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Samuel A. Sakyi
Department of Molecular,
Medicine School of Medical
Sciences KNUST, Ghana

Tel: 233244530214

Asymptomatic Bacteriuria among Type 2 Diabetics in the Sekondi-takoradi Metropolis, Ghana

¹Samuel A. Sakyi, ²Richard K.D. Ephraim, ²Bastu O. Adebisi,
¹James O-Yeboah and ³Gifty Osei-Berchie

Type 2 diabetes mellitus (T2DM) is associated with several overt and covert complications. The objective of this study was to establish the prevalence of Asymptomatic Bacteriuria (ASB), Antimicrobial Sensitivity pattern of the bacterial isolates and associated confounding factors leading to ASB in the Sekondi-takoradi metropolis. A cross-sectional, non-probability sampling technique was used to recruit 102 confirmed Type 2 diabetes mellitus and 23 healthy controls. Fasting blood samples were collected from both study and control participants for blood sugar analysis, midstream urine for microscopy, culture and Antibiotic Sensitivity Testing (AST). Total prevalence of ASB in this study was 26.4 with 41.9% of the total prevalence found in participants with 6-10 years of diabetic history yielding a comparative significant difference in ASB in patients with longer diabetic duration. *E. coli* was the most prevalent bacterial isolate (15.6%) and the most resistant (62.5-100%). Tetracycline and cotrimoxazole were the least effective drugs (0%) whilst nitrofurantoin, nalidixic and gentamicin were more effective. The peak incidence of ASB in T2DM occurs within age group 50-59 years and more frequently in female diabetics. T2DM patients significantly have abnormal Body Mass Index (BMI) ($p = 0.0022$). Age, sex and BMI predispose T2DM patients to prevalence of ASB with highly resistant isolates in the Sekondi-Takoradi metropolis. These must be factored in the treatment of T2DM complications.

Key words: Diabetes mellitus, asymptomatic bacteriuria, antibiotic sensitivity testing, urine culture

¹Department of Molecular Medicine, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

²Department of Laboratory Technology, Medical Laboratory Division, University of Cape Coast, Cape Coast, Ghana

³Department of Nursing, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana

INTRODUCTION

Diabetes mellitus is an endocrine disorder associated with micro and macro vascular complications. Additionally, diabetes are more susceptible to infections like Urinary Tract Infections (UTI) as the high glucose concentration in the urine of diabetics serves as a culture medium for pathogenic organisms (Carton *et al.*, 1992; Pozzilli and Leslie, 1994). UTI in diabetics has always been associated with serious kidney infections such as renal papillary necrosis, emphysematous cystitis and pyelonephritis (Nicolle, 2000).

UTI can be asymptomatic or symptomatic but as to which one takes precedence is still a matter of debate (Alebiosu *et al.*, 2003). Asymptomatic bacteriuria (ASB) is known to be higher in diabetics than non-diabetics (Zhanel *et al.*, 1991). A number of risk factors including age, sexual intercourse and duration of diabetes have been mentioned (Nicolle, 2000).

The prevalence of ASB ranges from 29% by Geerlings *et al.* (2000) in 639 diabetics, to 16.5% by Alebiosu *et al.* (2003) in South-west Nigeria. To date the prevalence of ASB among the Ghanaian diabetic population is unknown. This study therefore seeks to establish the prevalence of ASB, antimicrobial sensitivity patterns of isolates and to determine confounding factors in type 2 diabetes (T2DM) and ASB in the Sekondi-Takoradi metropolis.

MATERIALS AND METHODS

Study area/study design: The study was undertaken at Effia-Nkwanta Regional Hospital (ENRH), Sekondi-takoradi, capital of the Western region of Ghana, the main referral medical facility serving that part of the country and surrounding regions between November, 2011 to May 2012. A cross-sectional non-probability sampling method was adopted (T2DM patients coming first at the laboratory irrespective of sex and aged 20 years and above were recruited) after their consent had been sought.

Study population: A total of 125 participants made up of 102 confirmed T2DM under routine clinical review and 23 healthy controls were recruited from the diabetic clinic and the OPD of the ENRH respectively. T2DM and control patients not being treated for any infection and not on antibiotics were included whilst pregnant women and unconfirmed diabetics with elevated sugar levels were excluded.

Ethical consideration: Ethical clearance was sought for and granted by the ethics and research committees of the

ENRH and UCC. Permission was also obtained from the administrators of the hospital before the study began. Furthermore, all the participants after thorough explanation of the rationale of the study agreed to a written informed consent and were recruited into the study. All procedures followed were in accordance with the ethical standards of the Ministry of Health, Ghana as well as the Helsinki Declaration of 1975.

Collection of blood and urine samples: After an overnight fast (8-12 h) about 2 mL of venous blood was collected into fluoride oxalate bottles, centrifuged at 1500 g for 5 min and stored at -180°C until assayed.

Using sterile, dry, wide necked, leak proof screw capped containers, midstream urine (MSU) specimen were collected for urinalysis, microscopy, culture and sensitivity. The specimens were refrigerated immediately and cultured within 2 h.

Fasting blood sugar (FBS): The plasma glucose were measured for each of the samples using the chemistry analyzer Selectra Junior (Vital Scientific, The Netherlands).

Urine culture and sensitivity (C/S): A loopful (0.002 mL) of well mixed urine sample was taken from each case using a standard calibrated loop and inoculated on a Cysteine Lactose Electrolyte-Deficient (CLED) agar. This was incubated aerobically at 37°C for 18 -24 h in an IPF 400 Precision incubator (Mettler, Germany).

Identification and counting of bacterial isolates: Bacterial colonies were identified based on their colonial morphology (color, growth size and growth pattern). Standard biochemical tests including citrate, urease, indole, catalase and coagulase tests were used for further identification. The product of the loop volume and the colony count (on CLED) gave the bacterial count. Bacterial count $>1 \times 10^5$ CFU mL⁻¹ was considered significant whilst a bacterial count of between 1×10^4 - 10^5 CFU mL⁻¹ was considered as doubtfully significant. Bacterial count $<1 \times 10^4$ CFU mL⁻¹ was considered insignificant (Harding *et al.*, 2002).

Antimicrobial susceptibility test (AST): The Kirby Bauer method (Bauer *et al.*, 1966) was used to determine the susceptibility of the isolates to selected antimicrobial agents. Antibiotic-impregnated paper discs (Medical wire and Equipment Co. Ltd., Potley Corsham, England) containing the following antibiotics: nalidixic acid (NAL, 30 µg), gentamicin (GEN, 10 µg), tetracycline (TET, 30 µg), nitrofurantoin (NIT, 15 µg), cotrimoxazole (COT, 25 µg), ampicillin (AMP, 10 µg), cefuroxime (CRX, 30 µg) and pipemedic acid (PPA, 30 µg), were used.

Fresh isolates of pure colonies were emulsified in peptone water using a sterile straight wire loop and the turbidity was adjusted to the equivalent of 0.5 McFarland's standard. A portion of the emulsified suspension was obtained using a sterile cotton swab and a three dimensional streak was made on a Mueller Hinton agar plate. A sterile cotton swab was then used to obtain a portion of the emulsified suspension to make a three-dimensional streak on a Mueller Hinton agar plate. Based on the organism's Gram reaction an appropriate antibiotic disc was placed on the plated agar within 15 mins of seeding and then incubated at 37°C overnight (18-24 h). A caliper was used to determine the zone of inhibition which was then compared to a standard chart to determine susceptibility categorized as sensitive or resistant as previously described by Tagoe and Desbordes (2012). A Gram negative-organism *Escherichia coli* (NCTC 10418) and *Staphylococcus aureus* [National collection of type cultures (NCTC) 6571] a Gram-positive organism, were used as controls.

Statistical analysis: GraphPad Prism version 5.00 for windows was used for statistical analysis (GraphPad software, San Diego California USA, www.graphpad.com). The results were expressed as Means±SEM. Unpaired t-test was used to compare mean values of continuous variables and χ^2 was used to compare discontinuous variables. Odds ratio (OR, s) (with 95% CI) was calculated using chi-square statistical analysis.

RESULTS

Demographic and clinical features of diabetics: General characteristics of study population are shown in Table 1. Participants with ASB were older, had higher BMI, higher FBS and urine glucose levels compared to the controls ($p < 0.05$). The prevalence of ASB was higher in the

diabetic participants compared to controls though not significant ($p = 0.0613$). The male diabetic participants were older compared to the females ($p = 0.0031$) whereas the females were more obese compared to the males ($p = 0.0063$). The prevalence of ASB was higher in the females compared to the males though not significant ($p = 0.4613$). When the diabetics with ASB were compared to those without ASB, diabetics with ASB had a longer duration of diabetes compared to their counterparts without ASB ($p = 0.009$).

Age and sex distribution of ASB among diabetic participants: Table 2 shows the age and sex distribution of ASB among participants with diabetes. The peak incidence of ASB in males is in the sixth, seventh and eighth decade of life. In the females however, the peak incidence is found in the fourth decade of life. For the total diabetic population the peak incidence was found in the fifth decade of life.

Frequency of bacteria isolated from the urine of study participants: Table 3 represents the frequency of various bacteria isolated from the urine of study participants. *E. coli* were the most prevalent (15.6%) whereas *E. faecalis*, *P. aeruginosa* and *C. freundii* were the least prevalent (0.9%) in the diabetics with ASB. Gender wise, *E. coli* was more prevalent in females (16%) than in males (14.8%).

Prevalence of ASB in relation to glycaemic control: The prevalence of ASB in relation to glycaemic control is shown in Table 4. Twenty-five (30.8%) of the 81 participants with poor glycaemic control had ASB compared to 6/21 (28.6%) of subjects with good glycaemic control ($p = 0.840$).

The odds of developing ASB is the same in the good glycaemic and poor glycaemic groups (OR = 1.116; 95% CI = 0.3875-3.215).

Table 1: General characteristics of study participants

Parameter	Control (n = 23)	Diabetics (n = 102)	p value	Gender		p-value
				Male (n = 27)	Female (n = 75)	
Age (years)	65±2.68	57.25±1.14	0.0103	62.78±2.40	55.25±1.21	0.0031
Male	6/23 (26.0)	27/102	1	-----	----	----
Female	17/23 (73.9)	75/102 (73.5)	1	-----	-----	-----
Duration (years)	-----	7.931±0.60		7.67±1.09	8.03±0.73	0.7953
BMI (kg m ⁻²)	24.95±0.61	29.23±0.70	0.0022	25.94±0.81	30.32±0.85	0.0063
FBS (mmol L ⁻¹)	5.94±0.14	10.5±0.51	0.0001	9.90±0.94	10.72±0.61	0.4836
EC (HPF)	1.35±0.10	1.843±0.81	0.3608	1.519±0.16	1.96±0.24	0.2898
PC (HPF)	1.48±0.19	2.059±0.50	0.4992	1.33±0.10	2.32±0.67	0.2898
Nitrite	2/23 (8.7)	25/102 (24.5)	0.161	1/27 (3.7)	13/75 (17.3)	0.1056
Protein	4/23 (17.0)	32/102 (31.3)	0.2124	9/27 (33.3)	23/75 (30.6)	0.8124
Glucose	0/23 (0.0)	30/102 (29.4)	0.001	5/27 (18.5)	25/75 (33.3)	0.2179
ASB (%)	2/23 (8.7)	30/102 (29.4)	0.0613	6/27 (22.2)	24/75 (32.0)	0.4613

Data is expressed as Mean±SEM, BMI: Body mass index, ASB: Asymptomatic bacteriuria, PC: Pus cells, EC: Epithelial cells, FBS: Fasting blood glucose, HPF: High power field

Table 2: Prevalence of bacterial isolates from both controls and diabetics

Parameter	Control (%)	Total diabetics (%)	Diabetic males (%)	Diabetic females (%)
<i>E. coli</i>	2/23 (8.6)	16/102 (15.6)	4/27 (14.8)	12/75 (16.0)
<i>P. aeruginosa</i>	0/23 (0.0)	1/102 (0.9)	0/27 (0.0)	1/75 (1.3)
<i>Staph. aureus</i>	0/23 (0.0)	2/102 (1.9)	0/27 (0.0)	2/75 (2.6)
<i>K. pneumoniae</i>	0/23 (0.0)	7/102 (6.8)	2/27 (7.4)	5/75 (6.6)
<i>E. faecalis</i>	0/23 (0.0)	1/102 (0.9)	0/27 (0.0)	1/75 (1.3)
<i>C. freundii</i>	0/23 (0.0)	1/102 (0.9)	0/27 (0.0)	1/75 (1.3)
CON	0/23 (0.0)	2/102 (1.9)	0/27 (0.0)	2/75 (2.6)

CON: Coagulase negative *staphylococcus*

Table 3: Age and sex distribution of diabetics with ASB

Age (years)	Males with ASB (%)	Females with ASB (%)	Total no. with ASB%
30-39	0 (0.0)	3 (12.5)	3 (10.0)
40-49	0 (0.0)	3 (12.5)	3 (10.0)
50-59	0 (0.0)	10 (33.3)	10 (33.3)
60-69	2 (33.3)	3 (12.5)	5 (16.6)
70-79	2 (33.3)	3 (12.5)	4 (13.3)
80-89	2 (33.3)	2 (8.3)	4 (13.3)
Total	6 (20.0)	24 (80.0)	30 (100)

Table 4: Prevalence of bacteriuria in relation to blood glucose control

Blood glucose control	No.	Presence of ASB (%)
Good glycaemic control	21	6 (28.6)
Poor glycaemic control	81	25 (30.8)

Table 5: Antimicrobial sensitivity pattern of bacterial isolates of diabetics with ASB

Isolates	No. of Isolates	NAL (%)	NIT (%)	GEN (%)	TET (%)	CRX (%)	PPA (%)	COT (%)	AMP (%)
<i>E. coli</i>	16	4 (25)	6 (37.5)	9 (37.5)	0 (0.0)	2 (12.5)	1 (6.2)	0 (0.0)	0 (0.0)
<i>P. aeruginosa</i>	1	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Staph. aureus</i>	2	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>K. pneumoniae</i>	7	3 (42.8)	3 (42.8)	3 (42.8)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (0.1)
<i>E. faecalis</i>	1	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>C. freundii</i>	1	1 (100)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CoNS	2	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	30	9 (30)	14 (46.7)	13 (43.3)	0 (0.0)	2 (6.6)	2 (6.6)	0 (0.0)	1 (0.1)

ASB: Asymptomatic bacteriuria, NAL: Nalidixic acid, NIT: Nitrofurantoin, GEN: Gentamicin, TET: Tetracycline, CRX: Cefuroxime, PPA: Pipemidic acid, COT: Cotrimoxazole, AMP: Ampicillin

Participants with 6-10 years of diabetic history had ASB prevalence of 41.9%. However, participants who had been with diabetes for more than 20 years had the least prevalence of ASB (6.4%).

Antimicrobial sensitivity pattern of diabetic participants:

The antimicrobial sensitivity pattern of the isolates from diabetic participants with ASB is shown in Table 5. *E. coli*, the predominant isolate was most sensitive to GEN and NIT (37.5%) and least sensitive to PPA (6.2%). *K. pneumoniae* the second most common isolate was 42.8% sensitive to NAL, NIT and GEN and least sensitive to PPA and AMP (33.3%). *P. aeruginosa*, *S. aureus*, *E. faecalis* *C. freundii* and coagulase negative *staphylococcus* (CoNS) showed 100% sensitivity to NAL, NIT and GEN.

DISCUSSION

Historically, Diabetes mellitus has been considered as a risk factor for UTI. Most cases of UTIs are asymptomatic. Furthermore, complications of UTI mostly

associated with diabetics (Adeyeba *et al.*, 2007). In this study we sought to establish the prevalence of ASB and also the antimicrobial sensitivity pattern of the isolated organisms as well as the factors that promote ASB in T2DM in the Sekondi-takoradi metropolis. We achieved this by undertaking urine microscopy, culture and AST, measuring BMI and recording patient's history. The prevalence of ASB in this study was 26.4% and *E. coli* was the most prominent organism.

The 26.4% prevalence observed in this study is lower than the 31.7% reported by Makuyana *et al.* (2002) but comparable to the 26, 26.6% recorded by Geerlings *et al.* (2000) and Alebiosu *et al.* (2003), respectively. In contrast however, a lower prevalence (value) has been reported in other studies (Odetoyn *et al.*, 2008). These percentages are the ones generally reported among female diabetic patients. Furthermore, geographical considerations and the fact that only one urine sample was used compared to the two consecutive samples used in the other studies could account for the difference.

A number of studies have reported a linkage between the duration of diabetes and ASB (Zhanet *et al.*, 1995;

Geerlings *et al.*, 2000; Mendoza *et al.*, 2002; Lindsay and Nicolle, 2004). This relationship was confirmed by observations made in our study; furthermore, we established that ASB was more prevalent among participants with diabetes duration of 5-10 years. In this study, the peak incidence of ASB was found in the fifth decade of life for both the female diabetic participants and the total diabetic population. Furthermore, ASB was more prevalent in the males in the sixth to eight decade of life. This supports other studies where UTI was found more commonly in the elderly (Hooton *et al.*, 2004; Lindsay and Nicolle, 2004). Additionally, our findings suggested no significant relationship existed between ASB and glycaemic control in among our population (OR = 1.116) (Table 3), which is consistent with the findings of Odetoyin *et al.* (2008) and Makuyana *et al.* (2002) but contrary to the findings of Geerlings *et al.* (2000), Ishay *et al.* (2006) and Boroumand *et al.* (2006) who used HBA1c as a measure of glycaemic control.

A number of studies exploring the relationship between ASB and BMI among diabetics and non-diabetics have opined that no significant difference exists in BMI between the two groups (Geerlings *et al.*, 2000; Ishay *et al.*, 2006). Observations made in this study however contradict these reports. Ghanaian diet is predominantly carbohydrates in nature. The predominantly carbohydrate diet of Ghanaians.

The most prominent pathogen isolated in this study was *E. coli* (15.6%) (Table 4). This is in contrast to the findings of Alebiosu *et al.* (2003) where *K. pneumoniae* was the most frequent isolate from diabetics with ASB. However, this observation is in agreement with the majority of reports where *E. coli* had been found to be the major pathogen in ASB (Olaitan, 2006; Baqai *et al.*, 2008; Hajeri, 2008; Assel *et al.*, 2009). *E. coli* is known to be the commonest cause of community Acquired Urinary Tract Infections (UTI).

Multiple drug resistance involving AMP, COT, TET, is a common occurrence in diabetics with UTI as reported by a number of studies (Alebiosu *et al.*, 2003; Odetoyin *et al.*, 2008). Findings in our study confirm this observation. These antibiotics could easily be bought over the counter and that could account for their high resistance among this group of patients. A study by Tagoe and Attah (2010) found that the abuse of antibiotics is prevalent in Ghana and that AMP, COT and TET are among the highly abused drugs (Tagoe and Attah, 2010).

Most of the isolates from our cultures were susceptible to NAL, NIT and GEN (Table 5). This is in line with observations made in other studies (Alebiosu *et al.*, 2003). This implies that these antibiotics still remain the choice drugs for the treatment of ASB.

CONCLUSION

ASB is prevalent in 26.4% of the diabetic population in the Sekondi-Takoradi metropolis and *E. coli* is the most prevalent isolate. Furthermore, factors such as duration of diabetes and advanced age predispose diabetics to ASB even though GEN, NAL and NIT proved the drugs of choice for this diabetic population. It is therefore important for diabetologists to ensure that urine cultures are periodically requested for diabetics even when there are no symptoms. This will serve as a precautionary measure, especially for those at risk.

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