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RESEARCH ARTICLE OPEN ACCESS

ESCHERICHIA COLI BACTERIAL COLONIES IN PATIENTS WITH URINARY TRACT INFECTIONS USING FRESH AND STORED URINE SPECIMENS

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ABSTRACT

Urinary Tract Infection (UTI) is one of the most common bacterial infections that affects around 40% of women in their lives. Diagnosis of UTI is very necessary to determine the steps in treatment. Counting the number of bacteria is the gold standard for determining the severity of a UTI. In some hospitals, urine samples are often sent to the laboratory in conditions that are not fresh, even though the examination of bacteria in the urine must be carried out immediately, because storage of bacterial examination causes the development of bacteria, so that the growing colonies do not show the actual number of bacteria in the urine at the time of collection. to determine the results of examination of Escherichia coli bacteria on the urine of patients with Urinary Tract Infection (UTI) with urine that is examined immediately and stored. Urine culture on EMBA (Eosin Methylene Blue Agar) media , with a sample of 15. In this examination, an examination of the sample was planted on EMBA (Eosin Methylene Blue Agar) media and then viewed macroscopically. Then observed under a microscope with gram staining, after that it was planted on PCA (Plate Count Agar) media and then counted the number of colonies. The results of research that has been carried out on 15 urine samples of patients with a diagnosis of Urinary Tract Infection (UTI) which were examined immediately and stored for 15 and 30 minutes showed that the number of bacteria in the samples with variations in storage showed an increase at 30 minutes, whereas at 0 and 30 minutes 15 minutes there is no significant difference. Based on the research that has been done, it can be concluded that the number of bacterial colonies has increased, especially at the 30th minute, so it is hoped that the bacterial culture examination of UTI sufferers will be carried out before 30 minutes.

I. INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common bacterial infections that affects around 40% of women in their lives [1]. Although most UTIs are mild, these infections can also cause life-threatening sepsis. The most serious cause of UTIs is bacteria, but fungal, viral and parasitic infections can also be causes. Bladder infection or cystitis is the most common form of UTI but infection can occur in any part of the urinary tract, which can cause pyelonephritis, urethritis and prostatitis, among others.

Urinary Tract Infection (UTI) is an infectious disease that is often found in general practice. Several studies have shown that there are factors that can cause UTIs such as age, gender, long lying down, use of immunosuppressant and steroid drugs, catheterization, urinary retention habits, genital hygiene, and predisposing factors [2].

One important condition that needs to be considered in UTI symptoms is bacteriuria. Bacteriuria is a condition where bacteria can be found in the urine, but this condition does not always mean UTI. Bacteriuria is often asymptomatic. Bacteriuria is often known by another term, namely pyuria, which means a condition in which leukocytes are found in the urine. Leukocytes in the urine is a sign that there is an inflammatory response due to a bacterial infection. UTI clinical symptoms vary depending on age, the intensity of the inflammatory reaction and the location of the infection in the urinary tract. Children aged 2 months – 2 years who suffer from UTIs need special attention because of the atypical clinical symptoms, invasive methods of obtaining urine samples, and having the greatest risk of kidney damage. After the first episode, UTI can recur in 30-40% of patients, especially in patients with anatomic abnormalities, such as vesicoureteral reflux, hydronephrosis, urinary obstruction, bladder diveriticulum, and others. The main management of patients is to maintain urinary tract function and improve the patient's quality of life by immediately handling urination so that there is no interference with urine elimination [3].

According to the National Kidney and Urologic Diseases Information Clearinghouse (NKUDIC), UTI ranks second after upper respiratory infection (ARI) and as many as 8.3 million cases are reported per year. UTIs can affect patients of all ages, from newborns to the elderly. Women suffer from UTIs more often than men, approximately 50% of all women have suffered from UTIs in their lifetime. Even women often experience recurrent UTIs which can interfere with their social life [4].

According to the Ministry of Health of the Republic of Indonesia, the number of UTI sufferers in Indonesia is still quite a lot, reaching 90-100 cases per 100,000 population per year or around 180,000 new cases per year [5].

Diagnosis of UTI is very necessary to determine the steps in treatment. The bacterial count is the gold standard for confirming a UTI. The results of counting the number of bacteria in the urine are used to determine the degree of severity of UTI, namely mild severity of 10^3 CFU/mL, moderate around 10^4 CFU/mL, and severe if it reaches 10^5 CFU/mL. In several hospitals, urine samples are often sent to the laboratory in conditions that are not fresh, even though the examination of bacteria in the urine must be carried out immediately, because delays in the examination cause the development of bacteria, so that the colonies that grow do not show the actual number of bacteria in the urine at the time of collection [6].

According to the Clinical and Laboratory Standard Institute (CLSI) it is recommended that urine tests be carried out no later than 2 hours from the time urine is urinated. Urine delay for 2 hours without being stored at 2-8ºC and the addition of preservatives can reduce the quality of urine examination results. Urine examination results that change due to delays in examination cannot properly describe the patient's condition, so that it can be an error in diagnosis [7]. In Sirait's study, it showed that the bacteria numbered 7.5×10^4 CFU /ml was delayed for 6 hours, when the delay was 12 hours there was an increase of 1.2×10^5 CFU/ml, but when the delay was 18 and 24 hours it decreased to 8.0×10^4 CFU/ml and 7.8×10^4 CFU/ml. Examinations at the hospital are often delayed due to the delivery of urine specimens from the room for inpatients and the large number of patients is a concern for further research to determine the amount of Escherichia coli using urine that is checked immediately and stored tests [8].

II. MATERIALS AND METHODS

This type of research is an experimental research. The population in this study were sufferers who experienced urinary tract infections caused by Escherichia coli bacteria at Grandmed Lubuk Pakam Hospital, the number of samples that would be used as respondents in this study were 15 respondents. The research sample taken was urine found in people who had urinary tract infections.

III. RESULTS AND DISCUSSIONS

Culture specimen in Eosin Methylene Blue Agar media and incubated for 24 hours at 37 º C in an incubator with the following results (show in table 1):

Table 1: Culture Results on EMBA Media (Eosin Methylene Blue Agar).

Specimen No	Results		
	In the 0th minute	In the 15th minute	In the 30th minute
1	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
2	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
3	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
4	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
5	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
6	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
7	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
8	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
9	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
10	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
11	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
12	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
13	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
14	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color
15	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color	Round Colonies, Metallic Green Color

Source: Authors, (2023).

Based on the results obtained, specimens 1-15 actually cultured urine specimens at 0, 15 and 30 minutes showing round, metallic green colonies. Based on the culture results in the EMBA media, it shows that it is a colony of the Enterobacteriaceae family.

After culturing on EMBA media, microscopic examination was then carried out to ensure the growing bacterial colonies. the results can be seen in table 2.

Table 2: Gram Staining Results.

Specimen No	Results		
	In the 0th minute	In the 15th minute	In the 30th minute
1	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
2	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
3	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
4	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
5	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
6	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
7	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
8	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
9	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
10	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
11	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
12	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
13	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
14	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative
15	Basil, Gram Negative	Basil, Gram Negative	Basil, Gram Negative

Source: Authors, (2023).

Based on the results obtained, specimens 1-15 actually examined the bacterial colonies at 0, 15 and 30 minutes, showing positive gram-negative bacilli results. It can be seen in Bergey's book that the two results that have been carried out show that the bacterial colonies are Escherichia coli bacteria.

III.1 ON PCA MEDIA (PLATE COUNT AGAR)

Urine specimens as many as 15 specimens that have been obtained are immediately examined, then examination is carried out on storage specimens at the 15th and 30th minutes. The results of direct examination calculations, 15 and 30 minutes showed the number of bacteria in the specimen was $\geq 10^5$ CFU/ml. After that, the calculation of the average bacteria in units (10^5 CFU/ml) was carried out to find out the differences in the three storage treatments, the results of the calculations can be seen in Table 3. Then the results of the average calculation of the number of colonies on the media can be seen in table 4.

Table 3: Colony Count Results at 0 Minutes (Immediate), 15 Minutes and 30 Minutes.

Specimen No	Results		
	In the 0th minute	In the 15th minute	In the 30th minute
1	1.05	1.17	1.38
2	1.10	1.20	1.40
3	1.10	1.16	1.36
4	1.50	1.16	1.40
5	1.02	1.21	1.45
6	1.00	1.17	1.36
7	1.40	1.30	1.39
8	1.10	1.31	1.50
9	1.05	1.17	1.51
10	1.10	1.21	1.37
11	1.00	1.30	1.37
12	1.11	1.17	1.37
13	1.70	1.16	1.47
14	1.00	1.18	1.54
15	1.50	1.33	1.37

Source: Authors, (2023).

Table 4: The average value of bacteria in each variation of examination storage time.

No	Minute Storage	Number of Specimens	Bacterial mean value (10^5 CFU/ml)
1	0	15	1.18
2	15	15	1.21
3	30	15	1.42

Source: Authors, (2023).

The table above shows that there is a difference in the average value of the results of direct examination and storage at the 15th and 30th minutes. The longer the examination, the average number of bacteria increases in units of 10^5 CFU/ml.

In this study using the urine culture method, urine examination on direct examination showed the number of bacteria in each specimen was $\geq 10^5$ CFU/ml. When compared with the Guidelines on Urological Infection, all specimens examined had a UTI with a severe degree of severity, but supporting examinations

such as leukocytes in the urine must also show ≥10^5 white blood cells per high-power field (400x) for centrifuged urine specimens. Dipstick examination can be used for routine tests, such as leukocyte esterase, hemoglobin, and nitrite reactions. In complicated UTIs, there is usually an underlying disease. The bacteria that are often found are Gram negative bacteria such as *E.coli, Proteus, Klebsiella, Pseudomonas, Serratia, and Enterococci* [9-11].

The research that has been carried out is also supported by the research of Dewanti, which shows that there is an effect of delaying the 3-hour examination on the number of urinary leukocytes in UTI patients [12]. Likewise with research conducted by Nugraha, in his research it was shown that the duration of centrifugation of urine specimens affected the results of examination of urine leukocyte sediments in UTI sufferers, this was due to the large number of sediment deposits formed due to the centrifugation process and the high number of leukocytes [13].

The results of the delayed urine examination for each variation showed an increase in the number of bacteria, this was influenced by the composition in the urine. Urine is a liquid containing waste products from the body's metabolism, such as protein, dissolved salts such as sodium (Na+) and organic matter in the form of nitrates (NO_3) which are excreted through the urinary system [14,15]. These materials become nutrients or growth media, so that bacteria can grow and develop. Apart from nutrition, urine temperature which ranges from 32ºC-38ºC also supports bacterial growth, because bacterial metabolism is faster [16]. Nutritional content and temperature can cause bacteria to multiply if the inspection is delayed.

Another study conducted by Sirait [8], showed that at a delay of 6 hours, the number of bacteria was 75,050 CFU/ml, which increased to 128,000 CFU/ml at a delay of 12 hours, and at a delay of 18 hours, the number of bacteria began to decrease to 80,750 CFU/ml, then to 78,900 CFU. /ml on a 24 hour delay.

In this study using SPSS data analysis. Based on the Normality test with an error rate of 5%, a significance value of 0.909 was obtained for all study specimens, this value was <0.05 which indicates that the data is normally distributed. Specimen Test Paired T-test as a follow-up test for Normality was carried out to find out the significant differences from each treatment, so that it can be interpreted that each storage variation showed an increase in the 30th minute, while at 0th and 15th minutes there was no significant difference.

IV. CONCLUSIONS

Based on the research results obtained, it can be concluded that urine specimens that were examined immediately and postponed showed an increase in urine specimens at 0, 15 and 30 minutes. It is hoped that urine culture examination will be carried out immediately without delay. This is because the more specimens are postponed, the more bacteria will increase.

V. AUTHOR'S CONTRIBUTION

Conceptualization: Vincentia Ade Rizky and Saadah Siregar.
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Resources: Asvia Rahayu.
Supervision: Vincentia Ade Rizky.
Approval of the final text: Saadah Siregar and Asvia Rahayu.

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