 32	R	≥ 32	R	≤ 4	S	≥ 64	R	≥ 64	R	≥ 64	R	≥ 64	R	≤ 0,5	S	≤ 0,25	S

Based on the table above, 8 *Escherichia coli* isolates were ESBL positive, and 1 isolate was ESBL negative. The distribution of ESBL identification is shown in Figure 5.

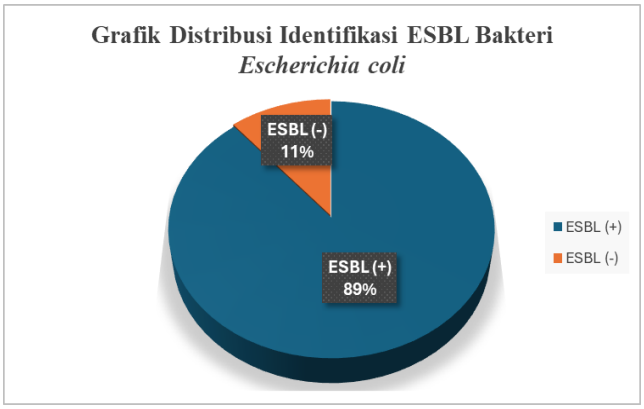

Figure 5. Distribution of ESBL Identification Results for Escherichia coli Bacteria

Resistance tests were conducted using Betalactam antibiotics, namely Penicillin (Ampicillin, Ampicillin/Sulbactam, Piperacillin/Tazobactam), Cephalosporin (Cefazolin, Ceftazidime, Ceftriaxone, Cefepime) Carbapenem (Ertapenem, Meropenem. The results of the resistance test are shown in the figure. The distribution graph of antibiotic resistance test results is shown in figures 6, 7 and 8.

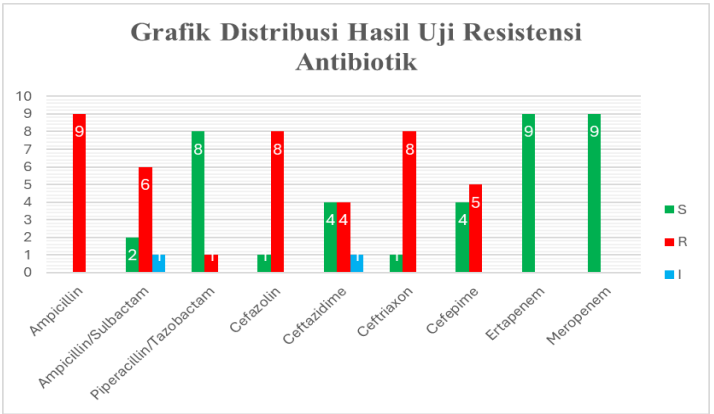

Figure 6. Distribution of Antibiotic Resistance Test Results

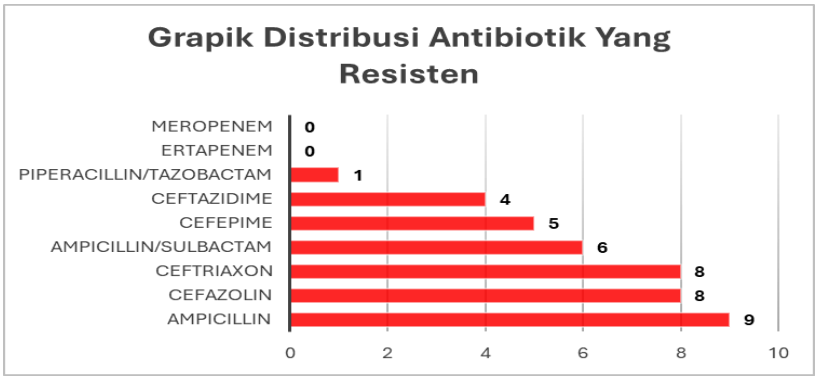

Figure 7. Distribution of Resistant Antibiotics

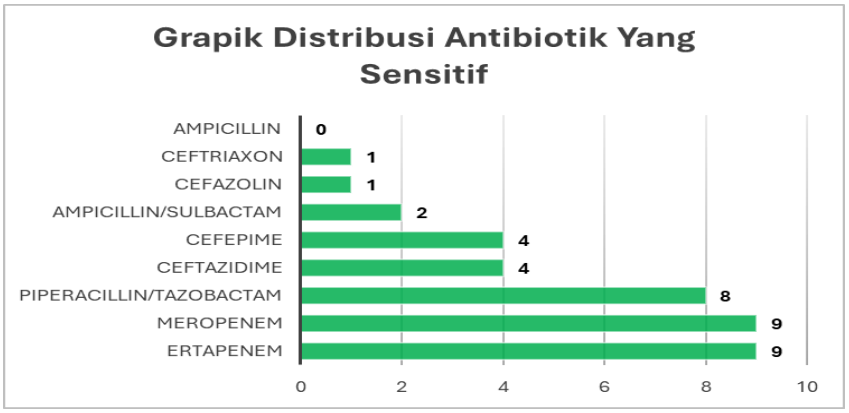

Figure 8. Distribution of Sensitive Antibiotics.

The next step in this research was the identification of ESBL (Extended Spectrum Beta-Lactamase) and testing *Escherichia coli* resistance using Beta-lactam antibiotics. This was done to determine whether the *Escherichia coli* bacteria isolated from urine cultures of UTI patients at RSUD Indramayu could produce ESBL enzymes and to identify which antibiotics were the most resistant and most sensitive by measuring the MIC (Minimum Inhibitory Concentration) values using the Vitec 2 Compact device.

Based on the bacterial identification results, 9 *Escherichia coli* isolates were obtained. Following this, ESBL identification revealed that 8 isolates (89%) were ESBL-positive, and 1 isolate (11%) was ESBL-negative. These results indicate that 89% of the *Escherichia coli* bacteria exhibited resistance by producing ESBL enzymes. *Escherichia coli* is among the bacteria with the highest production of ESBL enzymes (Prasetya et al., 2019). The ability to produce ESBL enzymes arises due to improper antibiotic use, which leads to genetic changes in *Escherichia coli* (Siregar et al., 2022). The ESBL gene in *Escherichia coli* initially appeared due to gene mutations mediated by plasmids, which act as a tool for transferring mutated genes (Masruroh et al., 2017).

The resistance testing was conducted using Beta-lactam antibiotics, specifically Penicillins (Ampicillin, Ampicillin/Sulbactam, Piperacillin/Tazobactam), Cephalosporins (Cefazolin, Ceftazidime, Ceftriaxone, Cefepime), and Carbapenems (Ertapenem, Meropenem). The MIC values obtained varied. For the Penicillin group, the MIC for sensitive results was ≤ 4, intermediate = 16, and resistant ≥ 32. The MIC for sensitive results was ≤ 4, intermediate = 8, and resistant ≥ 64 for the Cephalosporin

group. The MIC for sensitive results for the Carbapenem group was ≤ 0.5, intermediate = 8, and resistant ≥ 64.

Based on the resistance testing results, the Beta-lactam antibiotic most frequently showing resistance to *Escherichia coli* was Ampicillin, with 100% resistance across all examined isolates. Meropenem and Ertapenem most frequently showed sensitivity to *Escherichia Coli*, with 100% sensitivity across all examined isolates. This occurs because ESBL is an enzyme capable of hydrolyzing Penicillin, first, second, and third-generation Cephalosporins, and Aztreonam (except Cephamycins and Carbapenems) (Biutifasari, 2018). These findings are consistent with the research by Fitrianda et al. (2021), which explained that Carbapenems were the most sensitive antibiotics for UTI therapy, while Penicillins were the most resistant. This resistance phenomenon typically occurs when patients arriving at the hospital have already received antibiotic therapy from medical personnel at clinics or health centers or when antibiotics are taken without supervision, leading to incomplete or irregular medication intake.

Conclusion

Based on the results of the research, the following conclusions can be drawn:

1. The identification results show that Escherichia coli is the most prevalent bacterium, comprising 41% or 9 isolates out of 22 identified bacterial isolates from urine samples diagnosed with UTI at RSUD Indramayu.
2. The antibiotic resistance test for Beta-lactam antibiotics on the 9 Escherichia coli isolates revealed that Ampicillin was 100% resistant, while Meropenem and Ertapenem were 100% sensitive.
3. The MIC values for different antibiotic groups are as follows:

   Penicillin: Sensitive ≤ 4; Intermediate = 16; Resistant ≥ 32.

   Cephalosporin: Sensitive ≤ 4; Intermediate = 8; Resistant ≥ 64.

   Carbapenem: Sensitive ≤ 0.5; Intermediate = 8; Resistant ≥ 64.
4. The ESBL identification conducted on the 9 Escherichia coli isolates revealed that 8 isolates (89%) were ESBL-positive and 1 isolate (11%) was ESBL-negative.

Suggestion

Further research is needed on ESBL-producing *Escherichia coli* using different types of samples such as blood, sputum, and wound swabs. Additionally, studies should be conducted to examine the resistance profiles of ESBL-producing *Escherichia coli* when using penicillin-clavulanate antibiotics.

References

Biutifasari, V. (2018). Extended Spectrum Beta-Lactamase (ESBL). Oceana Biomedicina Journal, 1(1), 1. https://doi.org/10.30649/obj.v1i1.3

Darsono, P. V., Mahdiyah, D., & Sari, M. (2016). Gambaran Karakteristik Ibu Hamil Yang Mengalami Infeksi Saluran Kemih (Isk) Di Wilayah Kerja Puskesmas Pekauman Banjarmasin. *Dinamika Kesehatan*, 1, 162–170.

Dumaru, R., Baral, R., & Shrestha, L. B. (2019). Study of biofilm formation and antibiotic resistance pattern of gram-negative Bacilli among the clinical isolates at BPKIHS, Dharan. *BMC Research Notes*, 12(1), 1–6. https://doi.org/10.1186/s13104-019-4084-8

Erlinawati, Jumadewi, A., & Fitriana. (2024). Identifikasi bakteri gram negatif batang pada urine suspek infeksi saluran kemih mahasiswi di Poltekkes Kemenkes Aceh Identification of gram-negative rod bacteria in urine suspected of urinary tract infection female students at Poltekkes Kemenkes Aceh.

Fahirah Arsal, A. S. (2019). Deteksi dan Pola Kepekaan Antibiotik pada Extended Spectrum Beta Lactamase (Esbl) Eschericia Coli dari Sampel Urin Petugas Kesehatan di Rumah Sakit Ibnu Sina Makassar Tahun 2018. *UMI Medical Journal*, 3(2), 1–13. https://doi.org/10.33096/umj.v3i2.38

Fauzan, R., Harsono, K., Meisandy, R. P., Barokah, M., & Muhaimin, M. I. (2024). Optimising Human Resource Management as an Effort to Improve Employee Performance through Digital Attendance. *Riwayat: Educational Journal of History and Humanities*, *7*(1), 16-25.

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *HHS Public Access*, 13(March), 269–284. https://doi.org/10.1038/nrmicro3432.Urinary

Imaniah, B. A. (2015). Peta Kuman Dan Resistensinya Terhadap Antibiotika Pada Penderita Infeksi Saluran Kemih (ISK) Di RSUD Dr. Moewardi. Universitas Muhammadiyah Surakarta.

Imran, M. F. (2024). Criminological Examination of Physical and Psychological Violence Committed Against Children in the School Environment. *Riwayat: Educational Journal of History and Humanities*, *7*(1), 41-47.

Kemenkes. (2015). PERATURAN MENTERI KESEHATAN REPUBLIK INDONESIA NOMOR 8 TAHUN 2015.

Lia Yunita, S., Novia Atmadani, R., & Titani, M. (2021). PHARMACEUTICAL JOURNAL OF INDONESIA Faktor-faktor Yang Mempengaruhi Pengetahuan Dan Perilaku Penggunaan Antibiotika Pada Mahasiswa Farmasi Universitas Muhammadiyah Malang. *Pharmaceutical Journal Of Indonesi*, 2021(2), 188.

Masruroh, C. A., Sudarwanto, M. B., & Latif, H. (2017). Tingkat Kejadian Escherichia coli Penghasil Extended Spectrum B-Lactamase yang Diisolasi dari Feses Broiler di Kota Bogor. *Jurnal Sain Veteriner*, 34(1), 42. https://doi.org/10.22146/jsv.22813

Prasetya, Y. A., Nisya, K., & Amanda, E. R. (2019). Aktivitas Nanoemulsi Minyak Lengkuas (Alpinia galanga [L] Willd) Dalam Menghambat Bakteri Escherichia coli Penghasil Extended Spectrum Beta Lactamases (ESBLs). Seminar Nasional Pendidikan Biologi Dan Saintek (SNPBS), 4, 301–309.

Prihatini, Aryati, & Hetty. (2018). IDENTIFIKASI CEPAT MIKROORGANISME MENGGUNAKAN ALAT Vitek-2. *Indonesian Journal of Clinical Pathology and Medical Laboratory*, 13(3), 129–132. https://doi.org/10.24293/ijcpml.v13i3.915

Romulo, C. S., & Dalimunthe, Z. (2024). Effect of related party transaction and tax haven utilization on tax avoidance moderated by Country-by-Country reporting. *Riwayat: Educational Journal of History and Humanities*, *7*(1), 26-40.

Sari, R. P. (2019). Pengaruh Penggunaan Antibiotik Sefalosforin Generasi III Terhadap Munculnya ESBL (Extended-Spectrum Beta-Lactamase) Pada Persahbatan Jakarta Periode Januari-Desember 2017. UHAMKA JAKARTA.

Shah, C., Baral, R., Bartaula, B., & Shrestha, L. B. (2019). Virulence factors of uropathogenic Escherichia coli (UPEC) and correlation with antimicrobial resistance. *BMC Microbiology*, 19(1), 1–6. https://doi.org/10.1186/s12866-019-1587-3

Siregar, M. L., Nelwan, E. J., . E., Haryanto, B., Puspandari, N., Sinto, R., Nainggolan, L., Bonar, M., & Shatri, H. (2022). Risk factors and mortality outcomes of extended-spectrum beta-lactamase producing Escherichia coli bacteremia: A retrospective cohort study from two Indonesian referral hospitals. F1000Research, 11, 1449. https://doi.org/10.12688/f1000research.126345.1

Sugiyono. (2019). *Metode Penelitian Kuantitatif, Kualitatif, dan R&D*. Alfabeta.

Terlizzi, M. E., Gribaudo, G., & Maffei, M. E. (2017). UroPathogenic Escherichia coli (UPEC) infections: Virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. *Frontiers in Microbiology*, 8(1566), 1–23. https://doi.org/10.3389/fmicb.2017.01566