

Analysis of *Staphylococcus aureus* and *Escherichia Coli* and its Susceptibility to Antibiotic in Catheter-Associated Urinary Tract Infection Patients at Hospital in Province of West Nusa Tenggara

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Abstract

Objective – This study aimed to analyze the proportion of *Staphylococcus aureus* and *Escherichia coli* from urine samples of catheter-associated urinary tract infections (CA-UTI) patients at the General Hospital at Province of West Nusa Tenggara. Also measured the pattern of sensitivity to several antibiotics.

Methods – This study is a descriptive observational with a cross-sectional approach. A total of 60 samples were used in this study. Bacterial identification was carried out according to standard bacteriological culture techniques. Furthermore, the antibiotic sensitivity test following the Kirby-Bauer disc diffusion method using several antibiotics, including amoxycillin (AMP), ciprofloxacin (CIP), ceftriaxone (CRO), and sulphamethoxazole trimethoprim (SXT).

Results – The results showed that bacteria causing CA-UTI were *Staphylococcus aureus* (43.33%), *Escherichia coli* (21.67%), *Staphylococcus epidermidis* (10%), *Proteus mirabilis* (6.67%), *Enterobacter aerogenes* (6.67%), *Serratia marcescens* (5%), *Klebsiella sp* (3.33%), and *Pseudomonas sp* (3.33%). The antibiotic susceptibility test found that 4.58%, 11.25%, 9.16%, and 3% of bacteria were resistant to amoxycillin, ceftriaxone, ciprofloxacin, and sulphamethoxazole trimethoprim, respectively.

Conclusion – *Staphylococcus aureus* and *Escherichia coli* were the most gram-positive and gram-negative bacteria causing CA-UTI, respectively. The most resistant antibiotic was ciprofloxacin, and the most sensitive antibiotic is sulphamethoxazole trimethoprim.

Keywords: antibiotic-resistance, catheter-associated urinary tract infection, *Escherichia coli*, *Staphylococcus aureus*

Introduction

Urinary tract infection (UTI) is a common

bacterial infection. About 35% of nosocomial infections are urinary tract infections, and 80% of them are related to catheterization¹. UTI can progress to bacteremia, sepsis, and death². As many as 20% of hospital-acquired bacteremia with a mortality rate of about 10% are due to CA-UTI. CA-UTI is defined as a patient with urinary tract infection caused by a

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catheter³ and is one of the most common nosocomial infections worldwide that can increase morbidity and mortality^{4,5}. In the United States, about 11 million people have urinary tract infections⁶ and affect around 150 million people annually worldwide⁷. Based on data from the Indonesian Ministry of Health, around 90-100 cases of urinary tract infections in 100,000 population for each year in Indonesia⁸.

According to the CDC, 12-16% of hospitalized patients use a catheter and have about 3-7% risk for CA-UTI, increasing every day⁹. Important risk factors for CA-UTI are persistent catheterization and duration of catheterization¹⁰. Other factors include female gender, age, diabetes mellitus, impaired kidney function, and lack of antimicrobial therapy^{4,9}.

In normal conditions, the urinary tract is usually sterile, except for normal flora such as digestive flora, skin flora and genital flora¹¹. UTI is characterized by the presence of infection by pathogenic microorganisms in the urinary tract, including bacteria, fungi or parasites¹². However, it is usually caused by bacteria, both by gram-positive and gram-negative bacteria¹³. The most common bacteria found as the cause of CA-UTI were *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Providentiaertgeri*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Mycobacterium tuberculosis*^{11,12,13}.

UTIs are generally treated with antibiotics. Patients with catheterization have a three times greater risk for more extended hospitalization and prolonged use of antibiotics¹⁴. Inappropriate use of antibiotics creates problems, one of which is the emergence of microorganisms resistant to antibiotics^{15,16}.

Based on this description, that is important to know the pattern of bacteria and also the pattern

of antibiotic resistance, especially for a rational treatment of CA-UTI. For this reason, this study aimed to determine the proportion of *Staphylococcus aureus* and *Escherichia coli* that cause CA-UTI at the Hospital at Province of West Nusa Tenggara, also to analyze the pattern of resistance of these bacteria to antibiotics.

Materials dan Methods

2.1 Ethical clearance and study design

This research has been approved by the Ethics Committee of the Regional General Hospital of West Nusa Tenggara Province with reference number 070.1/05/KEP/2021. This research was conducted from January until March 2021 at Biomedical Research Unit, West Nusa Tenggara General Hospital, Indonesia. We made a descriptive observational with a cross-sectional approach.

2.2 Sample collection

Sample collection used a purposive sampling method. A total of 60 samples were collected. The inclusion criteria were patients with catheterization \geq 72 hours with a diagnosis of urinary tract infection, age $>$ 14 years, willing to provide urine (samples) and fill out an informed consent first.

2.3 Microbiological analysis

Sample (urine) was cultured on Mannitol Salt Agar (MSA) and Eosin Methylene Blue (EMB), incubated at 37° C for 18-24h. Bacterial colonies from media of MSA and EMB then inoculated into Nutrient Agar Plate (NAP). Then gram staining was performed. If gram-positive bacteria are found, it is continued with catalase and coagulase tests. Meanwhile, if gram-negative bacteria are found, it is followed by the biochemical test.

2.4 Antibiotics sensitivity testing

Kirby-Bauer disc diffusion method using several

antibiotics, including amoxicillin (AMC) 30 µg/disc, ciprofloxacin (CIP) 5 µg/disc, ceftriaxone (CRO) 30 µg/disc, and sulphamethoxazole trimethoprim (SXT) 25 µg/disc. The bacterial suspension was made from colonies bacteria on NAP was diluted with sterile physiological NaCl to make 0,5 McFarland standard. The bacterial suspension was cultured on Muller Hinton Agar media using a sterile cotton bud, and the antibiotic discs were placed on the agar surface then incubated at 37°C for 24h. Antibiotic inhibition zones formed were measured and adjusted to the Clinical and Laboratory Standards Institute (CLSI).

Results

A total of 60 subjects consisted of three categories: category of gender, age, and duration of catheterization. Table 1 shows the subjects consisting of 30 (50%) men and 30 (50%) women. Based on age category, UTI's patients in the range of 15-30 years were found 7 patients (11.67%), 31-45 years were 15 patients (25%), 46-60 years were 28 patients (46.67%), and range of 61-75 years were 10 patients (16.66%). Meanwhile, the duration of catheterization category consisted of 42 patients (70%) and 18 patients (30%) with a catheter duration of ≤7 days and ≥7 days, respectively (Table 1).

Table 1. General characteristics of subjects

Category		Total (n)	Percentage (%)
Gender	Men	30	50
	Women	30	50
Age	15-40 years	7	11,67
	31-45 years	15	25
	46-60 years	28	46,67
	61-75 years	10	16,66
Duration of catheterization	≤7 days	42	70
	≥7 days	18	30

Staphylococcus aureus (43.33%) was the main bacterial pathogen causing catheter-associated urinary tract infections, followed by *Escherichia coli* (21.67%), *Staphylococcus epidermidis* (10%), *Proteus mirabilis* (6.67%), *Enterobacter aerogenes* (6.67%), *Serratia marcescens* (5%), *Klebsiella sp* (3.33%), and *Pseudomonas sp* (3.33%) which can be seen in table 2.

Table 2. Bacterial identification that cause CA-UTI

Microorganism	Total (n)	Percentage (%)
Gram Positive		
Staphylococcus aureus	26	43,33
Staphylococcus epidermidis	6	10
Gram Negatif		
Escherichia coli	13	21,67
Proteus mirabilis	4	6,67
Enterobacter aerogenes	4	6,67
Serratia marcescens	3	5
Klebsiella sp	2	3,33
Pseudomonas sp	2	3,33
Total	60	100

Based on the results of the antibiotic sensitivity test in table 3, it was found that 63 isolates (26.25%) were resistant to antibiotics, of which 11 (4.58%), 27 (11, 25%), 22 (9.16%), and 3 (1.25%) were resistant to amoxicillin, ceftriaxone, ciprofloxacin, and sulphamethoxazole trimethoprim, respectively. Also,

90 bacterial isolates that were sensitive to antibiotics, as much as 27 (11.25%), 10 (4.17%), 10 (4.17%), and 43 (17,92%), were sensitive to amoxicillin, ceftriaxone, ciprofloxacin, and sulphamethoxazole trimethoprim, respectively.

Table 3. Antibiotic Sensitivity Test Results

Microorganism	n	Antibiotic Sensitivity Based on CLSI											
		Amoxycillin (AMC)			Ceftriaxon (CRO)			Ciprofloxacin (CIP)			Sulphamethoxazoletri methoprim(SXT)		
		S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
<i>S.aureus</i>	26	8 (7,72)	12 (11,54)	6 (5,77)	4 (3,85)	6 (5,77)	16 (15,39)	4 (3,84)	9 (8,65)	13 (12,5)	19 (18,27)	5 (4,80)	2 (1,92)
<i>S.epidermidis</i>	6	3 (12,5)	1 (4,16)	2 (8,34)	1 (4,16)	4 (16,67)	1 (4,16)	2 (8,34)	4 (16,67)	-	5 (20,84)	1 (4,16)	-
<i>E.coli</i>	13	6 (11,54)	5 (9,61)	2 (3,85)	2 (3,85)	8 (15,38)	3 (5,77)	2 (3,85)	7 (13,46)	4 (7,70)	9 (17,30)	3 (5,77)	1 (1,92)
<i>Klebsiella sp</i>	2	-	1 (12,5)	1 (12,5)	-	-	2 (25)	-	1 (12,5)	1 (12,5)	1 (12,5)	1 (12,5)	-
<i>Pseudomonas sp</i>	2	-	2 (25)	-	-	-	2 (25)	-	-	2 (25)	1 (12,5)	1 (12,5)	-
<i>Proteus mirabilis</i>	4	3 (18,75)	1 (6,25)	-	1 (6,25)	1 (6,25)	2 (12,5)	1 (6,25)	1 (6,25)	2 (12,5)	1 (6,25)	3 (18,75)	-
<i>Serratia marcescens</i>	3	3 (25)	-	-	1 (8,33)	1 (8,33)	1 (8,33)	1 (8,33)	2 (16,68)	-	3 (25)	-	-
<i>Enterobacter aerogenes</i>	4	4 (25)	-	-	1 (6,25)	3 (18,75)	-	-	4 (25)	-	4 (25)	-	-
Jumlah	60	27 (11,25)	22 (9,17)	11 (4,58)	10 (4,17)	23 (9,58)	27 (11,25)	10 (4,17)	28 (11,67)	22 (9,16)	43 (17,92)	14 (5,83)	3 (1,25)

S: Sensitive; I: Intermediate; R: Resistant

Discussion

Based on this study that has been conducted from January to March 2020, there were 60 subjects, consisting of 30 men and 30 women, UTI mostly occurred in the age range of 46-60 years, and the duration of catheterization was ≤ 7 days (Table 1). Women is a risk factor for CAUTI. Data from clinical epidemiological studies report that around 25-35% of adult women have experienced UTI at least once in their lives and have a risk for recurrent UTI¹⁷. UTI is more common in women than men, this is due to clinical factors such as hormonal, different anatomical variations, and different behaviour patterns between men and women¹⁸. Based on anatomical and physiological, women have a shorter urethra than men, drier urethral meatus, lack of personal care, especially intimate organs, decreased estrogen hormone, which causes an increase in vaginal pH, making it easier for microorganisms to colonize in the vagina, and the absence of antibacterial substances in vaginal fluids, that causes women more at risk for UTI^{19,20}. UTI can occur in teenagers about 1-3%, then increases with the

start of sexual activity, about 25-30% for age 20-40 years and up to 43% in women aged above 60 years²¹. Postmenopausal women and men over 50 years are at risk for CAUTI²².

Approximately 70-80% of complicated UTI are caused by catheters¹⁶. Catheterization is the most common cause of nosocomial infections because of its prolonged use and not under the indications²³ because of the risk of bacteriuria which increases by about 5% every day²⁴. Microorganism entry through the catheter with two routes; extraluminal route (through the lumen of the catheter) and intraluminal route (through the outer surface of the catheter). Microorganisms can attach to the catheter surface and colonize the bladder within three days from bacteria entry²⁵.

In this study, *Staphylococcus aureus* and *Escherichia coli* were the most common bacteria causing CA-UTI. The presence of *Staphylococcus aureus* in catheter urine can be caused by direct contact or through catheter tubes or other medical equipment used by nurses²⁶. Bacteriuria due to *S. aureus* can

occur in long-term care patients and is significantly associated with catheterization and antibiotic use²⁷. *Uropathogenic E. coli* (UPEC) is a strain of *E. coli* that often causes of urinary tract infections. This strain has the necessary factors for bacteria to colonize the urinary tract, overcoming the host defence system and causing more severe infections. These factors include fimbrial adhesin (fimbriae S, P and type 1), afimbrial adhesin, toxins (haemolysin and cytotoxic necrotizing factor), siderophores (aerobactin system) and capsular polysaccharide (group II capsules)²⁸.

Several types of antibiotics were used in this study, including amoxicillin, ceftriaxone, ciprofloxacin, and sulphamethoxazole trimethoprim. Ceftriaxone works by inhibiting the synthesis of mucopeptides in the bacterial cell wall²⁹. Ciprofloxacin by inhibiting the production of DNA gyrase and DNA topoisomerase IV enzymes that assist in the process of DNA replication, transcription, repair and bacterial recombination³⁰. Amoxicillin works by binding to penicillin-binding protein (PBP), which inhibits the transpeptidase process, thereby causing the activation of autolytic enzymes in the bacterial cell wall then causes lysis and death of bacteria³¹. Meanwhile, sulphamethoxazole trimethoprim which is a combination of sulphamethoxazole and trimethoprim, works by inhibiting the entry of PABA (paminobenzoid acid) molecules into the folic acid and inhibiting the formation of tetrahydrofolic acid³². From table 3, the bacteria were mostly resistant to ceftriaxone and sensitive to sulphamethoxazole trimethoprim.

Antibiotic resistance is still a problem in many countries. The existence of genetic mutations and the genetic transfer of microbes cause microorganisms to become more resistant. These resistance genes can be inherited or obtained from cellular genetic elements that can occur between bacteria³³. Several mechanisms are involved in antibiotic resistance,

(1) microorganisms produce enzymes that destroy the antibiotic substance, (2) microorganisms alter the permeability of the antibiotic substance, (3) microorganisms alter the structural target for the antibiotic substance, (4) microorganisms alter the metabolic pathways traversed by the reaction from inhibition of antibiotics, (5) microorganisms that change enzymes that can still carry out their metabolic functions are less affected by antibiotics³⁴.

Conclusion

CA-UTI are mostly caused by gram-positive and gram-negative bacteria; *Staphylococcus aureus* and *Escherichia coli*. Sulphamethoxazole trimethoprim is the antibiotic with the greatest sensitivity to bacteria that cause CA-UTI.

Authors statements

There is no conflict of interest to declare. All of the authors agreed that the manuscript is submitted to the Indian Journal of Forensic Medicine and Toxicology.

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