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Identification of Gram-Negative Bacteria in the Urine of Diabetes Mellitus Patients at Puskesmas X, Kediri City

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ABSTRACT

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by a steadily increasing prevalence both globally and nationally. Patients with DM are highly susceptible to various infections, with Urinary Tract Infection (UTI) being one of the most frequent complications. The hyperglycemic state in DM patients leads to glycosuria, a condition where the presence of glucose in the urine creates an ideal environment for the proliferation of pathogenic microorganisms. Microbiologically, Gram-negative bacteria are the primary etiology of UTIs in diabetic patients. Bacteria such as Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis possess specific virulence factors that facilitate adherence to the urinary tract epithelium. The objective of this research was to identify Gram-negative bacteria and determine their percentage distribution among Diabetes Mellitus patients at Public Health Center X in Kediri. This research employed a descriptive research method with an accidental sampling technique, utilizing 14 urine specimens from DM patients. The results revealed the presence of Klebsiella sp. (36%), Escherichia coli (50%), Salmonella typhi (7%) and Proteus sp. (7%) in the urine of the subjects. Bacterial identification was performed by culturing on Eosin Methylene Blue (EMB) agar, followed by Gram staining and biochemical tests including Triple Sugar Iron Agar (TSIA), Indole, Methyl Red, Voges-Proskauer, and Citrate, and Urea. Based on these findings, it can be concluded that urinary tract infections in Diabetes Mellitus patients at Public Health Center X, Kediri, are caused by several types of pathogenic bacteria, with the highest prevalence being Escherichia coli, followed by Klebsiella sp., Salmonella typhi., and Proteus sp. Early detection and management of bacterial infections are essential to prevent further complications in patients with Diabetes Mellitus

Keywords: *Klebsiella sp.; Diabetes Mellitus; Urine*

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by a steadily increasing prevalence both globally and nationally. Patients with DM are highly susceptible to various infections, with Urinary Tract Infection (UTI) being one of the most frequent complications. Hyperglycemic conditions in DM patients lead to glucosuria, the presence of glucose in the urine, which creates an ideal environment for the proliferation of pathogenic

microorganisms (Putri & Syahputra, 2022). Furthermore, autonomic neuropathy in diabetic patients often results in bladder dysfunction, leading to urinary retention that triggers bacterial colonization.

According to the 2021 International Diabetes Federation (IDF) data, the global prevalence of diabetes among adults aged 20–79 reached 536.6 million. This figure is projected to rise to 642.7 million by 2030 and is estimated to surge to 784 million by 2045, representing a 0.9% increase. Regionally, Indonesia ranks second highest in Southeast Asia, with the number of cases reaching 19.5 million (IDF, 2021). Meanwhile, at the local level, the East Java Provincial Health Office (2022) recorded 863,686 cases of diabetes mellitus. In Kediri City, 7,954 cases were identified in the same year, with the achievement of health services meeting national standards exceeding the target at 110.4%, covering 8,784 patients (Dinkes Jatim, 2022)

DM poses a risk of serious complications, including macrovascular diseases such as heart disease and stroke, as well as microvascular complications like retinopathy and diabetic nephropathy. Diabetic nephropathy damages the kidney's nephrons due to uncontrolled hyperglycemia and hypertension, while also increasing the risk of urinary tract infections (Cahyawaty, 2022). Patients with DM are susceptible to urinary tract infections caused by urinary retention and impaired immune systems. Microbiologically, Gram-negative bacteria serve as the primary etiology of UTI cases in diabetic patients. Bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* possess specific virulence factors that enable them to adhere to the urinary tract epithelium (Fauziah et al., 2021). The identification of Gram-negative bacteria is crucial, as this group frequently exhibits high resistance to first-line antibiotics, which can worsen the prognosis for diabetic patients (Ramadhani & Sari, 2023).

In Kediri City, particularly within the service area of X Public Health Center, monitoring infectious complications in patients enrolled in the Chronic Disease Management Program (Prolanis) for diabetes needs to be intensified. Early detection through the identification of bacteria in urine is a critical preventive measure to forestall more severe complications, such as pyelonephritis or sepsis (Hidayat & Utami, 2020). Although routine blood glucose monitoring is frequently conducted, specific microbiological urine examinations for this high-risk group remain underreported at this primary healthcare facility. Furthermore, to the best of the authors' knowledge, no similar study has specifically identified Gram-negative bacteria in the urine of diabetes mellitus patients within the Kediri City region; consequently, the local microbiological profile in this area remains poorly mapped. Based on these phenomena and the identified research gap, this study aims to investigate the identification of Gram-negative bacteria in the urine of Diabetes Mellitus patients at Puskesmas X, Kediri City.' This research aims to provide a profile of Gram-negative bacteria causing urinary tract infections, thereby serving as a foundational consideration for determining appropriate empirical therapy for diabetic patients

RESEARCH METHODS

This research employs a descriptive research design. The research population consisted of 14 respondents, and the samples utilized were urine specimens from patients with Diabetes Mellitus Puskesmas X in Kediri. The sampling technique employed was accidental sampling. The inclusion criteria comprised patients diagnosed with diabetes mellitus aged over 50 years, whereas the exclusion criteria involved patients who had already received treatment or antibiotic therapy. The research was conducted at the Bacteriology Laboratory of the Bhakti Wiyata Institute of Health Sciences, Kediri. The research was undertaken during December 2024.

The apparatus used included plates, test tubes, graduated cylinders, Pasteur pipettes, Erlenmeyer flasks, beakers, an oven, an autoclave, a centrifuge, centrifuge tubes, cotton swabs, stirring rods, parchment paper, an analytical balance, spatulas, inoculation loops (ose), lighters, spirit lamps, and aluminum foil, alongside sample containers and cool boxes. The materials and

media utilized were Eosin Methylene Blue (EMB) agar, Triple Sugar Iron Agar (TSIA), IMViC (Indole, Methyl Red, Voges-Proskauer, and Citrate) tests, and Urea media.

Mid-stream urine samples were collected in sterile urine containers and immediately transported to the Bacteriology Laboratory of the Bhakti Wiyata Institute of Health Sciences, Kediri, using a cool box. The urine specimens were then transferred into centrifuge tubes and centrifuged at 1.500-3000 rpm for 15 minutes. After centrifugation, the supernatant was discarded, and the resulting sediment was inoculated onto EMB agar and incubated at 37°C for 24 hours. Colonies growing on the EMB media were subjected to Gram staining and further subcultured into TSIA, IMViC, and Urea media for identification after a 24-hour incubation period.

RESULTS AND DISCUSSION

Based on the results of urinalysis conducted on Diabetes Mellitus patients at Public Health Center X in Kediri, several bacterial species were identified: *Klebsiella* sp. in 5 specimens (36%), *Escherichia coli* in 7 specimens (50%), *Salmonella typhi* in 1 specimen (7%), and *Proteus* sp. in 1 specimen (7%), as illustrated in Table 1.

Table 1. Observation of Colony Characteristics on EMB, IMViC, Urea, and TSIA Media

Specimen Code	EMB	Gram stain	IMViC	TSIA	Urea	Identification
P01	Transparent	rod-shaped Gram -	-+-+	Alkali/Acid Gas : + H2s : +	Positive	*Proteus* sp.
P02, P03, P04,P06, P10	Red 'bull's-eye center	rod-shaped Gram -	--++	Acid/Acid Gas : + H2s : -	Positive	*Klebsiella* sp.
P05,P07,P08, P11, P12, P13, P14	*Methalic Sheen*	rod-shaped Gram -	++--	Acid/Acid Gas : + H2s : -	Negative	*Escherechia coli*
P09	Transparent	rod-shaped Gram -	-+-+	Alkali/Acid Gas : - H2s : +	Negative	*Salmonella thypi*

Gram staining results of the 14 specimens revealed Gram-negative, red-colored bacilli with a scattered arrangement, as illustrated in Figure 1. Following the Gram stain, the isolates were inoculated into TSIA, IMViC, and Urea media, the identification of which is presented in Figure 2.

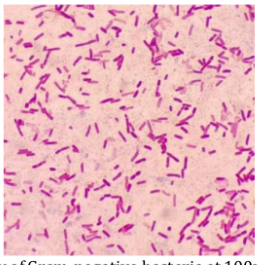

Figure 1. Microscopic view of Gram-negative bacteria at 100x objective magnification (research data)

Klebsiella sp. bacteria on EMB agar	*Escherichia coli* on EMB agar	*Salmonella typhi.* bacteria	*Proteus* sp. bacteria on EMB agar

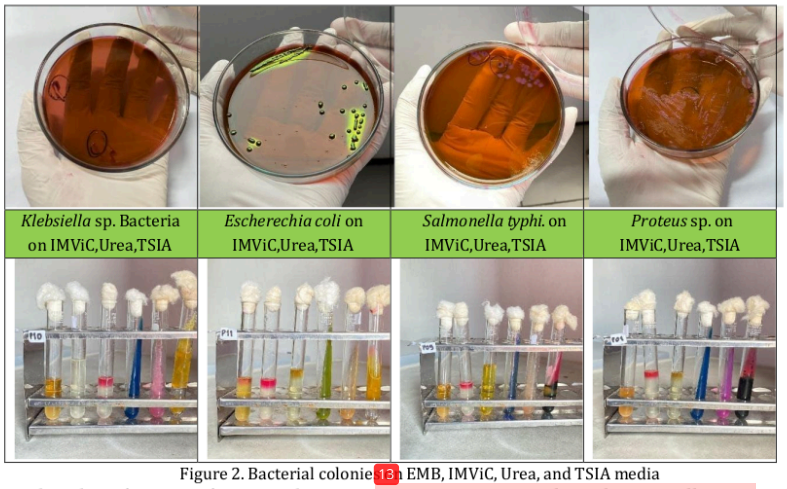

Figure 2. Bacterial colonies on EMB, IMViC, Urea, and TSIA media

The identification of urinary bacteria among patients with Diabetes Mellitus at Public Health Center X in Kediri revealed that *Escherichia coli* was detected in 50% of the specimens, followed by *Klebsiella sp.* at 36%, while *Proteus sp.* and *Salmonella typhi* each accounted for 7%. The presence of bacteria in urine, medically termed bacteriuria, is highly prevalent in patients with diabetes. *Escherichia coli* remains the most dominant pathogen causing urinary tract infections (UTIs) in this group. *E. coli* possesses specialized structures known as fimbriae or pili. In the nutrient-rich urine of diabetic patients, these fimbriae facilitate firm adhesion of the bacteria to the epithelial cells of the urinary tract, thereby preventing them from being flushed out during micturition. Patients with diabetes often experience glucosuria, a condition where elevated blood glucose levels exceed the renal threshold, resulting in the excretion of glucose into the urine. The high glucose content in the urine of diabetic patients creates an ideal microenvironment for the growth and replication of bacteria, particularly *E. coli*, as glucose serves as the primary nutrient source for these pathogens (Pratami et al., 2022). Furthermore, urinary stasis caused by neuropathy provides an extended window for *E. coli* to colonize and adhere to the urothelium (Hidayat & Lestari, 2023). Chronic hyperglycemia also impairs cellular immune responses, rendering the body's mechanism to eradicate bacteria entering the urinary tract ineffective (Sari & Ramadhani, 2021). The 50% prevalence observed in this study aligns with recent global analyses. A comprehensive meta-analysis conducted by Ayelign et al. (2025) indicated a pooled prevalence of approximately 52% for *E. coli*-induced UTIs among diabetic individuals. This consistently high rate is attributed to impairments in both humoral and cellular immunity in patients with diabetes mellitus, characterized by diminished neutrophil chemotaxis and phagocytic activity driven by glucose-induced oxidative stress.

Klebsiella sp. identified in this study is a Gram-negative bacterium and an opportunistic pathogen within the Enterobacteriaceae family, frequently associated with urinary tract infections (UTIs), particularly in immunocompromised patients such as those with Diabetes Mellitus. The virulence of this bacterium is facilitated by type 1 pili, which promote biofilm formation, and a thick polysaccharide capsule that enables the pathogen to evade phagocytosis. Patients with diabetes mellitus exhibit a heightened risk of developing UTIs caused by Gram-negative organisms like *Klebsiella* spp., due to impaired mucosal defense mechanisms and increased bacterial adherence to epithelial cells (Sari & Ramadhani, 2022). Consistently, a study conducted by Melaku et al. (2021) in Ethiopia demonstrated that Klebsiella pneumoniae represents the second most prevalent uropathogen (28.2%) after E. coli among diabetic

individuals. This elevated prevalence of Klebsiella sp. warrants clinical vigilance, as this genus possesses robust encapsulation mechanisms to evade host immunity and exhibits a strong propensity toward multidrug resistance (MDR). Consequently, these virulence factors can significantly complicate antibiotic therapeutic management for diabetes mellitus patients in both outpatient and hospital settings.

Proteus sp. was identified in one specimen (7%) and is a Gram-negative bacterium that flourishes in patients with elevated blood glucose levels due to a supportive growth environment. In line with these findings, Mulu et al. (2021) documented a relatively low prevalence of *Proteus* sp. (around 5–10%) among urinary tract isolates from patients with diabetes mellitus. Bacteria within the genus Proteus are opportunistic pathogens frequently isolated from the urine of diabetic patients. These bacteria exhibit a unique "swarming" motility facilitated by flagella, enabling them to migrate from the urethra toward the bladder and kidneys. Urinary tract infections in diabetic individuals are often polymicrobial, where species such as Proteus sp. demonstrate high adaptability to glucosuria environments through the formation of robust biofilms (Pratama et al., 2023).

Meanwhile, *Salmonella typhi* was also identified in one specimen (7%), representing a rare clinical occurrence. This bacterium typically infects immunocompromised individuals, such as the elderly or patients with renal impairment, and possesses the capability to disseminate from the gastrointestinal tract to the urinary system (Rizal & Hariandy, 2024). Furthermore, urinary tract infections caused by non-typhoidal *Salmonella* are frequently associated with comorbid conditions like diabetes mellitus, where a compromised immune status facilitates bacterial colonization outside the digestive tract" (Putra et al., 2022). The isolation of *Salmonella* from the urine of diabetic patients has been reported in a case study by Jan et al. (2023), which underscores the importance of monitoring for systemic complications when glycemic control is suboptimal.

In summary, the findings indicate that patients with Diabetes Mellitus (DM) are at a higher risk of developing bacterial infections. This vulnerability stems from chronic hyperglycemia, which impairs immune responses and causes neurological damage that hinders the early detection of infections. Consequently, it is imperative for DM patients to maintain a balanced diet, adhere strictly to prescribed medications, increase water intake to facilitate urinary tract clearance, and adopt a clean and healthy lifestyle to prevent more severe infectious complications.

CONCLUSION

Based on the research titled 'Identification of Gram-negative Bacteria in Diabetes Mellitus Patients at Public Health Center X, Kediri,' it can be concluded that the urine samples of Diabetes Mellitus patients contained *Klebsiella* sp. (36%, 5 specimens), *Escherichia coli* (50%, 7 specimens), *Salmonella typhi* (7%, 1 specimen), and *Proteus* sp. (7%, 1 specimen). These findings indicate that Diabetes Mellitus patients at Public Health Center X are at risk of infections caused by various bacterial species.

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