

Prevalence of Uropathogens in Diabetic Patients and their Antimicrobial Susceptibility Pattern.

*Akinnibosun, F. I and Iriakpe, H. J

Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B 1154, Benin City, Edo State, Nigeria.

Abstract: The prevalence of bacteria causing UTI in diabetic patients, as well as their susceptibility to commonly used antibiotics was investigated in Benin City. Bacteriological analysis involved standard biochemical tests and comparison of isolates' characteristics with known taxa. Species isolated from the urine samples analysed were *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Gram-negative isolates had a prevalence of 60 %, while Gram-positive isolates had 40 %. *E. coli* were found to be the most prevalence (44.4 %), while the least prevalent were *P. aeruginosa*(11.1 %), *S. aureus*(11.1 %) and *S. saprophyticus* (11.1 %). Isolates were found to be more in females than males. Three out of the six male diabetics sampled had uropathogens, while six out of the fifteen female diabetics sampled had uropathogens. Susceptibility tests were performed by Bauer-Kirby disc-diffusion method with standard antibiotics. The results were expressed as susceptible or resistant. All the isolates were found to be susceptible to ciprofloxacin and gentamicin. They were also found to be multi-drug resistant. This study showed that diabetic patients had increased risk of urinary tract infections due to the presence of uropathogens.

Key words: Diabetes, Urinary tract infection, Multi-drug resistance, Susceptibility pattern, Bacteria.

Introduction

Urinary tract infections (UTI) are serious health problems affecting millions of people each year. They are the second most common type of infections in the body (Stamm *et al.*, 2011). UTIs are one of the most common types of bacterial infections in humans occurring both in the community and the health care settings and ranks high amongst the most common reason that compel an individual to seek medical attention (Susman, 1998; Tice, 1999; El-Sweih *et al.*, 2008; Kolawole *et al.*, 2009).

It encompasses a spectrum of clinical entities ranging in severity from asymptomatic infections to acute cystitis, Prostatitis, Pyelonephritis and Urithritis (Fowler, 1986; Gluisier, 1991; Kunin, 1994; Kolawole, 2009). The structure of the female urethra and vaginal makes it susceptible to trauma during sexual intercourse as well as bacteria being massaged up the urethra and into the bladder during pregnancy and or child birth (El-Sweih *et al.*, 2008; Kolawole *et al.*, 2009; Jones *et al.*, 2006). In pregnancy the physiological increase in plasma volume and decrease in urine concentration develop glycosuria in up to 70% women which ultimately leads to bacterial growth in urine (Lucas and Cunningham, 1993). Worldwide about 150 million people are diagnosed with UTIs each year (Gupta, 2001). Majority of UTIs are not life threatening and do not cause any irreversible damage. However, when the kidneys are involved, there is a risk of irreparable tissue damage with an increased risk of bacteremia (Hvidberg *et al.*, 2000).

*Corresponding author:

fakinnibosun@yahoo.co.uk; *Akinnibosun, F. I

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Transmission occurs in four ways; namely through sexual intercourse, from mother to the foetus viaplacenta, through poor personal hygiene and via communal sponge and towel usage (Onifade *et al.*, 2011). The most commonly encountered microorganisms in UTI are Gram-negative bacteria including *Escherichia coli*, *Citrobacter sp.*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Proteus vulgaris* whereas *Klebsiella sp.*, *Staphylococcus aureus* and *Salmonella sp.* are found rarely (Foxman and Brown, 2003).

Diabetes mellitus is the most common endocrine disease and is associated with organ complications due to microvascular and macrovascular disease. People with diabetes also suffer from simple and complicated infections, although the association between diabetes and increased susceptibility to infection has been questioned (Wheat, 1980; Thornton, 1971). Nevertheless many specific infections are commoner in diabetes and some occur almost exclusively in diabetic subjects. Others may occur with increased severity and may be associated with greater risk of complications (Joshi *et al.*, 1999). UTIs fall into both these categories i.e. exclusive and more severe. Asymptomatic bacteriuria, acute pyelonephritis and complications of UTI are reported to be more common in patients with diabetes. Despite the clinical and economic significance, there is a paucity of research. 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.

Increasing multidrug resistance in bacterial uropathogens is an important and emerging public health problem. In the last three decades there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing urinary tract infections (Tenever and McGowan, 1996; Hryniewicz *et al.*, 2001; Kurutepe *et al.*, 2005). This is a serious issue particularly in the developing world where apart from high level of poverty, ignorance and poor hygiene practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation. Epidemiological surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance control measures and preventing the spread of antimicrobial-resistant microorganisms (Oteo *et al.*, 2005; Okonko *et al.*, 2009). The changing pattern in the etiology of UTI and their sensitivities to commonly prescribed antibiotics have been reported (Jacoby and Archer, 1991; Hryniewicz *et al.*, 2001; Kurutepe *et al.*, 2005; Mordi and Erah, 2006). The worldwide escalation in both community and hospital acquired antimicrobial resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control, and new treatment alternatives (Mulvey, 2004; Rhomberg *et al.*, 2006; Chikere *et al.*, 2008; Okonko *et al.*, 2009).

This study, therefore, was undertaken to determine the prevalence of uropathogens in diabetic patients in Benin City, Nigeria and to evaluate the antimicrobial susceptibility pattern of the bacterial isolates.

Materials and Methods

Sample Collection

Fresh mid-stream urine was collected from each patient into a 20 ml calibrated sterile screw-capped universal container which was distributed to the patients. The specimens were labelled transported to the laboratory and analysed within 6 hrs. In each container boric acid (0.2 mg) was added to prevent the growth of bacteria in urine samples. All patients were well instructed on how to collect sample aseptically prior to sample collection to avoid contaminations from urethra. Verbal informed consent was obtained from all patients prior to specimen collection. The study was conducted after due ethical approval. Twenty-one samples (six males and fifteen females) were collected.

Media Preparation

The media used in this study were nutrient agar and MacConkey agar. All media were prepared according to manufacturer's instruction.

Isolation and Identification of Isolates

The samples were inoculated on MacConkey agar and Nutrient agar. The inoculum on the plate was streaked out for discrete colonies with a wire loop following standard procedures (Cheesbrough, 2006; Mordi and Erah, 2006). The culture plates were incubated at 37 °C for 24 hrs and observed for growth through formation of colonies. Isolates were sub-cultured and pure cultures were identified using morphology, microscopy and biochemical tests following standard procedures described by Cowan and Steel (1974) and Cheesbrough (2006). 10^5 colony forming units per ml urine (cfu/ml) obtained in a clean midstream specimen is a sign of a clinically significant UTI.

Antibiotic Susceptibility Test

This was carried out using the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966), according to National Committee for Clinical Laboratory Standards (NCCLS, 2002). Multidrug resistance was defined as resistance to ≥ 3 of the antimicrobial agents tested (Oteo *et al.*, 2005). The antibiotics used for the isolates were: Amoxicillin (30µg), Chloramphenicol (30µg), Augmentin (30µg), zinnacef (20 µg), rocephin (25µg), ciprofloxacin (10 µg), streptomycin (30µg), erythromycin (10µg), gentamycin (10µg), septrin (30 µg), perfloxacin (10µg), ampiclox (30µg) sparfloxacin (30µg) and ofloxacin (30µg).

Results and Discussion

The study assessed the presence of bacteria in urines of diabetic patients. Twenty-one (21) samples for both male and female were collected (6 males and 15 females). Out of the 21 diabetic patients sample collected, only 9 (42.85 %) (3 male and 6 females) had growth of uropathogens (Table 1). Results revealed that 50 % of the diabetic males had UTI while 40 % of the diabetic females had UTI. *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* were isolated (Table 2). This agrees with the findings of Horcajada *et al.* (1999) that the incidence of bacteriuria is high in diabetics; and with that of Geerlings (2008) that diabetics are more prone to UTIs. This is because diabetes affects many systems that protect against infection in general, and against UTI specifically (Goswami *et al.*, 2001). Poor circulation in diabetes reduces the ability of infection-fighting white blood cells to get where they are needed, and even when they do, they are less able to ingest the offending bacteria and kill them than normal white blood cells. It may also be due to bladder dysfunction caused by diabetic neuropathy which allows urine to remain in static pools for long periods of time, providing luxurious ponds for bacteria to grow in. *E. coli* was the most commonly isolated organism

(44.4%) followed by *Proteus mirabilis* (22.2%), *Staphylococcus aureus* (11.1%) *Staphylococcus saprophyticus* (11.1%) and *Pseudomonas aeruginosa* (11.1%) (Table 2). The results of this study showed that the etiologic agents of UTIs mainly belonged to the Gram-negative bacteria, with *E. coli* being the most prevalent. This agrees well with the previous studies conducted (Braunwald et al., 2001; Connell et al., 2000; Johnson et al., 1999; Oelschlaeger et al., 2002). *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. 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. All the bacteria isolated occurred in the females except *S. aureus*, while *S. aureus* and *E. coli*, only occurred in the males (Table 3). This is similar to the findings of Braunwald et al., 2001. The uropathogens isolated and identified in this study, were similar to those of many other studies conducted in different countries, either in the region or internationally (Braunwald et al., 2001; Hooton et al., 2012). The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions, and host factors practices such as health care and education programmes, socio-economic standards and hygiene practices in each country. The enterobacteriaceae were the most common microorganisms isolated in this study. This is in agreement with the result of Dimitrov et al. (2004); Mordi and Erah, (2006); Dash et al. (2013); Abubakar, (2009); Johnson et al. (1999); Omigie et al. (2009). This high incidence of *E. coli* and other enterobacteriaceae could be attributed to the fact that they are commensals of the bowels and that infections is mostly by faecal contamination due to poor hygiene. This is owned to the fact that commensals of the intestines are more involved in the UTI because of the anatomy proximity to the genitourinary area (Hooton et al., 2012).

The results of antibiotic susceptibility test with Gram-negative bacteria (Table 4), showed that *E. coli*

was resistant to 7 (70 %) and susceptible to 3 (30 %) of the antibiotics. This goes in agreement with earlier studies by Wazait et al. (2003). *P. aeruginosa* was found to be resistant to 7 (70 %) and susceptible to 3 (30 %) of the antibiotics. This is similar to the results of Mazzulli et al. (2001). *P. aeruginosa* maintains antibiotic resistance plasmids and are able to transfer these genes by bacterial processes of transduction and conjugation (Nwanze et al., 2007). *Proteus mirabilis* showed resistance to 5 (50 %) and susceptible to 5 (50 %) of the antibiotics. *Proteus* sp has the ability to produce urease which helps them to survive in the urinary tract, and they have flagella that enable them to swim about their environment and also contribute to their virulence nature (Wilson and Gaido, 2004; Johnson et al., 1999; Braunwald et al., 2001). Antibiotic susceptibility test with Gram-positive bacteria (Table 5), showed that *S. aureus* was found to be resistant to 6 (60 %) and susceptible to 4 (40 %) of the antibiotics. *S. saprophyticus* was also found to be resistant to 6 (60 %) and susceptible to 4 (40 %) of the antibiotics. These findings were similar to those observed by Nkang et al. (2009) but contrary to Bischoff et al. (1999). This might be due to environmental differences. *S. aureus* has been reported to exhibit resistance to beta-lactam antibiotics of which benzyl penicillin is one. Outbreaks of *S. aureus* resistant to beta-lactam antibiotics have been frequently associated with devastating nosocomial infections (Depardieu et al., 2007; Buhlmann et al., 2008; Chikere et al., 2008). Both Gram-negative and positive bacteria were 100 % susceptible to ciprofloxacin and gentamicin as shown in Tables 4 and 5. This result is similar to that reported by Resih et al. (1993) and Okonko et al. (2009). Multidrug resistance was observed for some of the isolates as they were resistant to more than one drug (Wasfy et al., 2000; Akpomie and Akpan, 2013). Drug abuse and indiscriminate misuse of antibiotics among the general population has favoured the emergence of resistant strains.

Table 1: Incidence of Urinary tract infection among male and female diabetics

Sex	No. of diabetics sampled	No. of diabetics with UTI	% of diabetics with UTI
Male	6	3	50
Female	15	6	40

TABLE 2: Bacteria isolated and their percentage occurrence in the samples

Bacteria isolated	Percentage of Isolates
<i>Escherichia coli</i>	44.4%
<i>Proteus mirabilis</i>	22.2%
<i>Pseudomonas aeruginosa</i>	11.1%
<i>Staphylococcus aureus</i>	11.1%
<i>Staphylococcus saprophyticus</i>	11.1%

Table 3: Occurrence of isolated bacteria among male and female patients

Bacterial Isolates	Males	Females
<i>S. aureus</i>	+	—
<i>S. saprophyticus</i>	—	+
<i>E. coli</i>	+	+
<i>Proteus mirabilis</i>	—	+
<i>Pseudomonas aeruginosa</i>	—	+

Key: - = absent; + = present

Table 4: Antibiotic susceptibility pattern of Gram-negative (-ve) bacteria

Gram -ve	AM	AU	CN	PEF	OFX	S	SXT	CH	SP	CPX
<i>P. mirabilis</i>	S	S	S	R	S	R	R	R	R	S
<i>E. coli</i>	R	R	S	R	S	R	R	R	R	S
<i>P. aeruginosa</i>	R	R	S	R	S	R	R	R	R	S

Key: R = Resistant; S = Sensitive; AM= Amoxicillin; AU= Augmentin; CN= Gentamycin; PEF= Pefloxacin; OFX= Ofloxacin; S= Streptomycin; SXT= Septrin; CH= Chloramphenicol; SP= Sparfloxacin; CPX= Ciprofloxacin

Table 5: Antibiotic susceptibility pattern of Gram-positive (+ve) isolates

Gram+ve	AM	R	CPX	S	SXT	E	PEF	CN	APX	Z
<i>S. aureus</i>	R	S	S	R	R	S	R	S	R	R
<i>S. saprophyticus</i>	S	R	S	R	R	S	R	S	R	R

Key: R = Resistant; S = Sensitive; AM= Amoxicillin; R = Rocephin; CN= Gentamycin; PEF= Pefloxacin; APX= Ampiclox; S= Streptomycin; SXT= Septrin; E = Erythromycin; Z = Zinnacef; CPX= Ciprofloxacin

Conclusion

UTI may result as a complication of diabetes and this study confirms that diabetes predisposes humans to the risk of urinary tract infections due to the changes in bladder function and in circulation. The susceptibility and resistance profile of all isolates in this study have shown that ciprofloxacin and gentamicin are the most effective antibiotics of choice. Diabetic patients infected with UTIs should therefore be promptly treated with these antibiotics to prevent development of kidney damage or more serious infections. The findings of this study also confirm that some bacteria, still are resistant to antibiotics and frequently used drugs in many parts of the world.

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