

Original Research Article**Bacterial and drug susceptibility profiles of urinary tract infection in Diabetes Mellitus patients at Mbarara Regional Referral Hospital, Uganda****ABSTRACT****Introduction:**

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 for of infections.

Surveillance of urinary tract pathogens and their antibiogram is key to patient management. The main objective of this study was 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 patients (55 females and 50 males) that consented were recruited in the study. Mid stream urine samples were obtained 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 were 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 high resistance of 78.6%, and 64.3% against cotrimoxazole and Ampicillin respectively.

Conclusion: The isolation of bacterial pathogens that are resistant to the commonly prescribed drugs calls for an early screening of all diabetes mellitus patients with urinary tract infections.

Keywords: Bacterial, Urinary tract, Diabetes Mellitus.

INTRODUCTION

Urinary tract infections (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 (Rahimkhani, 2008; Okonko, 2009). 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 (Haider *et al.*, 2010).

An association between urinary tract infection and diabetes mellitus was first noted in an autopsy series reported in the 1940's (Janifer *et al.*, 2009). Bacterial infections are a major problem in diabetics (Pargavil *et al.*, 2011), and the risk of developing infection in diabetic patients is higher than in normal individuals and urinary tract is the most common site for infection (Gizachew *et al.*, 2012). (Not in reference) 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 Spp* specie.

Diabetes mellitus causes changes in host defense mechanisms, neuropathy which impairs 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 (Boyko, *et al.*, 2002).

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 is a major health problem in the treatment of UTI (Gupta *et al.*, 2001, Mordi *et al.*, 2006). There is growing concern regarding antimicrobial resistance worldwide, particularly to *E.coli* which is the dominant causative agent of UTIs (Chakupurakal *et al.*, 2010).

Therefore, this study was 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.

METHODS AND MATERIALS

A hospital based prospective study was conducted at Mbarara Regional Referral Hospital (Diabetes Clinic) from February to April 2014 to determine the profile of uropathogens and

method with 95% confidence and 5% precision taking as reference a study finding of 18.7% in Mulago-Kampala (Ouma, 2001). Study Participants who fulfilled the inclusion criteria were enrolled conveniently until the required sample size was achieved. Pregnant women who have taken antibiotics within seven days at the time of recruitment and who were not willing to participate were excluded from this study.

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 4⁰ 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 37⁰C for 24h. 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. (Not reference)

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. (Not reference)

The sterility of culture media were checked by incubating 3-5 % of the batch at 35 – 37⁰C for (expunge) overnight and observed for bacterial growth, and Those media which showed growth were discarded.

Antimicrobial susceptibilities of the bacterial isolates were performed according to the criteria of Clinical and Laboratory Standards Institute (CLSI, 2012) using the Kirby–Bauer disc diffusion

100 method on Muller-Hinton Agar (Oxoid, Ltd, England). A loop full of bacteria was transferred from

a pure culture colony to a tube containing 5ml 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. (Not reference)

The following antibiotics were used with their respective concentration: Ciprofloxacin (CIP, 5µg), Trimethoprim-sulfamethoxazole (SXT, 1.25/23.75µg), Ampicillin (AMP, 10µg), Gentamicin (CN, 10µg), ceftriazone (30µg). 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 h 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 (CLSI, 2012) criteria as sensitive, intermediate and resistant. (Not reference) The standard reference strains, *Staphylococcus aureus* (ATCC25923), and *Escherichia coli* (ATCC25922) were used to assure testing performance of the potency of antibiotic discs.

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.

RESULTS

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

The overall prevalence of UTI was 13.3 %, A total of 14 (from both asymptomatic and symptomatic patients) 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. *E. coli* was the most common bacteria accounting for *Escherichia coli* 7 (50.0%) was the highest uropathogen isolates followed by *Klebsiella pneumoniae* with (expunge) 4 (28.6%), isolates (expunge) , *Staphylococcus aureus* contributing (expunge) 2 (14.3%)

134 Isolates (expunge) and 1 (7.1%) unidentified coliform 1(7.1%) (Table1).

Table 1 Frequency of bacterial uropathogens isolated from pregnant women (Not all subject are pregnant) (N = 14) at Mbarara Regional Referral University Hospital, February to April 30, 2014 (Not necessary)

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

Antimicrobial susceptibility pattern of bacterial uropathogens

In general rates of susceptibilities Gram-negative isolates ranged from 0 % to 100%

(expunge) (Table 2 below). All isolates were sensitive to Gentamicin 12 (100%). Majority of the gram

negative isolates were sensitive to Ceftriaxone 11 (91.7%), Ciprofloxacin 8 (66.7%). All gram

negative isolates showed a resistance rate of 100 % to co-trimoxazole and 83.3% to ampicillin.

E.coli (58.3%) the highest which constituted for 58.3% of the Gram negative bacteria (50% of total isolates) showed

absolute resistance to ampicillin (%), and co-trimoxazole (%) (Table 2).

Gram positive isolates showed absolute sensitivity (100%) to all antibiotics tested

Table 2 Antimicrobial susceptibility pattern of isolated uropathogens. of Gram-negative bacteria isolated from urine

culture of Diabetes mellitus patients (N = 14) at Mbarara Regional Referral University Hospital, February to April 30, 2014 (expunge)

Bacterial Isolate	Total No.	Pattern	Antimicrobial agents tested				
			CN	CRO	CIP	AMP	SXT
<i>E.coli</i>	7	S	No (%) 7 (100)	No (%) 7(100)	No (%) 4 (57.1)	No (%) 0(0)	No (%) 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)
<i>Unidentified</i>							

Coliform	1	S	1(100)	1(100)	1(100)	1(100)	0(0)
		R	0(0)	0(0)	0(0)	0(0)	1(100)

153 AMP = Ampicillin CIP = ciprofloxacin SXT = co-trimoxazole.

154 CRO = ceftriaxone CN = gentamicin.

155

156 DISCUSSION

157

158 Our study prevalence of 13.3% correlates with a range obtained in study by Gizachew *et al*

159 (2012) (Not in reference) in Ethiopia that indicated showed the prevalence of UTI's among
diabetes patients to be

160 between 10.5 to 39.5%. However, the our study showed a is lower than prevalence compared to
that by

161 Ouma, 2001, carried out in Mulago hospital that showed a prevalence of 18.7%. The most

162 probable reason for the deviation of the results may have been due to the introduction of new

163 antibiotics that are more effective in treatment of the infection and (Statement not fact) proper
management of the

164 blood sugar by the patients. Also the differences in methodologies and study populations might

165 affect comparison of prevalence in different surveys.

166

167 The most common bacterial causative agent of UTI's in our study was The highest uropathogen
in our study was *Escherichia coli* 7(50%). (n=7)

168 contributing 50%. This agrees with (47%) obtained reported in Netherlands by (Hoepelman *et al.*, 2003) and Pargavi *et al.*, 2011, who reported (56%) in India.

169 obtained from India (Pargavi *et al.*, 2011). Our findings agree with those obtained from Yemen,

170 41.5 % (Al-Haddad, 2005), and Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia 44 %

171 (Assefa *et al.*, 2008) (Values not in agreement) that examined UTI in pregnant women. It is so
because both conditions are

172 associated with immune suppression

173

174 The second uropathogen *Klebsiella pneumoniae* 4(28.6%), our report is similar to Illamani *et al.*, 2013, who reported (25%) in Bharat University, in India. However our finding is lower than
Vandavasi, who reported (35%) in India. was the second most isolated bacteria (n=4) contributing
28.6%.

175 Comparable findings have been reported at Bharath University, India 25% (Illamani *et al.*, 2013)

176 *pneumoniae*. However our finding is slightly lower than that got in Vandavasi, India 35%

177 (Pargavi *et al.*, 2011). The most probable explanation for the slight discrepancy may be due to

178 differences in sample size used.

179

180 Gram-negative bacteria isolates were more prevalent (85.7 %) than Gram-positive bacteria

181 isolates (14.3 %). Comparable rate of isolation of Gram-negative and Gram positive bacteria, 60

182 % and 40 %, were reported in Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia

183 (Assefa *et al.*, 2008). This could be due to the presence of unique structure in Gram negative

184 bacteria which help for attachment to the uroepithelial cells and prevent bacteria from urinary
185 washing.
186

187 Antimicrobial resistance among uropathogens to the commonly used antibiotics is emerging and
188 this makes clinicians to have limited choices of drugs for the treatment of urinary tract infection

189 (Assefa *et al.*, 2008). In this study, susceptibility pattern of Gram-negative bacteria showed that
 190 most of the isolates were sensitive to Gentamicin (%) and ceftriazone (%) with moderate
 susceptibility to
 191 Ciprofloxacin (66.7%), but showed absolute resistance to Ampicillin and Co-trimoxazole (100%).
 192 Comparable Similar findings were was reported in Ethiopia by Gizachew *et al.*, 2012, (%)
 (Not in reference). except for
 193 Ciprofloxacin where it showed 92.3% sensitivity.

194
 195 The most probable explanation for the difference may be due to the increasing levels of
 196 resistance to the drug because of its erroneous use in empirical treatment. The easy availability
 197 and indiscriminate use of commonly used drugs such as co-trimoxazole and Ampicillin may
 198 explain the observed increase in resistance since antibiotic resistance has been recognized as
 199 the consequence of antibiotic use and abuse (Albrich, 2004)

200
 201 Also the increase in resistance may be attributed to the reasons for this alarming phenomenon
 202 might be inappropriate and incorrect administration of antimicrobial agents in empiric therapies
 203 and lack of appropriate infection control strategies, which may result in increased prevalence of
 204 resistant organism in the community.

205 Multi drug resistance (MDR= resistance in ≥ 2 drugs) was seen in almost 95 % of the isolated
 206 bacterial uropathogens (MDR not presented on table and result). Although this may not
 compare well with that reported in Tikur Anbessa

207 Specialized Hospital Addis Ababa, Ethiopia 74 % (Assefa, 2008) , it still indicates that multi drug
 208 resistance to commonly used antibiotics is an emerging challenge that should be addressed
 209 critically

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211 CONCLUSION

212 The study prevalence (13.3%) obtained is comparable to other previous studies and the study
 213 also agrees with other previous studies on the uropathogens associated with UTI's in diabetes
 214 patients and also noted isolation of bacterial uropathogens which are resistant to commonly
 215 used antimicrobial agents. (conclusion should be summary of the result and suggestion)
 Therefore, early screening of diabetics for UTI causing bacterial
 216 uropathogens and determining their antibiotic susceptibility pattern is an important intervention
 217 to prevent complications that may endanger the life of diabetics.

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219 REFERENCES

220

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