PREVALENCE OF URINARY TRACT INFECTION IN DIABETIC PATIENTS ATTENDING UMUAHIA HEALTH CARE FACILITIES

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ABSTRACT

Diabetes is a polygenic disease characterized by abnormally high glucose levels in the blood. There is evidence that patients with diabetes have an increased risk of Urinary Tract Infections (UTIs). UTI is the most common bacterial infection in diabetic patients. The study population included diabetic patients and non diabetic patients (attending five different Umuahia Health Care Facilities). Urine culture was carried out on the urine samples collected using MacConkey media. A total of four genera of bacteria were isolated namely; *Escherichia coli, Staphylococcus aureus, Klebsiella spp* and *Pseudomonas spp*. 70% of diabetics had positive urine cultures while 56% of non-diabetics had positive urine cultures. The most frequent bacteria isolated was *Escherichia coli* with a percentage value of 34.28%. The isolated micro-organisms were more sensitive to Gentamycin, Streptomycin and Ciproflox and more resistant to augmentin, ampicillin and ceporex. UTIs are frequent in diabetics, a great proportion of asymptomatic forms exist among diabetic patients therefore urine culture should be performed in all patients with diabetes.

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INTRODUCTION

The prevalence of diabetes mellitus has increased over the past decades, and it is now approaching epidemic proportions (International Diabetes Federation, 2012). Worldwide, 371 million people have diabetes and it is estimated that by 2030 this number will reach 552 million. Changes in lifestyle, aging of the population and the increasing prevalence of obesity are responsible for this dramatic situation (Ribera *et al.,* 2006). Diabetes is one of the top ten causes of death in the world and this fact is due especially to its complications. With the growing number of diabetic patients, the prevalence of urinary tract infections has also increased. Hyperglycemia and hypertension are the major risk factors for initiation of chronic kidney disease but other factors, such as repeated episodes of acute kidney injury (infections, drugs, or nephrotoxins) can also contribute to its progression (WHO, 2006). In diabetic patients, it is generally accepted that infections are frequent causes of morbidity and mortality. Immunologic defects contribute to the increased risk for infection: impaired neutrophil function, low levels of prostaglandin E, thromboxane B2,leukotriene B4, decreased T cell-mediatedimmune response, etc. (Geerlings, 2008). Other conditions such as incomplete bladder emptying due to autonomic neuropathy and high glucose concentration in the urine allow urinary colonization by microorganisms (Chin-Hong, 2006). The presence of bacteria in urine is bacteriuria.There is evidence that patients with diabetes have an increased risk of asymptomatic bacteria and urinary tract infections (UTIs) with UTIs being the most common bacterial infections in diabetic patients (Bonadio *et al.,* 2006). The increased prevalence of asymptomatic bacteria (ASB) and symptomatic UTI in diabetic patient may be the result of difference in host responses between diabetic and non-diabetic patients or to a difference in infecting bacterium itself (Greelings, 2006).Patients with diabetes have a 10-fold increased risk of UTI when compared to non-diabetics (Goswani *et al.,*2001) and diabetics have a longer hospitalization than non-diabetics (Moreno *et al.*, 1999). Diabetics are more prone to UTIs and to upper UTI (Geerlings, 2008). The reason for this predisposition is not completely understood, but the most important is likely to be bladder dysfunction caused by diabetic neuropathy. In diabetic women, there is higher incidence of bacteriuria and of asymptomatic kidney infection. UTIs are more common in women than men. Females are more commonly affected with UTI than males and are about thirty times more common among females than males (Geerlings, 2008). UTIs occur in females throughout life and tend to increase with age (Raz *and Stamm,*1992). Silent infections occur about 1% for each ten years of life. They can suddenly become symptomatic and produce considerable discomfort particularly among women prone to repeated infections and during the last three months of pregnancy (Stamm,1982). About half of adult women report that

they have had a UTI at some time during their life.

Diabetics as a whole suffer more UTIs than non-diabetics(Foxman, 2002). A study by Janifer et al.(2009) found that the prevalence of lower urinary tract infection was significantly higher in female patients than in male type 2 diabetic patients. Evidence from various epidemiological studies showed that UTI is more common in women with diabetes than those without diabetes (Janifer *et al.,* 2009). Urinary tract infection appears to be multifactorial in patients with diabetes and various diabetes related risk factors have been proposed. The study by Janifer*et al.,* (2015) on prevalence of urinary tract infection in patients with diabetes found that age, longer duration of diabetes, and poor glycemic control were significantly associated with urinary tract infection..

Bacteriological studies usually reveal the involvement of gram negative enteric organism that commonly cause urinary tract infections, such as *E. coli*, *Klebsiella spp*, and *Proteus spp*. Studies have shown that urinary tract infections due to Enterococci are quite common, particularly in patients who have received antibiotic treatment (Janifer *et al.,* 2009)

This increase is confined largely to those patients with long- standing diseases and neuropathic bladder dysfunction. Young diabeticsare not at risk of UTIs (Souhami and Moxham,1994). Moreover it is important to recognize and to treat UTIs in diabetic patients because of their possibly severe complications, including bacteremia, renal abscess, renal papillary necrosis.

The most common organisms causing UTIare *Escherichia coli (E. coli), Proteus, Klebsiella* spp*, Staphylococcus aureus, Pseudomonas* spp (American Diabetic Association, 2012).These organisms originate mainly from endogenous colonic flora. Pyuria itself is a poor indication of infection (Achary *and Jadan,*1980). In diabetic patients, screening for UTI is very important to enable it to be properly treated and to prevent the development of possible complications.

OBJECTIVE OF THE STUDY

a) To assess the prevalence of urinary tract infection among diabetic patients attending Umuahia health care facilities.
b) To isolate and characterize the micro-organisms responsible for UTI in diabetic patients.
c) To compare the frequency of UTI in diabetic and non-diabetic patients attending Umuahia health care facilities.
d) To identify the antibiotic sensitivity pattern of the various micro-organisms isolated.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in four different health care facilities, all in UmuahiaAbia State.

STUDY POPULATION

A total of100 urine samples were collected from 50 diabetic patients and 50 non-diabetic patients.

SPECIMEN COLLECTION

Patients were given sterile wide-mouth universal containers into which a clean catch (midstream urine)of about 10 – 20 ml urine was collected on the morning of test (Cheesbrough, 2010).

ISOLATION OF MICRO-ORGANISMS

Culture media (Nutrient agar and MacConkey agar) were prepared according to standard procedures (Cheesbrough, 2010). The urine was mixed by rotating the container. An inoculating loop of standard dimension was used to inoculate a loopful of the urine sample on MacConkey agar. It was incubated at 37 °C for 24 hours.

CHARACTERIZATION AND IDENTIFICATION OF ISOLATED MICRO-ORGANISMS

After 24 hours of incubation, the culture plates were examined and the appearance, size, color and morphology were observed. A Gram stain reaction, Catalase, Coagulase, Indole, Oxidase, Citrate utilization tests were carried out as described by Cheesbrough (2010).

GRAM STAIN

The test detects the type of micro-organisms isolated based on its staining reaction.

Procedure

1) A dried smear was made and fixed.
2) The fixed smear was covered with crystal violet for 30 seconds.
3) The stain was rapidly washed off with water.
4) The water was tipped off and the smear was covered with Lugol's iodine for 60 seconds.
5) The iodine was washed off with clean water.
6) The smear was decolorized rapidly for few seconds with acetone water. It was washed immediately with clean water.
7) The smear was covered with neutral red for few minutes.
8) The stain was washed off with clean water
9) The back of the slide was wiped clean and placed in a draining rack for the smear to air-dry.

Gram positive bacteria appeared purple while Gram negative appeared pale to dark red (Cheesbrough, 2010).

BIOCHEMICAL TESTS

Biochemical tests Including Catalase test, Coagulase test, Oxidase test, Indole test, Citrate as elucidated by Cheesbrough (2010) were carried out on the colonies to ascertain organisms isolated.

Catalase test

This test detects the presence of Catalase an enzyme that catalyses the release of oxygen from hydrogen peroxide.

Procedure

1) 2ml of hydrogen peroxide solution was poured into test tubes for each isolate.
2) Several colonies of the test organisms were removed using a sterile wooden stick and immersed into the hydrogen peroxide solution in the test tube.
3) Immediate bubbling was looked for.
   - Active bubbling indicates positive catalase test.

- No bubbles indicates negative catalase test.

Coagulase test

This test detects the presence of coagulase enzyme.

Procedure for Coagulase test

1) A drop of water was placed on the end of two separate grease-free slides for each isolate.
2) A colony of the test organism was emulsified in each of the drops to make suspensions.
3) A loopful of plasma was added to one of the suspensions. It was mixed gently and clumping of the organism was looked for within 10 seconds.
    - Clumping within 10 seconds indicates that the organism is *Staphylococcus aureus*growth.
    - No clumping within 10 seconds indicates that there is no bound Coagulase.

Oxidase test

This test detects the production of oxidase enzyme by some micro-organisms.

Procedure

1) A piece of filter paper was placed in a clean petri dish and 3 drops of freshly prepared oxidase reagent was added.
2) Using a piece of stick, a colony of the test organism was removed and smeared on the filter paper.
    - Development of blue-purple color within a few seconds indicates a positive oxidase test.
    - No blue-purple color indicates a negative oxidase test.

Indole test

The test detects the production of indole in tryptophan containing medium by some bacteria when Kovac's reagent is added to it.

Procedure

1) The test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water.
2) The bijou was inoculated at 37°C for up to 48 hours.
3) 0.5ml of Kovac's reagent was added to the bijou bottle. It was shaken gently.
    - A red color in the surface layer within 10 minutes indicates a positive indole test.
    - No red surface layer indicates a negative indole test.

Citrate test

This test detects the presence of *Klebsiella spp*

Procedure

1) The test organism was inoculated into sterile peptone water broth and incubated for few hours.
2) A sterile straight wire was then used to inoculate Simmons citrate agar with the broth culture.
3) It was incubated at 37°C for 48 hours.
    - Development of a blue color growth indicates a positive citrate test.

ANTIBIOTIC SENSITIVITY TEST

Antibiotic sensitivity test was carried out using paper disc diffusion technique. A total of 17 antibiotics were used as shown in Table 1.

Table 1: Antibiotics for sensitivity test

ANTIBIOTICS	CODE	CONCENTRATION
Travid	OF	100mcg
Reflacine	PEF	100mcg
Ciproflox	CPX	100mcg
Augmentin	AU	300mcg
Gentamycin	CN	100mcg
Streptomycin	S	30mcg
Ceporex	CEP	10mcg
Nalidixic acid	Na	30mcg
Septrin	SXT	30mcg
Amplicin	PN	30mcg
Norfloxacin	NB	10mcg
Amoxil	AML	10mcg
Rifampicin	RD	20mcg
Erythromycin	E	30mcg
Chloramphenicol	CH	30mcg
Ampiclox	APX	20mcg
Levofloxacin	LEV	20mcg

The organism isolated was inoculated on a dry sterile nutrient agar plate. This was spread over the entire surface of the nutrient agar. The antibiotic discs were placed on the agar using sterile forceps. The plates with the antibiotic discs were then incubated at 37°C for 24 hours to observe the zone of growth inhibition according to the diameter of the inhibition zone surrounding each antibiotic disc

STATISTICAL ANALYSIS

The results obtained in this study were subjected to frequencies and percentages to ascertain the level of variability that existed among diabetic and non-diabetic patients.

RESULTS

Out of the 100 urine specimen sampled for both diabetic and non-diabetic patients, a total of four different groups of organisms were isolated and characterized. The various groups identified were micro-organisms belonging to the genus; *Escherichia coli, Staphylococcus aureus, Pseudomonas spp, Klebsiella spp.*

Table 2 shows the various micro-organisms isolated, their appearance,

color, Gram stain reaction and the biochemical test results. *Escherichia coli, Pseudomonas spp and Klebsiellas spp* are Gram positive cocci *while Staphylococcus aureus* is Gram negative cocci. *Escherichia coli* was indole positive, *Pseudomonas spp* was oxidase positive and *Staphylococcus aureus* was Catalase and Coagulase positive.

Table 3 compared the frequency of isolated orgaanisms in diabetic patients to non-diabetic patients. Diabetic patients had 70% of the isolated organisms while non-diabetic patients had 56% of the isolated organisms.

Table 4 shows the percentage distribution of the isolated organisms in diabetic and non-diabetic patients. *E. coli* had the highest percentage distribution with 34.28% in diabetics and 42.85%non-diabetic patients; while *Pseudomonas spp* had the least percentage distribution with 11.42% and 10.71% respectively in diabetic and non-diabetic patients.

Fig. 1 shows comparison of frequency of the isolated organism according to gender. The diabetic and non-diabetic females had more isolates than the diabetic and non-diabetic males.

Table 5, 6 and 7 shows the resistance and sensitivity pattern of microorganisms to antimicrobial drugs. The four groups of organisms; *Escherichia coli, Staphylococcus aureus, Klebsiella spp, Pseudomonas spp,* were sensitive to Gentamycin, Streptomycin and Ciproflox. *Escherichia coli, Klebsiella spp, Psuedomona sspp were* resistant to augmentin, ampicillin, ceporex.

Table 2: Identification of isolates

	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas spp*	*Klebsiella spp*
Cultural examination	Smooth pink colonies	Colorless colonies	White or colorless colonies	Mucoid pink colonies
Gram stain reaction	Gram negative rods	Gram positive cocci	Gram negative rods	Gram negative rods
Catalase test	Negative	Positive	Negative	Negative
Coagulase	Negative	Positive	Negative	Negative
Oxidase	Negative	Negative	Positive	Negative
Indole	Positive	Negative	Negative	Negative
Citrate	Negative	Negative	Negative	Positive

Table 3: Frequency of isolated organisms in diabetics compared to non-diabetics

	Diabetics		**Non-diabetics**	
	No	**%**	**No**	**%**
Number of plates with isolates	35	70	28	56
Number of plates without isolates	15	30	22	44
Total	50	100	50	100

Table 4: Prevalence of organisms isolated from diabetics and non-diabetics

UTI Pathogens	Diabetics		Non-diabetics	
	No	%	No	%
E. coli	12	34.28	12	42.85
S. aureus	8	22.85	5	17.85
Pseudomonas spp	4	11.42	3	10.71
Klebsiellaspp	11	31.42	8	28.57

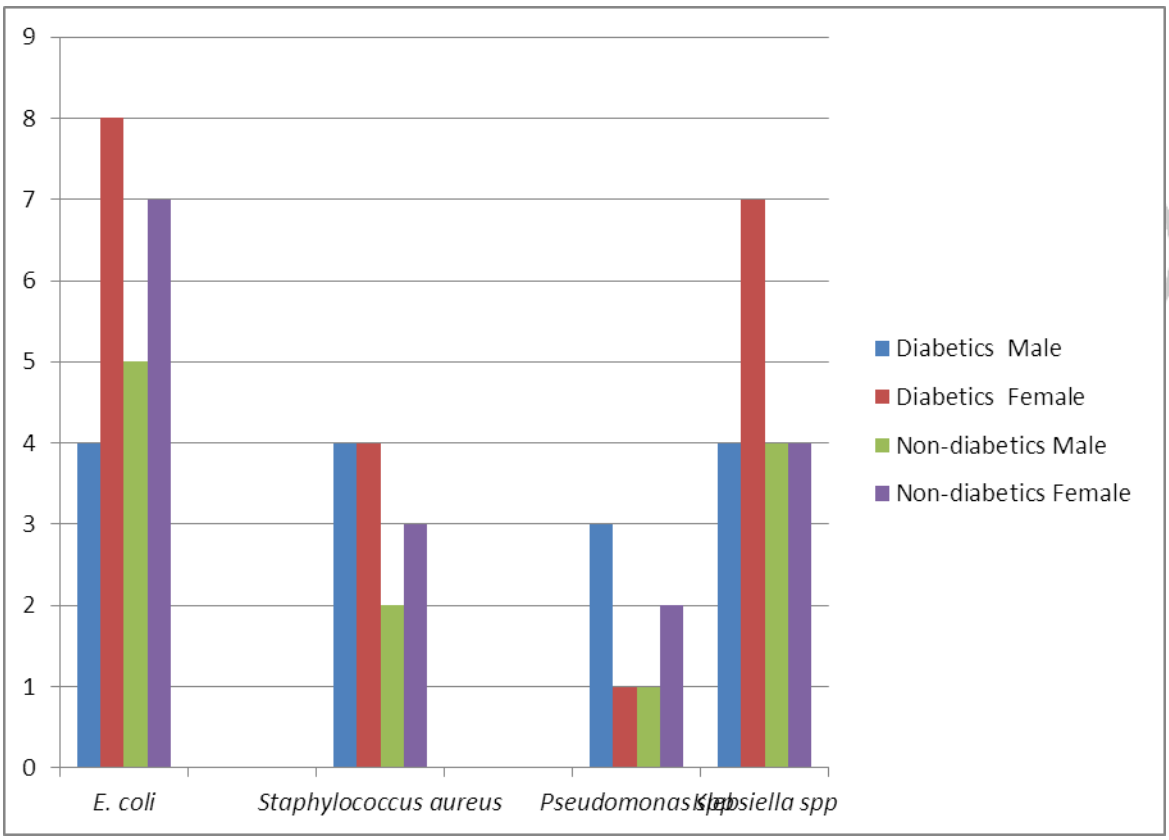

Fig. 1: Comparison of frequency of the isolated organisms according to gender

Table 5: Antibiotic Sensitivity/ResistanceProfile

Antibiotics (Gram positive disc)	***S. aureus*, n=8**		
	R (%)	**I(%)**	**S(%)**
Ciprofloxacin (CPX)	0 (0%)	0(0%)	8(100%)
Norfloxacin (NB)	0(0%)	0(0%)	8(100%)
Gentamycin (CN)	0(0%)	3(37.5%)	5(62.5%)
Amoxil (AML)	8(100%)	0(0%)	0(0%)
Streptomycin (S)	4(50%)	4(50%)	0(0%)
Rifampicin (R)	8(100%)	0(0%)	0(0%)
Erythromycin (E)	8(100%)	0(0%)	0(0%)
Chloramphenicol (C)	7(87.5%)	1(37.5%)	0(0%)
Ampiclox (A)	5(62.5%)	2(25%)	1(12.5)
Levofloxacin (L)	0(0%)	0(0%)	8(100%)

Key:
R= Resistant
I= Intermediate
S= Sensitive

Table 6: Antibiotic Sensitivity/Resistance Profile

Antibiotics (Gram negative disc)	*E. coli*, n=12			*Klebsiellaspp*, n=11		
	R (%)	I (%)	S (%)	R (%)	I(%)	S (%)
Travid (OFX)	0 (0%)	0(0%)	12(100%)	0(0%)	0(0%)	11(100%)
Reflacine (PEF)	0(0%)	4(33.3%)	8(66.7%)	0(0%)	4(36.4)	7(63.6)
Ciprofloxacin (CPX)	0(0%)	3(25%)	9(75%)	0(0%)	3(27%)	8(73%)
Augmentin (AU)	11(91.7%)	1(8.3%)	0(0%)	11(100%)	0(0%)	0(0%)
Gentamycin(CN)	0(0%)	0(0%)	12(100%)	0(0%)	0(0%)	11(100%)
Streptomycin (S)	0(0%)	3(25%)	9(75%)	0(0%)	4(36.4)	7(63.6)
Ceporex (CEP)	1(91.7%)	1(8.3%)	0(0%)	9(82%)	2(18%)	0(0%)
Nalidixic acid (Na)	0 (0%)	0(0%)	12(100%)	0(0%)	3(27%)	8(73%)
Septrin (SXT)	0(0%)	8(66.7%)	4(53.3%)	0(0%)	5(45.5%)	6(54.5%)
Ampicillin (PN)	12(100%)	0(0%)	0(0%)	11(100%)	0(0%)	0(0%)

Key:
R= Resistant
I= Intermediate
S= Sensitive

Table 7: Antibiotic Sensitivity/Resistance Profile

Antibiotics (Gram negative disc)	*Pseudomonas spp*, n=4		
	R (%)	I (%)	S (%)
Travid (OFX)	0 (0%)	0(0%)	4(100%)
Reflacine (PEF)	0(0%)	1(25%)	3(75%)
Ciprofloxacin (CPX)	0(0%)	0(0%)	4(100%)
Augmentin (AU)	4(100%)	0(0%)	0(0%)
Gentamycin(CN)	0(0%)	0(0%)	4(100%)
Streptomycin (S)	0(0%)	2(50%)	2(50%)
Ceporex (CEP)	3(75%)	1(25%)	0(0%)
Nalidixic acid (Na)	3(75%)	1(25%)	0(0%)
Septrin (SXT)	3(75%)	1(25%)	0(0%)
Ampicillin (PN)	12(100%)	0(0%)	0(0%)

Key:
R= Resistant
I= Intermediate
S= Sensitive

DISCUSSION

Urinary tract bacteria were isolated more in diabetics than in non-diabetics in the present study. This agrees with the findings of Horcajada *et al.* (1999) that the incidence of bacteriuria is higher in diabetics; and with that of Geerlings (2008) that diabetics are more prone to UTIs. People with diabetes have a higher risk of UTI because of changes in their immune system (National Kidney and Urologic Disease Centre, USA (2003). This study reveals a high prevalence of 70% and 56 % UTI in diabeticand non-diabetic patients respectively.

The bacteria associated with UTI in diabetics were predominantly *E. coli*

(34.28%), *Staphylococcus aureus* (22.85%),*Pseudomonas spp (11.42%)* and *Klebsiella spp (31.42%).E. coli* was the predominant organism 12(34.28%) and 12(42.85%) in diabetic and non-diabetic patients respectively in the study. Edward *et* al. (1999) reported a prevalence of *E. coli* 13.1% and 6.8% UTI respectively in diabetic and non-diabetic patients in USA. Mario *et* al. (1999) in Italy reported 18.1%UTI in diabetics. *E.coli* is a normal intestinal flora found in humans and is therefore expected to be more prevalent in urinary tract of immunologically suppressed patients.

Higher incidence of bacteriuria was also recorded in female diabetics (40%) than in female non-diabetic (32%) and the male diabetics (30%) respectively. Thiscorroborates the reports of Raz and Stamm (1992) that females are more commonly affected with UTI than males and with that of Geerlings et al.(2000) that women with *Diabetes mellitus* are about 2-3 times more likely to have bacteria in their bladders than women without D.M. The four groups of organisms; *Escherichia coli, Staphylococcus aureus, Klebsiella spp, Pseudomonas spp,* were sensitive to Gentamycin, Streptomycin and Ciproflox. *Escherichia coli, Klebsiellaspp, Pseudomonas spp were* resistant to augmentin, ampicillin, ceporex. There seems to be an increased risk of the infection spreading upwards into the kidneys in diabetic patients especially in those whose condition has lasted for long periods of time and in those poorly managed for the condition (Zimmet *et al.,* 2001).

CONCLUSION

In conclusion, prevalence of UTI among diabetics in this study was comparable to published literature. The commonly used antibiotics were sensitive to the urinary isolates.UTIs are frequent in patients with diabetes. The most frequent uropathogen is *E. coli*. Many UTIs are asymptomatic, especially in women. Because of the great proportion of asymptomatic UTIs among diabetic patients, urine culture should be performed in all hospitalized diabetic patients. In addition, considering the high prevalence of Asymptomatic Bacteriuria in diabetics, this condition could represent one of the causes leading to an unexplained worsening of the glycosuria in some patients. This study confirms that diabetes predisposes humans to the risk of urinary tract infections due to the changes in bladder function and in circulation.Diabetics infectedwith UTIs should therefore be promptly treated with the proper antibiotics to prevent development of kidney damage or more serious infections. However further studies with large sample size is highly recommended to authenticate the findings from this study.

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