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Bacterial and Drug Susceptibility Profiles of Urinary Tract Infection in Diabetes Mellitus Patients at Mbarara Regional Referral Hospital, Uganda

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Authors' contributions

This work was carried out in collaboration between all authors. Author LA participated in study design, data collection, data analysis, and wrote the first draft of the manuscript and managed literature searches and manuscript revisions. Author AB participated in study conception, design and data collection. Authors PO and OM participated in the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The risk of developing infection in diabetic mellitus patients is known to be higher than in normal individuals. The urinary tract is the most common entry point of infections. Surveillance of urinary tract pathogens and their antibiogram is key to patient management. The main objective of this study is to determine the prevalence of bacterial causative agents of urinary tract infections and their antibiogram in diabetes mellitus patients.

Methods and Materials: A hospital - laboratory based cross-sectional study was conducted from February to April 2014. A total of 105 asymptomatic and symptomatic diabetes patients (55 females and 50 males) that consented were recruited in the study. Mid stream urine samples were obtained

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for standard culture on CLED agar. Bacterial colonies were subjected to Gram stain and relevant biochemical tests were used for isolate identification and antibiogram determined using Kirby bauer disc diffusion method.

Results: Significant bacteriuria ($\geq 10^5$ Colonies/mL) was detected in 13.3% (14/105) of the participants. The common causative agents were *Escherichia coli* (50%), *Klebsiella pneumoniae* (28.6%), *Staphylococcus aureus* (14.3%) and unidentified coliform (7.1%). Majority of the isolates showed 92.9% and 85.7% sensitivity to Gentamicin and Ceftriaxone respectively but a relatively low susceptibility of 64.3% to ciprofloxacin and resistance of 78.6%, and 64.3% against co-trimoxazole and Ampicillin respectively.

Conclusion: Significant bacteriuria was obtained as 13.3% and *Escherichia coli* (50%) as was the highest uropathogen. Isolates showed high resistance to Co-trimoxazole (78.6%) and ampicillin (64.3%). The isolation of bacterial pathogens that resist the commonly prescribed drugs calls for an early screening of all diabetes mellitus patients with urinary tract infections.

Keywords: Bacterial; urinary tract; diabetes mellitus.

1. INTRODUCTION

Urinary tract infection (UTI) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which climb the opening of the urethra and begin to multiply to cause infection [1]. In contrast to men, women are more susceptible to UTI, and this is mainly due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora [2]. An association between urinary tract infection and diabetes mellitus was first noted in an autopsy series reported in the 1940's [3]. Bacterial infections are a major problem in diabetics [4], and the risk of developing infection in diabetic patients is higher than in normal individuals. Urinary tract is the most common site for infection [4]. The infection is caused by a number of microorganisms which include; *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* species, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter*, group *Streptococci*, *Enterococcus faecalis*, *Serratia* species. Diabetes mellitus causes changes in host defense mechanisms, neuropathy which impair bladder emptying and the presence of diabetic cystopathy and micro-vascular disease in the kidneys play a significant role in the higher incidence of UTI's in diabetic patients [5]. Current management of UTIs is usually empirical, without the use of a urine culture or susceptibility testing to guide therapy. However, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTIs is increasing and it is a major health problem in the treatment of UTI [6,7]. There is growing concern regarding antimicrobial resistance worldwide, particularly to *E. coli* which is the

dominant causative agent of UTIs [8]. Therefore, this study is designed to determine the bacterial profile and antibiotic susceptibility pattern of uropathogens among Diabetes mellitus patients in Mbarara regional Referral Hospital that will give area based prevalence and antibiotic sensitivity pattern for empirical therapy.

2. MATERIALS AND METHODS

A hospital based prospective cross sectional study was conducted at Mbarara Regional Referral Hospital (Diabetes Clinic) from February to April 2014 to determine the profile of uropathogens and their susceptibility pattern. The sample size (105) was determined using the standard proportion method with 95% confidence and 5% precision taking as reference a study finding of 18.7% in Mulago-Kampala [9]. Only consented participants who were diabetic regardless of UTI signs and symptoms were enrolled conveniently until the required sample size was achieved. Mid-stream urine samples were collected using sterile, wide mouthed plastic bottles with screw cap tops. On the urine sample bottles, patient's name, age, and time of urine collection were indicated. Female study participants were informed to clean their hands with water and their genital area before collection of the clean catch mid stream urine samples. Urine specimens were processed in the laboratory within 2 hours of collection and specimens that were not processed within 2 hours were kept refrigerated at 4°C until processed.

A calibrated sterile platinum wire loop that has a 4.0 mm diameter designed to deliver 0.01 ml was used for plating. A loopful of the well mixed urine sample was inoculated on Cystein Lactose

Electrolyte Deficient agar and Blood Agar (Oxoid, Ltd, England). All plates were then incubated aerobically at 37°C for 24 h. The plates were then examined macroscopically for bacterial growth. The bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria present per milliliter of urine. A significant bacterial count was taken for specimens that produced $\geq 10^5$ colonies per ml. Colony morphology, haemolytic pattern, Gram reaction and microscopic features were used as primary identification criteria. Biochemical tests, namely indole, citrate, catalase, coagulase, Methylred test, Voges Proskauer, lactose fermentation, were performed for identification of both Gram positive and Gram negative isolates [10]. The sterility of culture media were checked by incubating 3-5% of the batch at 35 – 37°C overnight and observed for bacterial growth, and media which showed growth were discarded. Antimicrobial susceptibilities of the bacterial isolates were performed according to the criteria of Clinical and Laboratory Standards Institute [11] using the Kirby–Bauer disc diffusion method on Muller-Hinton Agar (Oxoid, Ltd, England). A loop full of bacteria was transferred from a pure culture colony to a tube containing 5 ml of sterile physiological saline and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was adjusted to the turbidity of 0.5 McFarland standard tube and swabbed on Muller Hinton agar. The following antibiotics were used with their respective concentration: Ciprofloxacin (CIP, 5 µg), Trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), Ampicillin (AMP, 10 µg), Gentamicin (CN, 10 µg), ceftriazone (CRO, 30 µg). All the antimicrobials used for the study were obtained from Oxoid Ltd. The antimicrobial drug discs were selected based on Clinical and Laboratory Standards Institute (CLSI) and also based on their availability in the Hospital. Thereafter, the antibiotic discs were placed on Muller Hinton Agar and incubated at 37°C for 18 to 24 hr and the zones of inhibition were measured using caliber. The interpretation of the results of the antimicrobial susceptibility tests were based on Clinical and Laboratory Standards institute [11] criteria as sensitive, intermediate and resistant. The standard reference strains, *Staphylococcus aureus* (ATCC25923), and *Escherichia coli* (ATCC25922) were used to assure testing performance of the potency of antibiotic discs. Using Microsoft Excel, descriptive statistics in form of frequencies and percentages were generated.

Ethical clearance was obtained from Mbarara University Research and Ethical Committee and permission from Mbarara Regional Referral Hospital Executive director. Only participants who gave their consent before the sample collection were included in the study. Those who were positive for the test were referred to the physician for management of the infection.

3. RESULTS

A total of 105 diabetic patients with and without symptoms of UTI were investigated during the study period. The age of the participants was from 22 – 85 years, with a mean age of 53.9±12.063 years. Majority (52.4%) of the study participants were females.

The overall prevalence of UTI was 13.3%, total of 14 bacterial uropathogens were identified (Table 1). Out of the 14 bacteria isolated from the samples, 9 (64.3%) were from female participants and 5 (35.7%) were isolated from male samples. From the 14 isolates, 12 were Gram negative while 2 were Gram positive bacteria. *Escherichia coli* 7(50.0%) was the highest uropathogen followed by *Klebsiella pneumoniae* 4 (28.6%), *Staphylococcus aureus* 2 (14.3%) and unidentified *coliform* 1 (7.1%) (Table 1).

Table 2 showed antimicrobial susceptibility pattern of uropathogens; all isolates were sensitive to Gentamicin 12 (100%). Majority of the Gram negative isolates were sensitive to Ceftriazone 11 (91.7%), Ciprofloxacin 8 (66.7%). All Gram negative isolates showed a resistance of 100% to co-trimoxazole and 83.3% to ampicillin. *E. coli* (58.3%) the highest Gram negative bacteria (50% of total isolates) showed 100% resistance to ampicillin and co-trimoxazole (Table 2). Gram positive isolates showed 100% sensitivity to all antibiotics tested.

Table 1. Bacterial uropathogens isolated (N = 14)

Bacterial isolate	Total (%)
<i>Escherichia coli</i>	7 (50.0)
<i>Klebsiella pneumoniae</i>	4(28.6)
<i>Staphylococcus aureus</i>	2(14.3)
Unidentified coliform	1(7.1)
Total	14 (100)

Table 2. Antimicrobial susceptibility pattern of isolated uropathogens

Bacterial isolate	Total No.	Pattern	Antimicrobial agents tested				
			CN No (%)	CRO No (%)	CIP No (%)	AMP No (%)	SXT No (%)
<i>E. coli</i>	7	S	7 (100)	7(100)	4 (57.1)	0(0)	0(0)
		R	0 (0)	0(0)	3(42.9)	7(100)	7(100)
<i>Klebsiella pneumoniae</i>	4	S	4(100)	3(75)	3(75)	1(25)	0(0)
		R	0(0)	1(25)	1(25)	3(75)	4(100)
<i>S. aureus</i>	2	S	2(100)	2(100)	2(100)	2(100)	2(100)
		R	0(0)	0(0)	0(0)	0(0)	0(0)
Unidentified Coliform	1	S	1(100)	1(100)	1(100)	1(100)	0(0)
		R	0(0)	0(0)	0(0)	0(0)	1(100)

AMP = Ampicillin CIP = ciprofloxacin SXT = co-trimoxazole. CRO = ceftriaxone CN = gentamicin. S= Sensitive R= Resistant

4. DISCUSSION

Our study prevalence of 13.3%, this correlates with a range obtained in study by in Ethiopia that showed the prevalence of UTI's among diabetes patients to be between 10.5 to 39.5% [12]. However, our study is lower than Ouma, 2001 carried out in Mulago hospital that showed a prevalence of 18.7% [13]. The highest uropathogen in our study was *Escherichia coli* 7(50%). This agrees with (47%) reported in Netherlands and India (56%) [4,14]. However, our finding is higher than (41.5%) reported in Yemen and Ethiopia [9,15]. The second uropathogen was *Klebsiella pneumoniae* 4(28.6%). Our report is comparable to findings reported in India 35% [8] and Ethiopia 14% [11]. In general, the present study confirms that almost no difference exist in the type and frequency of bacteria isolated in diabetic and non-diabetic patients [4,12,16]. Gram-negative bacteria isolates were more prevalent (85.7%) than Gram-positive bacteria isolates (14.3%). Our report is similar to study done in Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia that reported Gram negative (60%) and Gram positive (40%) isolation [15]. This could be due to the presence of unique structure in Gram negative bacteria which help for attachment to the uroepithelial cells and prevent bacteria from urinary washing.

Antimicrobial resistance among uropathogens to the commonly used antibiotics is emerging and this makes clinicians to have limited choices of drugs for the treatment of urinary tract infection [15]. In this study, susceptibility pattern of Gram-negative bacteria showed that most of the isolates were sensitive to Gentamicin (100%) and ceftriazone (>75%) with moderate susceptibility to Ciprofloxacin (66.7%), but

showed 100% resistance to Ampicillin and Co-trimoxazole. Our findings are comparable to resistance reported in a study done in Ethiopia that showed *Klebsiella* species resistance to ampicillin (100%) and co-trimoxazole (83.3%), and *Escherichia coli* resistance to ampicillin (61.5%) [12]. The low level resistance to ciprofloxacin reported in the present study (33.3%) is comparable to that reported in Ethiopia (<25.0%) and Kampala, Uganda [12,13]. The probable explanation for the difference may be due to the increasing levels of resistance to the drug because of its erroneous use in empirical treatment. The easy availability and indiscriminate use of commonly used drugs such as co-trimoxazole and Ampicillin may explain the observed increase in resistance since antibiotic resistance has been recognized as the consequence of antibiotic use and abuse [17].

Also the increase in resistance may be attributed to the reasons for this alarming phenomenon might be inappropriate and incorrect administration of antimicrobial agents in empiric therapies and lack of appropriate infection control strategies, which may result in increased prevalence of resistant organism in the community.

5. CONCLUSION

Over all prevalence rate of (13.3%) urinary tract infections in diabetes mellitus in this studied population is alarming, and the predominant uropathogens is *Escherichia coli*. and *Escherichia coli* the highest pathogen associated with UTI in diabetic patients. Therefore, early screening of diabetics for UTI causing bacterial uropathogens and determining their antibiotic susceptibility pattern is an important intervention to prevent complications that may endanger the life of diabetic patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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