

Research Article

**Identification of Gram Negative Bacteria in the Urine of Catheterized Patients
at Referral Hospital in Ternate**

**Identifikasi Bakteri Gram Negatif pada Urine Pasien Pengguna Kateter
di Rumah Sakit Rujukan Ternate**

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ABSTRACT

Infections can occur anywhere and often occur usually sourced from hospitals. Nosocomial infection is an infection caused by various microorganisms, one of which is bacteria originating from the hospital environment. Gram-negative bacteria, especially the Enterobacteriaceae family, are the main bacteria that cause nosocomial infections. One of the common infectious diseases in health care is urinary tract infection and some UTIs are caused by catheter insertion. This research aimed to identify gram-negative bacteria in the urine of catheterized patients at RSUD Dr. H. Chasan Boesoirie, a referral hospital in Ternate. The research method is descriptive observational with a cross sectional approach using a total sampling technique obtained 83 samples from November 14, 2023 to December 17, 2023. The results showed that 7 samples (8.4%) were identified as positive for gram-negative bacteria with 7 types of bacterial species and the highest percentage of bacteria was *Escherichia coli* (33.4%). The use of urinary catheters was mostly female (55.4%) with the highest age in the age group 56-65 years (34.9%) and the duration of urinary catheter use was mostly in samples with a duration of <72 hours (90.4%). In conclusion, gram-negative bacteria were identified in the urine of catheterized patients.

Keywords: Catheter urine, gram negative bacteria, Ternate

ABSTRAK

Infeksi bisa berlangsung di mana saja dan sering terjadi biasanya bersumber dari rumah sakit. Infeksi nosokomial adalah infeksi yang dikarenakan oleh berbagai macam mikroorganisme salah satunya yaitu bakteri yang berasal dari lingkungan rumah sakit. Bakteri gram negatif, terutama famili *Enterobacteriaceae* merupakan bakteri utama penyebab infeksi nosokomial. Salah satu penyakit infeksi yang umum terjadi di tempat pelayanan kesehatan adalah infeksi saluran kemih dan sebagian ISK diakibatkan oleh pemasangan kateter. Tujuan penelitian ini yaitu untuk mengidentifikasi bakteri gram negatif pada urine pasien pengguna kateter di RSUD Dr. H. Chasan Boesoirie yang merupakan rumah sakit rujukan di Ternate. Metode penelitian berupa deskriptif observasional dengan pendekatan secara *cross sectional* menggunakan teknik *total sampling* didapatkan sebanyak 83 sampel dari 14 November 2023 sampai 17 Desember 2023. Hasilnya didapatkan sampel yang teridentifikasi positif bakteri gram negatif sebanyak 7 sampel (8,4%) dengan 7 jenis spesies bakteri dan persentase bakteri terbanyak yaitu *Escherichia coli* (33,4%). Penggunaan kateter urine terbanyak yaitu pada perempuan (55,4%) dengan usia terbanyak pada kelompok usia 56-65 tahun (34,9%) dan durasi pemakaian kateter urine paling banyak pada sampel dengan durasi <72 jam (90,4%). Kesimpulannya yaitu teridentifikasi bakteri gram negatif pada urine pasien pengguna kateter.

Kata Kunci: Bakteri gram negatif, kateter urine, Ternate

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DOI: <http://dx.doi.org/10.21776/ub.jkb.2024.033.02.5>

INTRODUCTION

Infections can occur anywhere; the most common ones are usually sourced from hospitals. Hospital infections or nosocomial infections are infections experienced by patients while undergoing treatment in the hospital. This infection can be caused by a variety of microorganisms, one of which is bacteria originating from the hospital environment (1). Some gram-negative bacteria, especially bacteria from the *Enterobacteriaceae* family, are the main bacteria that cause nosocomial infections or what is now known as *Healthcare-Associated Infection* (HAIs) (2). Urinary tract infection or UTI is a common infectious disease in health care settings, which can occur in both outpatients and inpatients (3). Urinary tract infections of concern stem from nosocomial infections. These UTIs occur in patients in healthcare institutions and in those who frequently take antibiotics. The irrational use of antibiotics can lead to bacterial resistance to antibiotics (4). The most important risk factor for bacteriuria is catheterization of the urinary tract. Most UTIs result from catheter insertion. Catheter-induced UTI is the leading cause of nosocomial infections, accounting for 80% of cases obtained from the use of urinary catheters (5).

Urinary catheterization is a medical procedure that inserts a tube through the urethra into the bladder to help pass urine. The use of a urinary catheter increases the risk of UTI by about 5% every day (6). Catheter use stimulates inflammation, traumatizing the urethral mucosa, and bladder neck. Inflammation and mechanical damage to the urinary tract epithelium not only increase the risk of UTI, but also affect the patient's ability to immune response to bacteria in the bladder (7). To diagnose urinary tract infections in patients undergoing periodic catheterization, urine sampling from the catheter is an important step in the diagnostic process. A urine culture result of $>10^4$ cfu/ml is considered to be significant bacteriuria. Bacteria commonly found in short-term catheterization are *Escherichia coli*, *Citrobacter*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Staphylococcus aureus*, and *Faecalis*, while *Acinetobacter* is a bacterial species often found in long-term catheterization (8).

Based on the background above and because there has been no research on the identification of gram-negative bacteria in the urine of catheterized patients at RSUD Dr. H. Chasan Boesoirie Ternate. Therefore, this encourages researchers to study the identification of gram-negative bacteria in the urine of catheterized patients at RSUD Dr. H. Chasan Boesoirie Ternate. This study aims to identify gram-negative bacteria in the urine of catheterized patients and to determine the characteristics of catheter users divided into gender, age, and duration of catheter use at RSUD Dr. H. Chasan Boesoirie Ternate.

METHOD

Research Type and Design

The type of research used in this study was descriptive observational research with a cross sectional approach. Inclusion criteria were patients who used urine catheters ≥ 48 hours, and patients who are treated at the age of 17-65 years.

Place and Time of Research

The research location includes sampling conducted at the Surgical Inpatient Installation (IRDA) A and B, Obstetric

Care Room, and Neurology Room of Dr. H. Chasan Boesoirie Ternate Hospital. Bacteriological examination of samples was carried out at the Pharmaceutical Microbiology Laboratory, Faculty of Medicine, Khairun University Ternate. The research was conducted from November 14, 2023, to December 17, 2023.

Tools and Materials

The instruments used in this study were primary data obtained based on the results of catheter urine culture, gram staining, and biochemical tests. Secondary data of catheter user patients were obtained from medical records. The tools and materials used in this study, namely:

a. Tools

Autoclave, beaker glass, stirring rod, petri dish, porcelain cup, cover glass, hot plate magnetic stirrer, incubator, label paper, erlenmeyer flask, laminar air flow, spirit lamp, micropipette, microscope, analytical balance, glass object, disposable ose, pH meter, dropper pipette, sterile urine pot, tube rack, horn spoon, marker pen, and test tube.

b. Materials

Materials for urine culture tests were urine samples of catheterized patients, Nutrient Broth (NB) media, Mac Conkey Agar (MCA) media, Triple Sugar Iron Agar (TSIA) media, Sulfide Indole Motility (SIM) media, Citrate media, MR and VP media, distilled water, gentian violet carbol, lugol, 96% alcohol, safranin, and immersion oil (9).

The Procedure

The procedure of the primary data began with catheter urine sampling using a needle which was then inserted into a sterile container and coded. After that, bacterial isolation was carried out on Nutrient Broth media in a test tube that had been prepared and incubated. After incubation, proceed with making Mac Conkey Agar media according to the number of petri dishes and identifying colonies on Mac Conkey Agar. After identifying the colonies on Mac Conkey Agar, a Biochemical Reaction Test consisting of TSIA, Citrate, SIM, MR, and VP was performed. Gram staining was then done to identify gram-negative bacteria.

Data Processing Plan

The data obtained from the catheter urine examination were further categorized based on gender, age, and length of catheter use. The results obtained were presented as a frequency distribution table and analyzed descriptively to draw conclusions.

Research Ethics

The research was conducted after submitting and obtaining research approval from the Health Research Ethics Committee (KEPK) UIN Alauddin Makassar with No: S.004/KEPK/FKIK/I/2023, then the researcher submitted a letter requesting permission to conduct research to the Director of RSUD Dr. H. Chasan Boesoirie Ternate, the researcher gave informed consent and consent sheets to respondents before conducting research sampling, and maintaining the confidentiality of patient identity.

RESULTS

Obtained 83 samples met the inclusion criteria and exclusion criteria. The results of this study are displayed in several frequency and percentage tables with the content

of the discussion as follows:

Table 1. Variable table distribution

Variables	N	%
Gender		
Male	37	44.6
Female	46	55.4
Age		
17-25 years old	16	19.2
26-35 years old	14	16.9
36-45 years old	12	14.5
46-55 years old	12	14.5
56-65 years old	29	34.9
Duration of Catheter Use		
<72 hours	75	90.4
72-168 hours	8	9.6
>168 hours	0	0
Total	83	100

Note: N (number of data)

Based on the distribution of gender variables in Table 1, there are 46 samples of female gender with a percentage of 55.4% and 37 samples of male gender with a percentage of 44.6%. Age variable distribution in Table 1, ages 17-25 years were 16 samples with a percentage of 19.2%, ages 26-35 years were 14 samples with a percentage of 16.9%, ages 36-45 years were 12 samples with a percentage of 14.5%, ages 46-55 years were 12 samples with a percentage of 14.5%, and ages 56-65 years were 29 samples with a percentage of 34.9%. It can be seen that the duration of use of the urinary catheter <72 hours was 75 samples with a percentage of 90.4%, and the duration of use of the urinary catheter with a range of 72-168 hours was 8 samples with a percentage of 9.6%.

Based on the distribution of gender variables in Table 2, in catheter user patients identified with gram-negative bacteria, female gender was obtained in 5 samples and male in 2 samples. The distribution of age variables in Table 2, in patients using catheters identified with gram-negative bacteria with ages 26-35 years as many as 2 samples, ages 36-45 years as many as 1 sample, ages 46-55 years as many as 1 sample, and ages 56-65 years as

many as 3 samples. The distribution of the duration of catheter uses in Table 2, the duration of catheter use <72 hours was 6 samples, and the duration of catheter use 72-168 hours was 1 sample.

Table 3. Results of gram negative bacteria identification

Gram Negative Bacteria	N	%
Available	7	8.4
None	76	91.6
Total	83	100

Note: N (number of data)

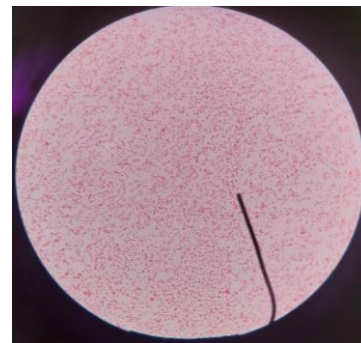


Figure 1. Gram staining results of gram negative bacteria that are red and rod-shaped (Bacillus)

Based on the results of urine culture that has been done to get isolates of gram-negative bacteria presented in Table 3, the results of gram-negative bacteria identified were 7 samples with a percentage of 8.4%, and samples that were not found gram-negative bacteria were 76 samples with a percentage of 91.6%.

Table 4. Results of species identification of gram negative bacteria

Species of Bacteria	N	%
<i>Citrobacter diversus</i>	1	11.1
<i>Escherichia coli</i>	3	33.4

Table 2. Characteristics of gram negative bacteria from urine culture

Samples	Karakteristik					Type of Bacteria
	Gender	Age	Duration of Catheter Use	Diagnose	The Room	
H ₄ P ₁	Female	53	<72 hours	Malignant Neoplasm of Thyroid	IRDA B	<i>Salmonella paratyphi A</i> , <i>Escherichia coli</i>
H ₉ P ₁	Female	57	<72 hours	Hemiplegia Dextra due to Susp. NHS	Neurology	<i>Escherichia coli</i>
H ₉ P ₂	Male	29	<72 hours	Susp. Tetanus	Neurology	<i>Salmonella typhi</i> , <i>Citrobacter diversus</i>
H ₉ P ₃	Male	56	72-168 hours	Hemiparese Sinistra due to Susp. NHS	Neurology	<i>Pseudomonas aeruginosa</i>
H ₁₂ P ₂	Female	35	<72 hours	Postpartum Hemorrhage	Obstetric Care	<i>Escherichia coli</i>
H ₁₈ P ₂	Female	65	<72 hours	Closed Fracture Later-trochanter	IRDA B	<i>Shigella dysenteriae</i>
H ₁₈ P ₃	Female	36	<72 hours	Peritonitis due to Perforated Appendicitis + Grade III Adhesion	IRDA A	<i>Neisseria gonorrhoeae</i>

Note: Susp. (Suspect), NHS (Non Haemorrhagic Stroke), IRDA (Surgical Inpatient Installation), H...P... (Day...Patient...).

Table 4. Results of species identification of gram negative bacteria (Cont.)

Species of Bacteria	N	%
<i>Pseudomonas aeruginosa</i>	1	11.1
<i>Salmonella paratyphi A</i>	1	11.1
<i>Salmonella typhi</i>	1	11.1
<i>Shigella dysenteriae</i>	1	11.1
<i>Neisseria gonorrhoeae</i>	1	11.1
Total	9	100

Based on the results of identification of gram-negative bacterial species in Table 4, it can be seen that gram-negative bacterial species were found, namely *Escherichia coli* with a frequency of 3 samples with a percentage of 33.4% and other species, namely *Citrobacter diversus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi A*, *Salmonella typhi*, *Shigella dysenteriae*, and *Neisseria gonorrhoeae* with a frequency of 1 sample each with a percentage of 11.1%.

DISCUSSION

Characteristic Distribution of Gender Variables

The distribution of gender variables obtained more female gender who used urine catheters, namely 46 samples (55.4%) compared to male gender, namely 37 samples (44.6%). It can also be seen from the results of this study that the samples suspected of being infected with UTI were more female as many as 5 samples than male as many as 2 samples. These results are in line with Gilang's research (2014) with a total of 30 samples, it was found that the incidence of urinary tract infections in catheter patients occurred mostly in female, namely as many as (60%) (10). (10). These results are also in line with research conducted by Ratna (2023) with a total of 43 respondents, 25 female respondents (58%) were suspected of UTI (11).

Women tend to be more at risk of bacteriuria because they have a short urethra and are located closer to the vagina, periurethral glands, and rectum anatomically and physiologically. Women have a urethra that is about 3-4 cm long and is located near the anus (12). The entry of microorganisms through the catheter surface then onwards causes extraluminal contamination; women experience this most often. The most common difficulty in performing catheterization especially in women is finding the urethral opening (11). Lesions in the urinary tract are easy to occur if catheter insertion is not done carefully, which can increase colonization and infection in the urinary tract. In contrast, men have a longer urethra and prostate gland secretions that are able to fight bacteria (13).

Variable Distribution Characteristics of Age

The distribution of age variables showed that patients aged 56-65 years or the elderly were more likely to use urinary catheters, namely 29 patients (34.9%). It can also be seen from the results of this study that patient aged 56-65 identified gram-negative bacteria in as many as 3 patients (42.9%). The results of this study are in accordance with the research of Rosa and Ulfa (2017) with a total of 57 respondents, found that most patients with catheters were at the age of 61-75 years, namely 19 patients (33.34%) followed by the age of 46-60 years as many as 22.81% (5). The results of this study are also in line with research conducted by Vellyana and Gunawan

(2020), with a total of 28 respondents who found that the frequency of respondents characteristics based on age was mostly the late elderly as much as 46.4% (14).

Increasing age and reduced ability to care for oneself can increase the incidence of infections. A frequent case, whose prevalence increases with age is urinary tract infection (15). Although UTIs can occur in all age groups, the patients at highest risk are infants and geriatric patients. This is because it is related to the immune system's ability to fight infection (16). The high prevalence of UTIs in the elderly can be caused by increased residual urine in the bladder due to ineffective bladder emptying, decreased mobility, or both cellular and humoral immune systems (15). Urinary tract infection is the most common case of acute bacterial sepsis in patients aged >65 years. The average increase in CAUTI infections increases with old age. Chronic illness, antimicrobial use, presence of decubitus infection, immobility, and incomplete bladder emptying are the causes of bacteriuria in the elderly population (17).

Variable Distribution Characteristics of Catheter Use Duration

The distribution of catheter usage duration variables obtained by patients with catheter usage duration <72 hours as many as 75 patients with a percentage of 90.4% and catheter usage duration 72-168 hours as many as 8 patients with a percentage of 9.6%. It can be seen from the results of this study that 7 patients were positive for gram-negative bacteria with a duration of urine catheter use <72 hours (3 days) as many as 6 patients (85.7%). The results of this study stated that the respondents who were catheterized at the time of the study were mostly in a relatively short period of time. This may be due to the indication of urethral catheter installation which requires a short duration. The results of this study are in accordance with research conducted by Nurdin with a total of 30 respondents found that 17 patients (56.7%) had a duration of urine catheter use ≤ 3 days (18). The results of this study are also in line with research conducted by Ana with a total of 38 respondents, it was found that most respondents experienced a long catheter insertion time of two days, namely 10 respondents (26.3%) and a small proportion of respondents experienced a long catheter insertion time of six days and seven days, each of which was 3 respondents (7.9%) (19). Almost 26% of patients with catheter use for 2-10 days had bacteriuria and all patients with catheter use for a month had bacteriuria (17). However, this study is slightly different from Putri's research which states that patients have a risk of 56.07 times getting UTI with a duration of catheter use >3 days compared to patients who use catheters ≤ 3 days (20).

Identification of Bacteria

The results of bacterial identification in this study from 7 patients who were positive for gram-negative bacteria, obtained 7 types of bacterial species with the highest percentage of bacteria namely *Escherichia coli* (33.4%), and other species found were *Citrobacter diversus*, *Salmonella typhi*, *Salmonella paratyphi A*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, and *Neisseria gonorrhoeae* with a percentage of 11.1% each. The results of this study are in accordance with research conducted by Gilang (2014) with the results obtained, namely 6 types of bacteria that cause urinary tract infections in catheter users with the highest percentage of bacteria being *Escherichia coli* bacteria (26.7%) (10). The results of this study are also in line with research conducted by Ratna with a total of 43 urine samples that were examined

showing colony growth and 9 types of bacterial species have been identified with the highest percentage of bacteria, namely *Escherichia coli* bacteria (60%) (11).

Based on the results of bacterial identification in samples H4P1, H9P1, and H12P2, the samples identified *Escherichia coli* bacteria. *Escherichia coli* bacteria are a type of coliform bacteria that belong to the Enterobacteriaceae family and are often found in the digestive tract (21). Although some strains of these bacteria are beneficial to humans, there are so-called pathogenic *E. coli* that cause disease in humans. This bacteria can grow rapidly in aerobic and anaerobic environments (22). The onset of UTI can be caused by uropathogenic strains of *E. coli*. The binding elements possessed by these uropathogenic strains are known as P fimbriae or pili that will bind to P blood group antigens. These pili function as mediators in the process of *E. coli* adherence to uroepithelial cells, so the risk of UTI infection is higher in patients infected with *E. coli* with P fimbriae (23).

The results of bacterial identification in sample H4P1 identified *Salmonella paratyphi A* bacteria, and sample H9P2 identified *Salmonella typhi* bacteria. The typhoid-causing bacilli *Salmonella typhi* and *paratyphi* are members of the genus *Salmonella*. These bacilli are gram-negative, mobile, non-encapsulated, do not form spores but have fimbriae, are aerobic, and facultative anaerobes. It can live in water, ice, garbage, and dust. The only reservoir is humans, namely someone who is sick or a carrier (24). *Salmonella* infections of the urinary tract are uncommon infections that tend to be encountered sporadically for which a high index of suspicion is required. Generally, *Salmonella* infection does occur via the ingestion of contaminated food or water via the faecal-oral route. Generally *Salmonella* urinary tract infections do manifest with symptoms that simulate the symptoms of more common causes of urinary tract infection. Occasionally individuals who have *Salmonella* urinary tract infection could be asymptomatic. The symptoms of *Salmonella* urinary tract infection may be present contemporaneously with or develop after symptoms of enteric *Salmonella* infections with diarrhoea, abdominal discomfort, as well as other symptoms. Nevertheless, some individuals who have *Salmonella* urinary tract infections do not have any preceding or concurrent symptoms of enteric *Salmonella* infections. The clinical findings of *Salmonella* urinary tract infections are non-specific or any different from the clinical findings of more common urinary tract infection (25).

Based on the results of bacterial identification, *Pseudomonas aeruginosa* was identified in sample H9P3. This bacterium is an opportunistic pathogen that causes nosocomial infections, especially in patients who have a decreased immune system. It does this by relying on endogenous infection by natural bacteria in the body that can cause infection because it is in a place that is not its natural environment (26).

The results of bacterial identification in sample H18P2

identified *Shigella dysenteriae* bacteria. Humans are used as hosts for *Shigella sp* bacteria to depend on for life and reproduction. Transmission of bacteria from one person to another can occur through contact or touch. Transmission can also occur through fecal-oral transmission by flies, drinks, and contaminated food. *Shigella* is a type of gram-negative bacteria that is bacillus-shaped, single, has no flagellum, facultative aerobic, and does not form spores. Its optimal growth temperature is 37°C, its habitat is in the gastrointestinal tract, and infection occurs through the oral phase. This microorganism can produce toxic (thermolabile) LT that will invade into the epithelial cells of the small intestinal mucosa and continue to develop in the invasion area (27). *Shigella* and *Escherichia coli* are closely related phenotypically and their genetic makeup is about 80–90% similar. These similarities may also be reflected in their virulence factors as some studies have shown that *Shigella flexneri* also possesses the *sat* gene found in uropathogenic *Escherichia coli* (UPEC) and known to code for a secreted autotransporter toxin that elicits cytopathic effect on bladder and kidney cells in the course of a urinary tract infection. This may explain why a UTI initially caused by *Shigella flexneri* could easily undergo a swift and unnoticeable change in uropathogen to *Escherichia coli* as both bacteria probably affect the urinary tract in a similar way due to some similarities in their pathogenicity and virulence (28).

Based on the results of bacterial identification, *Neisseria gonorrhoeae* was identified in sample H18P3. *Neisseria gonorrhoeae* is a diplococcus and is acid resistant. The parts that will be infected with *N. gonorrhoeae* are mucous membranes such as cuboid epithelial mucosa (flat layer) which is still immature (undeveloped) in the genitourinary tract, eyes, rectum, and throat. First, the bacteria will penetrate the mucosal surface, then multiply in the subepithelial tissue, and successfully produce extracellular products that can destroy cells. Infections caused by these bacteria will form subepithelial microabscesses that, when ruptured, release PMNs and gonococci (29).

Based on the characteristics of the H9P2 sample, a sample aged 29 years was found and diagnosed with suspected tetanus. Several factors are associated with urinary tract infections at a young age, including intimate hygiene, sexual intercourse, and contraceptive use (30). The results of bacterial identification in this sample identified not only *Salmonella typhi* bacteria, but also *Citrobacter diversus* bacteria. *Citrobacter* consists of a group of aerobic gram-negative bacilli found in soil, water, food, human and animal intestines. The genus *Citrobacter* consists of three species, one of which is *Citrobacter diversus* (also known as *Citrobacter koseri*). *Citrobacter* species are low virulence organisms that are rarely reported to cause serious infections in humans involving the central nervous system, gastrointestinal tract, urinary tract, and respiratory tract. *Citrobacter diversus* can also cause sepsis (21,31).

The conclusion is that gram-negative bacteria were identified in the urine of catheterized patients at RSUD Dr. H. Chasan Boesoirie Ternate.

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