Bacteriuria Among Diabetic and Non-diabetic Patients in some Hospitals in Rivers State, Nigeria

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Abstract: Among the great diversity of etiological agents attributed to urinary tract infections, bacteria are the major causative organisms that are responsible for more than 95% of Urinary Tract Infections (UTIs). This study aimed at the isolation and identification of bacteria in urine from diabetics and non-diabetics. A total of 300 mid-stream early morning urine specimens were collected from diabetic and non-diabetic patients attending some Hospitals in Rivers State. The specimens were inoculated aseptically onto CHROMagar Orientation plates and incubated aerobically for 24-48 hours at 37^{o}C. Glucose in urine was also measured using uristrip (Combi 9). A total of 95 isolates belonging to 12 genera were isolated. Some of the isolates include *Staphylococcus sp., Tatumella sp., Enterobacter sp., Klebsiella sp., Kluyvera sp., Rauotella sp., Proteus sp., Serratia sp., Enterococcus sp., Escherichia sp., Pragia sp.*, and *Photorhabdus asymbiotica* with *Staphylococcus aureus* having the highest occurrence of 19%. Others include *Escherichia coli* 9%, *Kluyvera ascobata* 7%, *Klebsiella aerogenes* 8%, *Serratia liquefactens , Enterococcus sp., Pragia fontium* constituted 5% each*, Enterobacter cloacea , Enterobacter pyrinus, Rauotella planticola, Serratia ficana, Tatumella terrea* constituted 4% each*, Enterobacter cancergenes, Enterobacter hormaechie* constituted 3% each, *Klebsiella oxytoca, Proteus mirabilis, Serratia odorifera, Serratia fonticola* and *Photorhabdus asymbiotica* constituted 2% each while *Kluyvera intermedia* constituted 1% respectively. The study showed different types of bacteria isolated which in most cases were significantly higher in diabetic than non-diabetic patients.
Keywords: Diabetic, Non-Diabetic, Urine*, Klebsiella aerogenes, Staphylococcus aureus*

INTRODUCTION

Urinary tract infection (UTI) is an infection in any part of the urinary system including the kidney, ureters, bladder and urethra. Most infections involve the lower urinary tract- the bladder and urethra (Mayo, 2019). Bacteriuria is the presence of bacteria in the urine. Bacteriuria accompanied by symptoms is a urinary tract infection while that without is known as asymptomatic bacteriuria. Asymptomatic bacteriuria is the presence of bacteria in the properly collected urine of a patient that has no signs or symptoms of a urinary tract infection. Asymptomatic bacteriuria is very common in clinical practice. While few infants and toddlers have asymptomatic bacteriuria, the incidence increases with age (Givler and Givler, 2021).

Among the great diversity of etiological agents attributed to Urinary tract infections (UTIs), bacteria are the major causative organisms that are responsible for more than 95% of urinary tract infections (UTIs). Urinary Tract Infections are one of the most common microbial diseases encountered in medical practice affecting people of all ages (Bonadio *et al.,* 2001; Mayo, 2019). Worldwide, urinary tract infection prevalence was estimated to be around 250 million persons per year (Gezinu *et al.,* 2016). The most common bacterial species that are implicated in UTIs are *Escherechia coli*, *Klebsiella sp*, *Enterobacter sp*, *Pseudomonas aeruginosa* and *Proteus mirabilis* (Flores-Mireles *et al*., 2015). Although *E. coli* is the most frequent bacterium of UTI, other aggressive pathogens are highly prevalent in diabetic UTIs such as fungal infections and some Gram-negative rods (Aswani *et al.,* 2014). Community-acquired prevalence of UTI in Nigeria is 12.3% while nosocomial acquired UTI is 9.3% (Jombo *et al*., 2006).

The incidence of urinary tract infections depends upon diverse risk factors such as diabetes mellitus (DM), advanced age, urinary tract obstructions, immunosuppression and neurological disorders.

Women are at greater risk of developing UTIs than men. Infections limited to the bladder can be painful (Redder *et al.,* 2016). In most parts of Sub-Saharan Africa as well as other developing parts of the world, UTIs are among the most common finding in everyday clinical practice (Jombo *et al.*, 2011). Urinary tract infections are among the causes of morbidity among the general population and the second most common cause for people visiting the hospital. The occurrence of UTI worldwide is related to social class and age of patients. Diabetes patients have a higher incidence of UTI than their non-diabetic counterparts (de lastours and Foxman, 2014) with a higher severity of UTI which can be a cause of complications ranging from dysuria (pain or burning sensation during urination) to organ damage and sometimes even death due to complicated UTI (Pyelonephritis) (Saleem and Daniel, 2011).

In 2012, the direct medical cost associated with managing UTIs in the 22 million diabetic patients in the USA was estimated to be more than 2.3 billion dollars (Ward *et al.,* 2014). The national direct estimate cost of diabetes in Nigeria was estimated in the range of 3.5-4.5 billion per annum (Ismail and Joseph, 2015). Moreover, diabetic patients encounter further urinary urgency and incontinence during the night, a condition often manifested by painful urination and retention of urine in the bladder (Johnson *et al*., 2013). Furthermore, those patients frequently suffer from bacterial cystitis with a higher prevalence in diabetic women including a higher prevalence of both asymptomatic bacteriuria and symptomatic UTI added to recurrent complications as compared to healthy women.

Increased UTI in diabetic patients might be a result of the nerve damage caused by high blood glucose levels, affecting the ability of the bladder to sense the presence of urine, thus, allowing urine to stay for a long time in the bladder and increasing the infection probability. Another explanation is that high glucose levels in urine improve the growth of the bacteria in the urine (Johnson *et al.*, 2013). Again the reduced blood circulation due to prolonged diabetes mellitus may result in abnormalities of the lost system as reflected for instance by the decrease in certain cytokines such as IL-6 and other proinflammatory cytokines in the urine of diabetic patients which may increase the risk of developing an infection.

The struggle of mankind against infectious diseases is well known and the prevalence of urinary tract infections among diabetics keeps increasing with different species of bacteria aggravating the infection rate. This study was therefore designed to isolate and identify etiological agents of urinary tract infections of bacterial origin among diabetic and non-diabetic subjects in some hospitals in Rivers State, Nigeria.

MATERIALS AND METHODS

Study Area

The study areas were three hospitals from the three senatorial districts of Rivers State. These include: General Hospital, Ogale, Nchia, Eleme, Model primary Healthcare centre, Abonnema and Model Primary Healthcare centre, Mgbundukwu, Port Harcourt. Eleme is a local government area in rivers state, Nigeria. It is a part of the port Harcourt metropolitan city, covering an area of 138 km2 (1) (en.m.wikipedia.org). It has an estimated population of about 68,591 people and located at latitude 442’39,996”N and longitude 647’27,996”E.

Akuku-toru local government area is typically riverine with a land of 4,350 square kilometre. It is bounded on the north by degema local government area, on the south of the Atlantic ocean, on the east by the asari-toru local government area and on the west by nembe local government area in bayelsa state (m.facebook.com). Mgbundukwu is a ward in Port Harcourt local government area of Rivers State, Nigeria. It is located in Mile II Diobu area of Port Harcourt (www.manpower.com.ng).

Study Design

A cross-sectional study was conducted in Model Primary Healthcare centre, Mgbundukwu, Port Harcourt in Rivers East Senatorial District, General Hospital, Ogale Nchia, Eleme in Rivers South East Senatorial District and Model Primary Healthcare centre, Abonnema located in Rivers West Senatorial district, all in Rivers State. Male and female patients with type I or type II diabetes and non-diabetics attending these three hospitals were included in the study. Written informed consent, relevant clinical and socio-demographic characteristics were collected from patients using pre-tested questionnaires. Blood glucose levels were measured with spectrometric method using fasting blood samples in fluoride oxalate anti-coagulated bottles and glucose in urine was also measured using unstrap (combi 9).

Ethical Consideration

Ethical approval was obtained from the Rivers state Hospitals Management Board. Consent forms were also given to participants for their consent.

Inclusion criteria

Diabetic patients attending Model Primary Health care centre, Mgbundukwu, General Hospital, Ogale nchia, Eleme and Model Primary Health care centre, Abonnema, regardless of the presence or absence of UTI symptoms. Control subjects were recruited using apparently healthy individuals without any known illness. Samples of non-diabetic patients were used as the egative control

Exclusion Criteria

Patients with known underlying renal pathology and those on antimicrobial therapy in the previous month were excluded from the study.

Sample collection

Three hundred urine samples were collected randomly for this study. Participants provided a mid-stream first early morning urine sample according to the clean-catch procedure into a sterile universal container as described by Herrero *et al.* (2015) **.**

Cultivation and identification of isolates

A standard wire loop was used to inoculate urine samples to McConkey agar, Mannitol salt agar, CLED agar and CHROmagar orientation plates which were incubated aerobically for 24-48 hrs. at 37^0C and growth was judged as significant or not significant or even contaminated. Symptomatic bacteriuria was defined as significant bacteriuria in addition to symptoms related to urinary tract infection while asymptomatic bacteriuria was defined as significant bacteriuria in the absence of urinary tract infection symptoms. Inspection and standard microbiological identification of isolates was also done based on morphology, colour, shape, size etc. Nutrient agar was also used for subculture to obtain pure colonies and then preserved in 10% glycerol for further genotypic identification. Isolates were also Gram-stained and confirmed by biochemical tests. Biochemical tests include: coagulase, catalase, oxidase, citrate, motility, indole, methyl-Red, Voges-Proscauer, glucose, lactose, sucrose and Mannitol. Blood glucose levels were measured with spectrometric method using fasting blood samples in fluoride oxalate anti-coagulated bottles and glucose in urine was also measured with combi 9 using the dipstick method. These procedures have been described by Ochei and Kolhatkar (2000).

Statistical Analyses

Data collected were analysed for statistical significance using Chi-square test and percentages with GraphPad Prism. Values ≤0.05 were considered statistically significant.

RESULTS

Social Demographic information and the prevalence of bacteria in the Study Population

The result of the study revealed that out of 300 urine samples studied 95 (31.7%) had bacterial growth (Table 1) of the 60 samples from the non-diabetic subjects 27 (45%) had bacterial growth while 68 (28.3%) of the the 240 samples from the diabetic patients were also positive with bacteria (Table 1).

Table 1: Prevalence of Bacteriuria among Diabetic and non Diabetic Studied Subjects

Category	Number studied	Number positive with bacteria	Percentage prevalence
Non Diabetic	60	27	45
Diabetic	240	68	28.3
Total	**300**	**95**	**31.7%**

Table 2 shows the social demographic percentage population and bacteria prevalence of study population of diabetic - 240(80%) and non-diabetics - 60(20%). The bacteria prevalence of subjects based on occupation shows professional 3(15%), skilled 11(30%), and unskilled 13(34%) for non-diabetic subjects and for diabetic subjects; professional 17(85%), skilled 26(70%) and unskilled 25(66%). There was statistically significance difference on the prevalence of bacteria between non-diabetic and diabetic subjects based on the occupational type although supported by weak relationship between bacteriuria and occupation showed by Cramer's V= 0.1868.

The bacteria prevalence of subjects based on level of education shows non-professional 9(39%), primary 8(35%), and secondary 6(17%) and tertiary 4(31%) for non-diabetic subjects and for diabetic subjects into non-professional 14(61%), primary 15(65%), and secondary 30(83%) and tertiary 9(69%). The prevalence of bacteria differ significantly between non-diabetic and diabetic population based on the educational level (P=0.0047) and a Cramer's V= 0.1801, showing a moderate relationship.

The bacteria prevalence of subjects based on religion shows, Christianity 20(34%), Islam 7(25%), and others 0(0%) for non-diabetic subjects and for diabetic subjects, Christianity 38(66%), Islam 21(75%), and others 9(100%). This shows a significant moderate relationship between the bacteriuria and religionis as depicted by the Cramer's V= 0.3619 and p=<0.0001.

Percentage Distribution of Bacterial Isolates

Table 2 shows the percentage distribution of bacterial isolates. From all bacterial isolates obtained, *Staphylococcus aureus* was highest with a percentage distribution of 20% and least frequent was *Kluyvera ascobata* with 1%. The prevalence of the isolated bacteria in ascending order are: *Kluyvera ascobata* (1%), *Klebsiella oxytoca* (2%), *Photorhabdus asymbiotica* (2%), *Proteus mirabilis* (2%), *Serratia fonticola* (2%), *Serratia odorifera* (2%), *Enterobacter cancergenes* (3%), *Enterobacter hormaechie* (3%), *Enterobacter cloacea* (4%), *Enterobacter pyrinus* (4%), *Rauotella planticola* (4%), *Serratia ficana* (4%), *Tatumella terrea* (4%), *Enterococcus sp* (5%), *Pragia fontium* (5%), *Serratia liquefactens* (5%), *Kluyvera ascobata* (7%), *Klebsiella aerogenes* (8%), *Escherichia coli* (9%) and *Staphylococcus aureus* (20%).

Prevalence of Bacterial Isolates among Diabetics and Non-Diabetics

Table 3 shows the percentage prevalence of bacterial isolates among diabetic and non-diabetic subjects. Highest percentage prevalence is seen with *Klebsiella aerogenes* (8.4%) among diabetic subjects and *S. aureus* (14.7%) among non-diabetic patients. Statistical significance was observed among diabetic and non-diabetics when compared and is seen among; *Enterobacter pyrinus* (Diabetic 4 (4.2%), non-dabetic 0 (0.0%), P=0.0286), *Klebsiella aerogenes* (Diabetic 8 (8.4%), non-dabetic 0 (0.0%), P=0.0002), *S. aureus* (Diabetic 5 (5.3%), non-dabetic 14 (14.7%), P=0.0086), *Rauotella planticola* (Diabetic 4 (4.2%), non-dabetic 0 (0.0%), P=0.0286), *Serratia ficana* (Diabetic 4 (4.2%), non-dabetic 0 (0.0%), p=0.0286), *Enterococcus sp* (Diabetic 5 (5.3%), non-dabetic 0 (0.0%), P=0.0079), *Pragia fontium* (Diabetic 5 (5.3%), non-dabetic 0 (0.0%), P=0.0079) and *Tatumella terrea* (Diabetic 4 (4.2%), non-dabetic 0 (0.0%), P=0.0286).

Table 2: Social Demographic data and prevalence of Bacteriuria in the Study Population

Demographics	Population			Bacteria Prevalence (%)		
	Non-Diabetic Subject n=60 (%)	Diabetic Subject n=240 (%)	*p*-value	Non-Diabetic Subject n=27 (28.4%)	Diabetic Subject n=68(71.6%)	*p*-value
Occupation						
Professional	25 (19)	105 (81)	0.7520	3 (15)	17 (85)	0.0057
Skilled	19 (21)	70 (79)		11 (30)	26 (70)	
Unskilled	16 (20)	65 (80)	Cramer's V=0.0447	13 (34)	25 (66)	Cramer's V=0.1857
Level of Education						
Non-professional	23 (61)	15 (39)	<0.0001	9 (39)	14 (61)	0.0047
Primary	18 (34)	35 (66)		8 (35)	15 (65)	
Secondary	10 (11)	85 (89)		6 (17)	30 (83)	
Tertiary	9 (8)	105 (92)	Cramer's V=0.4716	4 (31)	9 (69)	Cramer's V=0.1801
Religion						
Christianity	35 (15)	203 (85)	<0.0001	20 (34)	38 (66)	<0.0001
Islam	16 (37)	27 (63)		7 (25)	21 (75)	
Others	9 (47)	10 (53)	Cramer.s V=0.2843	0 (0)	9 (100)	Cramer's V=0.3619
Gender						
Female	30 (20)	120 (80)	0.8624	15 (24)	48 (76)	0.0469
Male	30 (20)	120 (80)	Cramer's V=0.0000	12 (38)	20 (62)	Cramer's V=0.1513

Table 3: Distribution of Bacterial Isolates Among Diabetics and Non-Diabetics Subjects

Bacteria	Diabetic Subject n (%)	Non-Diabetic Subject n (%)	*p*-value
Enterobacter cloacea	1 (1.1)	3 (3.2)	0.4857
Enterobacter pyrinus	4 (4.2)	0 (0.0)	0.**0286**
Enterobacter cancergenes	3 (3.2)	0 (0.0)	0.1000
Enterobacter hormaechie	3 (3.2)	0 (0.0)	0.1000
Klebsiella aerogenes	8 (8.4)	0 (0.0)	0.**0002**
Klebsiella oxytoca	2 (2.1)	0 (0.0)	0.3333
Kluyvera intermedia	1 (1.1)	0 (0.0)	>0.9999
Kluyvera ascobata	5 (5.3)	2 (2.1)	0.2861
S. aureus	5 (5.3)	14 (14.7)	0.**0086**
Rauotella planticola	4 (4.2)	0 (0.0)	0.**0286**
Proteus mirabilis	2 (2.1)	0 (0.0)	0.3333
Serratia ficana	4 (4.2)	0 (0.0)	0.**0286**
Serratia odorifera	2 (2.1)	0 (0.0)	0.3333
Serratia fonticola	2 (2.1)	0 (0.0)	0.3333
Serratia liquefactens	1 (1.1)	4 (4.2)	0.2063
Escherichia coli	5 (5.3)	4 (4.2)	>0.9999
Enterococcus sp	5 (5.3)	0 (0.0)	**0.0079**
Pragia fontium	5 (5.3)	0 (0.0)	**0.0079**
Tatumella terrea	4 (4.2)	0 (0.0)	**0.0286**
Photorhabdus asymbiotica	2 (2.1)	0 (0.0)	0.3333

Prevalence of Subjects with Bacteriuria Based on Age

Table 4 shows the percentage prevalence of subjects with bacteriuria based on age ranges. Prevalence of bacteriuria according to age ranges are; 21-30 (Diabetic 9 (53%), non-diabetic 8 (47%), P=0.4796), 31-40 (Diabetic 11 (65%), non-diabetic 6 (35%), P=<0.0001), 41-50 (Diabetic 13 (72%), non-diabetic 5 (28%), P=0.7773), 51-60 (Diabetic 16 (76%), non-diabetic 5 (24%), P=<0.0001), 61-70 (Diabetic 19 (86%), non-diabetic 3 (14%), P=<0.0001). Statistical significance was observed within age ranges 21-30 (P=<0.0001) and 61-70 (P=<0.0001), others showed no significance. A moderate association was observed between bacteriuria and age ranges of subjects showed by Cramer's V=0.25.

Table 4: Distribution of Bacteriuria Based on Age

Age Range (Years)	Non-diabetic Subjects n (%)		Diabetic Subjects n (%)		*p*-value
	No studied	No positive	No studied	No positive	
21-30	12	8 (47)	48	9 (53)	0.4796
31-40	12	6 (35)	48	11 (65)	<0.0001
41-50	12	5 (28)	48	13 (72)	<0.0001
51-60	12	5 (24)	48	16 (76)	<0.0001
61-70	12	3 (14)	48	19 (86)	<0.0001
Total	**60**	**27**	**240**		**34.76, *p*=<0.0001**

n represents the number of persons that have significant bacteriuria. n=48 for each age range in diabetic subjects, n=12 for each age range in non-diabetic subjects. Cramer's V = 0.25 (shows moderate association).

DISCUSSION

Findings from this study provided information on the prevalence of bacterial isolates from the urine specimens of diabetic and non-diabetic subjects attending some Hospitals in Rivers State, Nigeria. It was discovered from this study that *Staphylococcus aureus* has the overall highest percentage prevalence of 20%, followed by *Escherichia coli* and *Klebsiella aerogenes* with percentage prevalences of 9% and 8% respectively, though *E. coli* and *S. aureus* have the same prevalence of 5.3% among diabetics This is however contrary to the studies done by other investigators which recorded *E. coli* as the most prevalent bacterial isolate with 41.9% (Martins *et al.,* 2019) and a% (Mohammedaman *et al*., 2019). The high prevalence of *S. aureus* in this study agrees with the findings of Martins *et al*., 2019 that had 31.4% and Ekwealor *et al.,* 2016 that had a prevalence of 28% however differs from the findings of previous studies (Ochada *et al*., 2014 and Prakash and Saxena, 2013). The high prevalence of *S. aureus* in this study may be due to increased use of instrumentation such as bladder catheters. The isolation of *Klebsiella sp. Proteus sp. Serratia sp. Enterobacter sp.* and other Gram negatives which are mostly members of the *enterobacteriaceae* family in this study is similar to other studies (Yismaw *et al*., 2012, Kibret and Abera, 2014). Of the eight (8) species that showed significant difference in occurrence (Table 2), only *S. aureus* was significantly higher (p=0.05) in non-diabetics while others: *Enterobacter pyrinus, Klebsiella aerogenes, Rauotella Planticola, serratia ficana, Enterrea* were significantly higher in diabetes (P<0.05). this is of great public health concern and measures such as regular UTI and blood glucose screening should be practiced to reduce the incidence rates.

Similar rates of isolates with different frequency of isolation were also reported form previous studies (Yismaw *et al.,* 2012, Yeshitela *et al.,* 2012, Kibret and Abera, 2014) among diabetics and non-diabetics. Highest percentage prevalence is seen with *Klebsiella aerogenes* (8.4%) among diabetic subjects and *S. aureus* (14.7%) among non-diabetic patients. The overall bacteriuria prevalence in the present study was found to be 31.7% (table 1). This is slightly lower than the discovery of Martins et al., 2019 (32.2%), but higher than studies conducted by Tibyangye *et al.,* 2015 (22.3%), Odongo *et al*., 2013 (24.2%) and Mwaka *et al*., 2011 (10%). Out of this bacterial UTI prevalence, symptomatic and asymptomatic bacteriuria contributed to 53.7% and 46.3% respectively which agrees with the findings of previous studies (53.5% and 46.5% respectively Martins *et al*., the diabetic and non-diabetic bacteriuria). Findings of asymptomatic bacteriuria in this study is however contrary to the findings of Mwaka *et al*. (2011) that had a prevalence of 72.5%.

Total percentage prevalence of bacteriuria among diabetics and non-diabetics was found to be 45% and 28.3% respectively in this study. This is however higher in contrast to the studies conducted by Handan *et al*., (38%), mohammedaman *et al.,* 2019 (33.9%), Sewify *et al.,* 2016 (35%), Jha *et al.,* 2014 (54.6%) and Chukwuocha *et al.* 2012 (17.3%) among diabetics. Diabetic patients have a higher prevalence of bacteriuria as compared to their non-diabetic counterparts. The stastistically significant relationship between UTI and diabetes could be due to altered immunity in diabetic patients which include depressed plymorphonuclear leucocyte functions, altered leucocyte adherence, chemotaxis, phagocytosis, impaired bactericidal activity of the antioxidant system. A higher glucose concentration in the urine may create a culture medium for pathogenic micro-organisms in diabetic subjects that may result in UTIs (Stapleton 2002, Hopps *et al.,* 2008). This study demonstrated that occupation, level of education, religion, age and gender bear statistically significant relationship with urinary tract infections among diabetic and non-diabetic subjects. It was observed from this study that professionals in the occupation category had lesser bacteriuria as compared to the skilled and unskilled. This could be as a result of the professionals being more knowledgeable than others (skilled and unskilled) in this category. This trend also follows for the category of level of education as those who have attained tertiary education had lesser bacteriuria prevalence among diabetics and non-diabetics. The higher bacteriuria prevalence among Christians in the religion category in this study may probably be as a result of the large number of Christians that were involved in the study. Older adults (61-70yrs) had highest prevalence of bacteriuria as demonstrated by this study among diabetics (which is in agreement with the discovery of Al-Rubeaan *et al.,* 2013, Brown *et al*., 2005, Lin *et al.,* 2012) while age range 21-30yrs had the highest bacteriuria prevalence among non-diabetics. This could be as a result of factors other than diabetes. This is in agreement with others studies demonstrated my Mohammedaman *et al.,* 2019 and Hamdan *et a*l., 2015. Female gender were found to have statistical significant relationship with UTIs parts in this study as demonstrated in similar studies conducted by Kabugo *et al.,* 2016 and Chukwuocha *et al*., 2012. Statistical significance was observed. Prevalence of bacteriuria according to location showed statistical significance in Abonnema, Mgbundukwu and Eleme showed no statistical significance.

CONCLUSION

Findings of the study have shown a high prevalence of bacteriuria among diabetic and non-diabetic subjects attending in some selected Hospitals in Rivers State, Nigeria. Moreover, prevalence of bacteriuria was higher in the diabetic patients.

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