

# Correlation of Leukocyte Esterase Positivity Levels Against the Results of Several Examination Parameters Laboratory Urinary Tract Infection in Patients at Mataram City Regional Hospital

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## ABSTRACT

*Background* : Urinary tract infection (UTI) is caused by the multiplication of bacteria in the urinary tract. The standard proving procedures for UTI are urine culture examination and urine dipstick examination (leukocyte esterase and nitrite). *Objective* : This study aims to measure leukocyte and nitrite values, calculate the total number of bacteria, identify Gram type, species and antibiotic sensitivity and analyze the correlation of leukocyte esterase positivity to the results of several UTI examination parameters. *Research Methods* : The method used is an analytic observational design with cross sectional design using primary data and analyzed with the Spearman rank test. *Results* : The results showed that in 198 patients with suspected UTI, 123 (62%) were positive for leukocyte esterase, 16 (8.1%) were positive for nitrite. The total number of bacterial count showed 82 people (41%) with bacteriuria  $\geq 10^5$  CFU/mL. Found 61 Gram-negative bacilli and 21 Gram-positive cocci with 21 different bacterial species. Antibiotic sensitivity test showed 51% sensitive, 6% intermediate and 43% resistant. Correlation of leukocyte esterase with nitrites ( $p 0.023 < 0.05$ ), total bacteria count ( $p 0.00 < 0.05$ ), Gram negative bacilli ( $p 0.00 < 0.05$ ), and Gram positive cocci ( $p 0.533 > 0.05$ ), antibiotic sensitivity test ( $p 0.794 > 0.05$ ) and bacterial species ( $p 0.316 > 0.05$ ). *Conclusion* : The results showed that there was a correlation between the level of positivity of leukocyte esterase and nitrite, total bacteria count and Gram-negative bacilli, but there was no correlation between Gram-positive cocci, bacterial species and antibiotic sensitivity tests.

## INTRODUCTION

Infectious disease is one of the major health problems in developing countries, including Indonesia. One of the diseases that often attacks the urinary system is Urinary Tract Infection (UTI). The prevalence of UTI in Indonesia is still quite high, according to estimates by the Ministry of Health of the Republic of Indonesia, the number of UTI sufferers in Indonesia is 90-100 cases per 100,000 population per year or around 180,000 new cases per year (Ministry of Health of the Republic of Indonesia, 2014). The standard protocol for the UTI diagnosis approach is routine urine analysis, microscopic examination of fresh urine, urine culture, and the bacterial colonization count. The diagnosis of UTI is made based on clinical findings and laboratory examinations, one of which is the dipstick test. This test is a practical

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choice in terms of time and cost for diagnosing UTI. Urine testing with a dipstick is an alternative to testing microscopy urinalysis for the diagnosis of uncomplicated acute cystitis (Seputra et al , 2020).

The parameters observed on the urine dipstick to determine the diagnosis of UTI are leukocyte esterase and nitrite. Leukocyte esterase positive is a condition where there are leukocytes that secrete the esterase enzyme caused by the presence of Enterobacteriaceae bacteria (Sabriani et al, 2021). An increased number of leukocytes in the urine indicates inflammation that can occur in the urinary tract system from the kidneys to the lower urinary tract (bladder and urethra) (Brunzel, 2018). According to research by Inayati and Falah (2014), the sensitivity of leukocyte esterase was 83.3% and the specificity was 72%. Urine nitrite examination is used to determine bacteriuria, because nitrite is the result of nitrate metabolism which is reduced by bacteria that have reductase enzymes such as bacteria in the Enterobacteriaceae group . Nitrite has a specificity of 98% and a sensitivity of 53% for diagnosing UTI (Trinadi et al, 2016).

A more specific examination to identify UTI uropathogenic bacteria is a microbiological examination that is useful for seeing the growth of uropathogens that cause UTIs. Urine culture is used not only for identification of bacteria but also for counting bacteria in urine. UTI is diagnosed when there are more than 100,000 CFU/ml of bacteria of the same species. Pure growth of Enterobacteriaceae is commonly found in urine specimens containing it  $>10^5$  bacteria/ml (Darsono et al, 2016) . Gram staining for identification of bacteriuria can be performed using centrifuged or non-centrifuged urine specimens. One bacterium per immersion field with Gram stain in uncentrifuged urine, corresponding to  $>10^5$  bacteria/ml of middle portion of urine (Sobel and Kaye, 2015). The bacteria that most often cause urinary tract infections in Gram-negative bacteria are Escherichia coli, Klebsiella sp , Proteus sp , Enterobacter, Providencia, Citrobacter, Pseudomonas. While Gram positive bacteria are Staphylococcus and Streptococcus (Sukandar, 2014). The sensitivity and specificity of Gram staining compared to urine culture is 47.8% and 97.2% with an accuracy of 78% (Trihono et al, 2019). The advantage of urine culture in the diagnosis of UTI is that it can determine the pattern of bacteria and antibiotic sensitivity which are important factors in determining appropriate therapy for an infectious disease, especially those caused by bacteria. Urine culture is a very reliable test with a sensitivity of 95% and a specificity of 85% (Seputra et al, 2020).

The operation of the dipstick is very easy, the cost of examination is not expensive, so it is the choice as a screening tool for UTI (Mambatta, 2015). Until now, urine culture examination is the gold standard for diagnosing UTI. However, the weakness of this culture examination is that it requires a long time (3-5 days) and high costs, as well as a special laboratory to carry out the examination (Najeeb, 2015).

Many studies have been conducted to determine the diagnostic value of leukocyte esterase urinalysis in diagnosing UTI. From the studies that have been conducted by previous researchers, no one has examined the level of positivity of leukocyte esterase on laboratory examination parameters in the diagnosis of UTI patients, namely nitrite test and urine culture (bacterial count, type of gram of bacteria, identification of bacterial species and antibiotic sensitivity test).

## METHODS

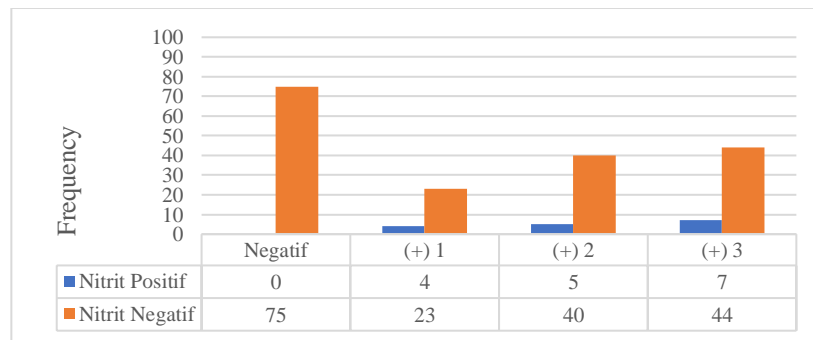
This study was a cross-sectional study conducted from January to March 2023 at the Mataram City Hospital. This study included patients at the Mataram City Hospital with a diagnosis of UTI, while the exclusion criteria were urine culture results that were too long and contaminated with outside air, patients who had received treatment with antibiotics and adult patients in a pregnant condition. The study involved 198 patients who met the inclusion criteria. The research data used were primary data with an analytical observational research design with diagnostic tests of dipstick examination of leukocyte esterase parameters associated with nitrite tests, urine culture which includes identification of bacterial Gram types, bacterial counts, identification of bacterial species, and bacterial antibiotic sensitivity tests. The dipstick examination method is colorimetry. Urine was examined using the Combostick R-300 Urine Analyzer. The results of the leukocyte esterase examination on the urine dipstick were reported as negative, +1, +2, and +3 and the nitrite results were reported as negative and positive.

Identification of the Gram type of bacteria is done by Gram painting as a presumptive test to see the morphology of bacteria and bacterial staining properties, then conducting a confirmation test by quantifying the number of bacteria on CLED and MCA media that have been incubated at 37 °C for 1x24 hours. The number of colonies that grow is multiplied by 1000 CFU/ml. Identification of bacterial species found in patient urine samples and antibiotic sensitivity testing using the semiautomatic Technical Dedicated Reasonable (TDR)-300B method. Microorganism identification examination through 20-24 biochemical tests and antibiotic sensitivity tests based on colorimetric principles. The identification test in the TDR method uses the appropriate type of reagent card including TDR STAPH-64, TDR STR-64, TDR ONE-64, TDR NF-64. Selection of reagent cards based on bacterial morphology through colony identification on agar media and Gram painting Antibiotic sensitivity test results can be obtained automatically using the TDR-300B. Results in the form of Minimal Inhibitory Concentration (MIC) values and interpreted as sensitive, intermediate and resistant.

Ethical clearance No.LB.01.03/6/013/2023 was obtained from the Health Research Ethics Committee of the Health Polytechnic of the Ministry of Health Mataram. Analysis of data obtained using a computer. Data correlation test using Spearman Rank test. Statistical analysis was processed using the SPSS computer program. The p value is significant if <0.05 and 95% confidence interval.

## **RESEARCH RESULTS AND DISCUSSION**

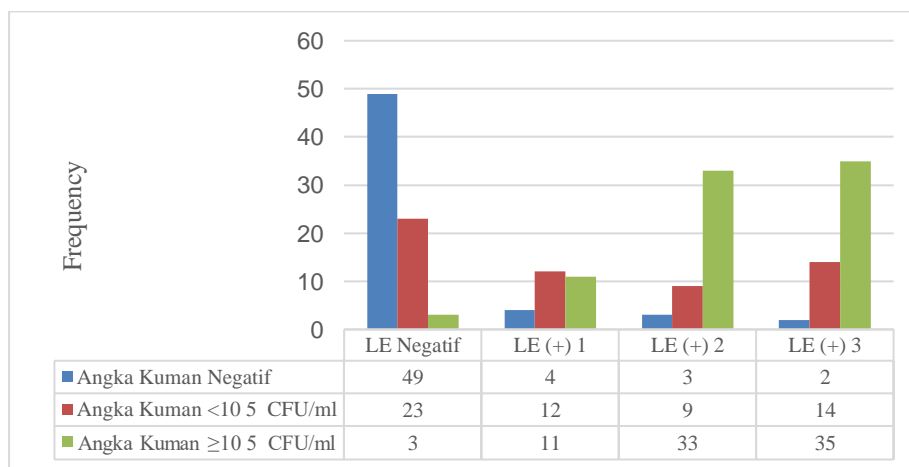
From the research that has been done, the results of leukocyte esterase positivity level examination were obtained using several laboratory examination parameters for urinary tract infections, nitrite, number of bacteria count, Gram type of bacteria, type of bacterial species and antibiotic sensitivity test. The research results are presented in a figure accompanied by the following explanations



**Figure 1.** Graph of Esterase and Nitrite Leukocyte Urinalysis Results

The results of urine leukocyte esterase examination and the nitrite test are shown in Figure 1.1. The most positive nitrite examination results were found in leukocyte esterase (+) 3 results and the least in negative leukocyte esterase results, followed by negative results in the nitrite test.

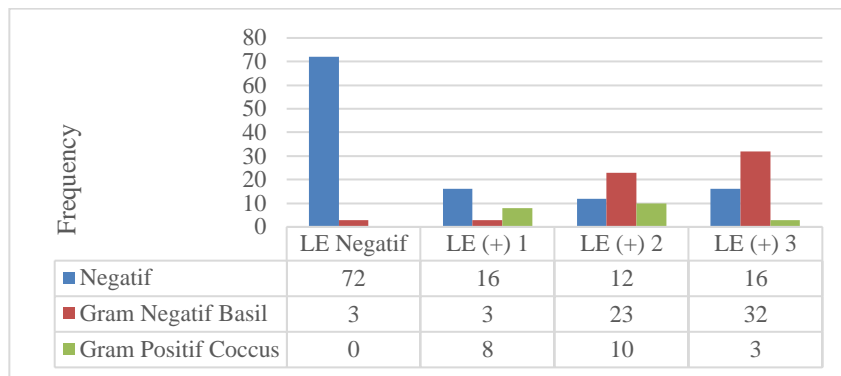
The results of colony counting for the number of germ numbers are calculated using the formula for the number of colonies divided by the number of samples inoculated (planted) or the number of colonies multiplied by 1000 with units of CFU/ml. The results of the urine leukocyte esterase examination and the total number of bacteria count are shown in Figure 1.2 below.



**Figure 2.** Leukocyte Esterase Urinalysis Examination and Number of Bacterial count

The examination results displayed in Figure 1.2 show the number of urine samples that were cultured to see the presence of bacterial growth. A total of 198 urine samples had different bacterial growth values. A total of 82 samples showed bacterial growth results of  $\geq 100,000$  CFU/ml, this value indicates positive UTI which is most often found at the leukocyte esterase (+)3 positivity level. The other 116 urine samples which were cultured showed negative results.

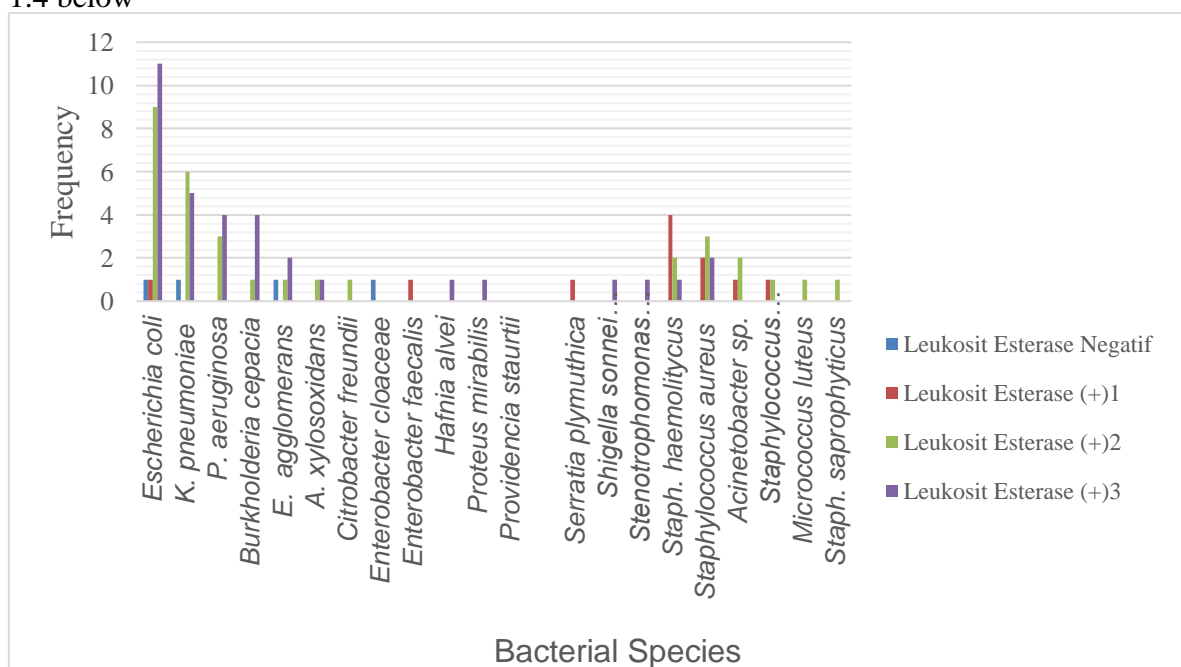
The results of the examination of the Gram type of bacteria is carried out using Gram staining. The purpose of Gram staining is to differentiate Gram positive and Gram negative bacteria. The results of urine leukocyte esterase examination and Gram types of bacteria are described in Figure 1.3 below



**Figure 3** Leukocyte Esterase Urinalysis Examination Results and Gram Types of Bacteria

The examination results shown in Figure 1.3 Gram staining show that the bacteria that cause UTI can be caused by Gram negative bacteria in 61 samples (74%) or Gram positive bacteria in 21 samples (26%).

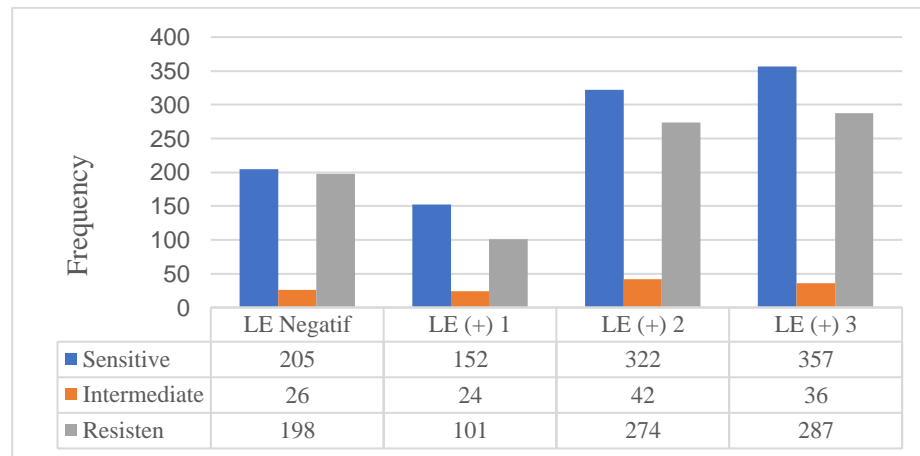
Gram-negative bacillus bacteria were most commonly found in the leukocyte esterase examination results with a positivity level of (+)3 in 32 examination samples, while Gram-positive coccus bacteria were most often found in the leukocyte esterase examination results with a positivity level of (+)2 in 10 examination samples. The results of urine leukocyte esterase examination and bacterial species in urine culture are explained in Figure 1.4 below



**Figure 4.** Urinalysis Results for Leukocyte Esterase and Bacterial Species

In the examination of the identification of bacterial species, the results showed that the bacterial species as the cause of UTI with 21 different bacterial species. In the leukocyte esterase examination which was connected to the bacterial species identification examination, the highest results were found in leukocytes with a positivity level (+) 3. The results showed that 35 bacterial isolates were positive as the cause of UTI with 13 different bacterial species, where *Escherichia coli* was found most frequently in 11 test samples .

To test bacterial antibiotic sensitivity, the TDR-300B Microorganism Analysis System is used. The antibiotic sensitivity test uses the broth microdilution principle based on standards from the Clinical and Laboratory Standard Institute (CLSI) which produces sensitive, intermediate and resistant results. The results of urine leukocyte esterase examination and antibiotic sensitivity test in urine culture are described in Figure 1.5 below



**Figure 5.** Results of Leukocyte Esterase Urinalysis and Antibiotic Sensitivity Test

In the examination of leukocyte esterase associated with the examination of bacterial sensitivity tests, the results of sensitive, intermediate and antibiotic resistant tests were found to be mostly found at the level of leukocyte esterase positivity (+) 3 and the least found at the level of positivity test results (+) 1.

The reagent strips detect leukocyte esterase found in granulocytes (neutrophils, eosinophils and basophils). The presence of esterase is used as a clue to the presence of neutrophils in the urine although microscopic examination often does not find leukocytes. An increase in the number of neutrophils in the urine can be a clue to UTI. A positive result of leukocyte esterase has a significant relationship to the number of neutrophils, both in the intact and lysed state. So leukocyte esterase can describe the presence of pyuria. The advantage of this test is its ability to detect both intact and lysed leukocytes.

The results of leukocyte esterase and nitrite look significant from the comparison of negative leukocyte esterase and negative nitrite results which show 100%. While the positive leukocyte esterase results were not followed by the same large positive nitrite results. This is in line with the research of Brilian F, (2017) where the results showed that the leukocyte esterase test on the dipstick eight times indicated bacteriuria as a risk of urinary infection compared to nitrite. So it can be concluded that diagnosing UTI with nitrite is not very significant, because even though the nitrite results are negative, it cannot rule out the absence of bacteriuria because it can be caused by urinary tract infections by bacteria that do not produce nitrite. Nitrite-forming bacteria are *E. coli*, *Proteus*, *Klebsiella*, *Aerobacter*, *Citrobacter*, *Salmonella*, some *Enterococci*, *Staphylococci*, *Pseudomonas*, *Serratia marcescens* while bacteria that do not form nitrite are *Streptococcus faecalis*, *Gonococcus*, *Tuberculosis*, *Shigella*, urine has not been in the bladder long enough, or excessive diuresis (Rosida and Pratiwi, 2019). In this study, a weak and unidirectional significant relationship was found between the results of the examination of leukocyte esterase and nitrite. The reason for the weak correlation value of leukocyte esterase and nitrite in this study is because not all urine samples examined used morning urine specimens

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so that the urine has not been long enough in the bladder. Bacterial enzymes will convert nitrate to nitrite within a minimum of 4 hours in the bladder (Bellazreg, 2019). The metabolic process of nitrate to nitrite is very fast, so it only stays in the urine for a short time. Consumption of vegetables such as lettuce and spinach can give false positive results in urine nitrite tests, because these vegetables contain nitrite, but the culture results are negative (Tuntun and Aminah, 2021). So it can be biased when interpreting urine nitrite results.

In this study, the results obtained a significant correlation with a strong and unidirectional correlation between variable leukocyte esterase and the total number of bacteria count which can be interpreted that the level of positivity of the leukocyte esterase test results as it increases, the number of bacterial count also increases. The higher the level of leukocyte esterase positivity the greater the chance of suffering from a UTI is greater. The reagent strip detects leukocyte esterase found in granulocytes (neutrophils, eosinophils and basophils). The presence of esterase is used as an indication of the presence of neutrophils in the urine although on microscopic examination often no leukocytes are found. An increase in the number of neutrophils in the urine can be an indication of a UTI. A positive result of leukocyte esterase has a significant correlation to the number of neutrophils, both in intact and lysed states. So leukocyte esterase can describe the presence of pyuria. An increase in the number of neutrophils indicates a urinary tract infection, which is indicated by positive results on leukocyte esterase examination and a significant number of bacterial count ( $> 10^5$  CFU/ml) (Malau and Adipereno, 2019). The leukocyte esterase test has low specificity but has good sensitivity for diagnosing UTI (Sabriani et al, 2021). In positive one (+1) the results showed that 59.3% did not suffer from UTI. It is possible that the one positive bacterial result (+1) seen in routine urine does not represent the count number bacterial on inspection culture urine which can cause UTI, so that the urine culture examination is said to be negative or there is no growth of pathogenic aerobic bacteria. Bacterial results were positive one (+1) seen in the urine is also due to pooling samples that are not sterile so that the visible bacterial count are only bacterial contaminants (flora normal) from rectum or tool genitals. In the negative routine urine bacteria results, 4.0% of urine culture results were positive. It is possible that the sample was collected before urinate or the patient drinks water so that urine occurs dilution in bladder so that pathogenic bacteria when examination were not detected on examination. The container for urine routine examination and culture urine are different, so that some get diluted urine (last discharge before running out) (Sulistiani, et al, 2021).

There is a significant relationship with a moderate and unidirectional correlation between the variable level of leukocyte esterase positivity and the results of Gram-negative bacilli examination (p-value 0.000) and there is no significant relationship between the variable level of leukocyte esterase positivity and the results of Gram-positive cocci bacteria examination (p-value 0.533). The most commonly encountered bacteria in urine are rodshaped (bacilli), but coccus forms are also found. These microorganisms vary in size from long, thin, short rods, fat rods. The number of Gram negative bacteria found compared to Gram positive is in line with the research of IImasari et al (2023) which states that there is a correlation between Gram negative rod bacteria and leukocyte esterase levels, and Gram negative rod bacteria were found in 29 samples and positive leukocyte esterase levels in 30 samples. The main cause of more than 85% of UTI cases are Gram-negative bacilli that are normal inhabitants of the gastrointestinal tract, usually *E. coli*, followed by *Proteus*,

Klebsiella, and Enterobacter. Streptococcus faecalis, which also originates from the gastrointestinal tract, staphylococci and almost all bacteria and fungi can also cause lower and renal UTIs. From the examination data, Gram-positive bacteria that cause UTIs are also obtained, with a smaller number than Gram-negative bacteria, so they are not found to be the cause of urinary tract infections. In this study, Staphylococcus aureus and Staphylococcus haemolyticus were found to cause the most UTIs from Gram-positive bacteria. In a study conducted by Malau and Adipireno in 2019, it was found that the Gram-positive bacteria that caused the most UTIs were Staphylococcus haemolyticus as many as 6 samples (17.15%) of a total of 35 samples showed bacterial growth results  $\geq 100,000$  CFU/ml.

There was no significant relationship between the variable level of leukocyte esterase positivity and antibiotic sensitivity test results (p-value 0.794). Antibiotic sensitivity of the bacteria that cause Urinary Tract Infection (UTI) has a different inhibition zone in each test which indicates that each bacteria has a different resistance to the antibiotics given. There is no relationship between the results of the leukocyte esterase examination and the antibiotic sensitivity test, this can be caused because in principle the antimicrobial sensitivity test is the determination of disease-causing bacteria that may show resistance to antimicrobials or the ability of an antimicrobial to inhibit the growth of bacteria that grow in vitro, so that it can be selected as a potential antimicrobial for treatment. Some factors that play an important role from the patient in addition to things that can affect the results of sensitivity testing are the site of infection and severity of disease, virulence and pathogenicity of the infecting bacteria, having several diseases simultaneously, the status of the patient's immune system and immune system, drug interference and interactions, antimicrobial-binding serum proteins, diffusion of antimicrobials in host cells and tissues. (Siregar, NS. 2021). Several factors can influence bacterial resistance to antibiotics. Overuse of antibiotics, irrational use of antibiotics, excessive use of antibiotics, long-term use of antibiotics (Siregar, NS. 2021).

There is no significant relationship between leukocyte esterase variables and bacterial species in urine culture (p-value 0.316) so that the results of the examination of leukocyte esterase positivity levels cannot be used to determine bacterial species at any positivity level. Microorganisms that cause UTIs are generally single, such as Gram-negative Escherichia coli bacteria, which are the most common cause of UTIs (Rosida and Pratiwi, 2019). Escherichia coli when present on the skin or near the anus easily reaches the urinary tract, especially based on the anatomy of the urinary tract and the female anus is very close which can result in a higher risk of infection. In addition, Escherichia coli can enter the urinary tract through the use of catheters in medical therapy. Klebsiella pneumonia is a frequent cause of nosocomial infections in patients with weakened immunity (Black 2012). Klebsiella pneumoniae is one of the causes of nosocomial infections with its main transmission originating from the gastrointestinal tract and is also closely related to the hand hygiene of medical personnel on duty, Klebsiella pneumoniae in the hospital area has the ability to spread rapidly, making it the most common cause of nosocomial infections (Andari et al., 2021). Pseudomonas sp which often infects patients with urinary catheters (Rosida and Pratiwi, 2019). Gram-positive bacteria that cause UTI in this study are Staphylococcus haemolyticus, Staphylococcus aureus. Gram-positive bacteria play a lesser role in UTIs, however Staphylococcus saprophyticus can be found in 10-15% of UTIs. Patients with a history of kidney stones, use of instrumentation, or surgery commonly get UTIs due to Enterococcus and Staphylococcus aureus.



From the results of this study, it was found that the most common bacteria causing UTIs were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. These results are similar to the results of research conducted on UTI patients at Sanglah General Hospital from January to June 2019, that Gram-negative bacteria are the most common bacteria that cause UTI, namely *Escherichia coli* (51.21%), *Klebsiella pneumoniae* (8.54%) and *Acinetobacter baumannii* (7.31%). Likewise, Nazrih Ulmas' research (2016) at the Hajj Adam Malik Hospital obtained that the most common bacteria causing UTIs were *Escherichia coli* as much as 42% followed by *Klebsiella pneumoniae* 30% *Acinetobacter baumannii* 10% and Fitrianda et al's research in 2021 the results of urine isolation of UTI patients in the Internal ward of Dr. M. Djamil Padang Hospital, obtained three types of bacteria, namely *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus sp.*

### **Conclusion**

Based on the research that has been done, it can be concluded that there is a significant correlation with a weak and unidirectional correlation between the results of the examination of the level of positivity of leukocyte esterase and nitrite, a significant relationship with a strong and unidirectional correlation between the results of the examination of the leukocyte esterase test and the number of germs, a significant relationship with a moderate and unidirectional correlation between the variable level of positivity of leukocyte esterase and the results of the examination of Gram-negative bacilli and there is no significant relationship between the variable level of positivity of leukocyte esterase and the results of the examination of Gram-positive cocci bacteria, the results of antibiotic sensitivity tests and bacterial species in urine culture.

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