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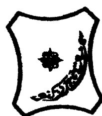

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PREVALENCE OF BACTERIURIA AMONG DIABETIC PATIENTS ATTENDING GENERAL HOSPITAL KATSINA

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ABSTRACT
Diabetes mellitus (DM) is a group of metabolic disorders categorized by hyperglycemia resulting from defects in insulin secretion, body inability to respond to insulin or when there is inadequate secretion of insulin by the pancreas. This study was aimed at determining the prevalence of bacteriuria among diabetic patients attending General Hospital Katsina and course of urinary tract infection in diabetic patients the urine samples of diabetic patients and urinary tract infection patients were collected from the study site between July-October, 2017. One hundred and twenty five (125) samples were processed. Bacterial isolates were identified by standard microbiological procedures. A total of 82 bacteria samples were positive for P. aeruginosa (24.3%), Streptococcus spp (18.3%), Klebsiella spp (22.1%), E. coli (15.8%) and S. aureus (7.3%). Urinary Tract Infections are frequent in patients with diabetes and the most frequent uropathogens are P. aeruginosa, Klebsiella spp, |E. coli, Streptococcus spp and S. aureus. Considering the high prevalence of Asymptomatic Bacteriuria in diabetics, this condition could represent one of the causes of an unexplained worsening of the glycemic control in some patients.
Keyword: Diabetes Mellitus, Bacteria, Urine, Prevalence, Urinary Tract, Infection.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders categorized by hyperglycemia resulting from defects in insulin secretion, body inability to respond to insulin or when there is inadequate secretion of insulin by the pancreas. It has been estimated that about 347million people worldwide suffer from diabetes and Nepal with the mortality rate of 300 (Banadio, 2001*).* The chronic hyperglycemia in diabetes is related with long term damage, dysfunction, and Failure of several organs especially the eyes, genitourinary system, nerves, heart, and blood vessels (Caihong *et al.,* 2012).

Evidence suggested that the incidence of UTI in diabetes patients is four times higher comparing to non-diabetes patients (Banadio, 2001). It has been reported that the occurrence of UTI in diabetic patients were different to the non-diabetic patients (Adeyeba *et al*., 2007). Urinary tract infections (UTIs) are some of the most common infections experienced by humans surpassed in frequency among ambulatory patents only by respiratory and older men are the most susceptible to UTIs and they usually have weaken immune system. Every woman has a 60% lifetime risk of developing bacterial cystitis that develops before the age of 24. By contrast, men have a lifetime risk of only 13% (Caihong *et al*., 2008). In children approximately 5% of girls and 1% of boys have a UTI by 11 years of age (Banadio*,* 2006). It is also the most common cause of nosocomial infections in adults. Urinary tract infection is said to exist when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney, or prostate with or without the presence of specific symptoms (Caihong *et al*., 2012).

The vast majority of uncompilicated UTIs are caused by the Gram negative Bacillus spp; *Escherichia coli*, with other pathogens including *Enterococcus* spp, *Staphylococcus Saprophyticus, Klebsiella* Spp and *Proteus mirabilis* (Adeyeba *et al*., 2007). The wide spread and unsuitable use of antimicrobial agents has invariably resulted in the development of antibiotic resistance which in recent years has become a major problem worldwide (Caihong *et al*., 2012).

Most patients with diabetes usually develop cytopathynrphrophaty and renal papillary necrosis, complications which may predispose them to urinary tract infection. Long term effects of diabetic had developed a serious damage to human especially the female recorded about 30% of diabetic patients. Complicated UTIs in patients who have diabetes include renal and perirenalanscesses, dysuria and hematuria (Adeyeba *et al*., 2007). The aim of this study is to determine the prevalence of bacteriuria among diabetic patients attending general Hospital Katsina.

MATERIALS AND METHODS

Study area

The samples were collected from patients attending Katsina state General Hospital, along Muhammad Dikko road Katsina State.

Study duration

The study was conducted from the month of July to October, 2017.

Sample Size

A minimum sample size of 125 was used. Using prevalence rate of 8.8% It was calculated to be 125 at 95% confidence level.

Samples Collection

A total of 125 urine samples of diabetic patients were collected in a sterile universal bottles and transferred to microbiology laboratory of Umaru Musa Yar'adua University Katsina. The patients were given a sterile, dry, wide-necked leak proof container and requested to provide early morning clean catch mid-stream urine. The urine sample were then immediately transferred to the laboratory. The urine samples were then immediately transferred to the laboratory for analysis.

Sample Analysis

The method of Cheesbrough (2009) was adopted in carrying out the analysis of the urine sample. Urine specimens were collected and examined macroscopically for the appearance and microscopically for the presence of pus cells, casts as well as epithelial cells which signifies a urinary tract infection. Urine specimens were then inoculated on Blood Agar, Mac Conkey Agar and Cystein Lactose Electrolyte Deficient (CLED) Agar and Manitol Salt Agar and incubated at 37°C for 24 hours under aerobic condition. After 24 hours of incubation, the culture plates were examined macroscopically and their appearance, size, color and morphology of the colonies and bacterial isolates were examined using standard biological procedures including Gram staining and biochemical tests.

Macroscopic Analysis of the urine sample

The urine sample was examined macroscopically for its appearance (Clearity and Turbidity).

Microscopic Analysis of the Urine Sample

Ten ml of well mixed urine was aseptically transferred into a centrifuge tube and centrifuged for 5 minutes. After being centrifuged the supernatant was poured away and the pellet was placed on a clean grease-free glass slide, covered with a cover slip and viewed under 10 X and 40 X objective lenses with the condenser iris closed sufficiently to give a good contrast (Cheesbrough, 2006).

Culturing of the urine sample

Blood Agar, Cystein Lactose Electrolyte deficient (CLED) agar, MacConkey Agar and Chocolate Agar were prepared based on the manufacturers direction each and was poured into a sterile petri dishes aseptically near to a burning flame and allowed to cool. A loop full of the urine was inoculated onto the culture media by the use of a sterile wire-loop. The inoculated culture media was incubated at 37°C in the incubator for 24 h in an inverted format.

Macroscopic Examination of Culture Plates

After 24 h the culture plates were examined macroscopically for growth of the microorganisms and the characteristics of each colon was noted for further analysis.

Gram staining of the microorganisms

A discrete colony was smeared on a clean grease free glass slide by the use of a wire loop and then allowed to air dry before heat fixing. The heat fixed bacterial smear was floored with crystal violet stain for 1minute and washed with water after which iodine was applied for another 1 minute and washed with water. Acetone was flooded on the smear and quickly washed with water. Finally the smear was counterstained with safranin for 30 seconds and washed with water. Excess water along the edges of the glass slide were blotted and allowed to air dry on a rack. The glass slide was then examined under the microscope for possible results (Acharya, 2013)

Biochemical identification

In order to confirm the identity of bacteria, routine conventional biochemical tests were carried out to further ascertain the organism. Such include:

Catalase test

Few drop of hydrogen peroxide solution were poured into glass slide. Using a sterile wooden stick several colonies of the test organism was removed and immersed in the hydrogen peroxide solution. Bubble were being observed for (Cheesbrough, 2006).

Citrate test using Simmom's Citrate agar.

Slopes of the medium were prepared in test tubes. Using a sterile wooden stick several colonies of the test organism was removed and immersed in the hydrogen peroxide solution. Bubble were being observed for (Cheesbrough, 2006).

Coagulase test

A drop of distilled water was placed on a slide. A colony of the test organism was emulsified on the drop to make a thick suspension. A loopful of plasma was added and gently mixed. Clumping was observed for within 10 seconds (Cheesbrough, 2006).

Indole test using Tryptone Water

The test organism was inoculated in a test tube containing 4ml of sterile peptone water. It was incubated at 37°C for 24hours kovac's Indole reagent (0.5ml) was added and gently shaken. A

red color was observed for in the surface layer within 10 minutes (Cheesbrough, 2006).

Motility

The triple iron agar was prepared in a test tube. After sterilization the isolate was stabbed into motility media with inoculating needle and incubated at 35°C for 24 hours. Motility of the bacterium was identified by the presence of growth going out away from the stab line and test is positive. If bacterium is not motile , there will only be growth along the stab line (Acharya, 2013).

Methyl red test

The MR-VP broth was prepared in a test tube. After sterilization, a loop full of colonies was inoculated in to the tube. After stwas incubated for 5 days at 37°C. After the incubation periods 5 drops of methyl-red reagent (for methyl red test) and Barrits reagent (for voges-proskauer) were added in each of the tube. Change of color was observed for pink and yellow color indicates positive and negative result respectively for methyl red test and vice-versa for vogesproskauer test (Acharya, 2013).

Urease test using christensen's urease broth

The test organism were heavily inoculated in a bijou bottle containing 3 ml sterile christensen's urea broth. It was incubated at 35°C for 12 hours. A pink color in the medium was observed for (Cheesbrough, 2006).

RESULTS

Table 1 highlighted the physical appearance of the suspected organisms and Table 2 illustrate the biochemical reactions of the suspected organisms are used to confirm the identified bacteria. Table 3 indicated that, the results of urine culture analysis indicated that 65.6% were recorded with positive growth, 28% negative growth and 6.4% with no growth. Table 4.4 showed the identity of bacteria isolated from significant growth. *Pseudomonas aeruginosa* were found to be the most prevalent organism (24.3%) followed by *Streptococcus* species (18.3%) and the least was *Staphylococcus aureus* (7.3%).

Out of 125 sample examined, 82 (65%) emerged positive and 43 (34.4%) emerged negative. Therefore the prevalence of bacteriuria in this study was 65.6% while Table 6 showed the percentage of bacteriuria in relation to sex of the study subjects which reveal that female patient has the highest prevalence with 55 (44%) while male with the lowest rate of 27 (21.6%).

Table 1: Colony Morphology, Microscopy and Gram Stain of the Suspected Bacteria.

Media (Agar)	Colony morphology	Microscopy	Gram stain	inference
MSA	Yellow colonies with zones	Cocci, adhere in clusters	Positive	*S.aureus*
EMB	Green metallic sheen colonies	Rod-shaped, arranged singly	Negative	*E.coli*
MacConkey	Pale yellow colonies	Small rod-shaped cells	Negative	*P. aeruginosa*
Blood	Alpha hemolysis	Coccus in chains	Positive	*Streptococcus*
CLED	Golden yellow colonies	Coccus in chains	Positive	*C.N Staphyloccus*
MacConkey	Pinkish mucoid	Road-shaped	Negative	*Klebseilla* spp

Key: MSA: Mannitol Salt Agar EMB= Eosin Methylene Blue Agar
CLED= Cysteine Lactose Electrolyte Deficient Agar C.N= Coagulase Negative.

Table 2: Biochemical reaction of the Organisms

Organism	Motility	Cat	Cit	Coa	Ind	MR	Urease
Pseudomonas	Motile (unipolar)	+	+	-	-	-	-
Klebseilla spp	Non motile	+	+	-	-	-	+
E.coli	Motile	+	-	-	+	+	-
Streptococcus spp	Non motile	+	+	-	-	-	-
S. aureus	Non motile	+	+	+	-	-	-
C.S *Streptococcus*	Non motile	-	-	-	-	-	+

Key: Cat-Catalase test, Ind- Indole Test, Cit- Citrate Test, Coa- Cagulase Test, C.N- Coagulase Negative, MR- methyl-red Test, Urease- Urease Test

Table 3 : Urine Culture Analysis

Cases	Number	(%)
Positive Growth	82	65.6
Negative Growth	35	28.0
No Growth	8	6.4
Total	125	100

Table 4: Occurrence of Bacteria Isolated From Significant Urine Samples

Bacteria	Number Isolated	(%)
Staphylococcus aureus	6	7.3
C.N Staphylococcus	10	12.1
Klebsiella species	18	22.1
Escherichia coli	13	15.8
Streptococccus species	15	18.3
Pseudomonas aeruginosa	20	24.3
Total	82	99.9

Keys : C.N.S: Coagulase negative staphylococcus

Table 5: Prevalence of Bacteriuria among Diabetic Patients

Total Number Examined	Number of Positive result	(%)	Number of Negative Result	(%)
125	82	65.6	4.3	34.4

Table 6: Percentage of Bacteriuria in relation to sex of study subjects

Gender	Total number examined	Number of positive result	(%)	Number of negative result	(%)
Female	74	55	44	19	15.2
Male	51	27	21.6	24	19.2
Total	125	82	65.6	43	34.4

DISCUSSION

The following organisms, *Staphylococcus aureus, C.N Staphylococcus, Klebsiella* species, *Escherichia coli, Streptococccus* species and *Pseudomonas aeruginosa* are the prominent in urine sample of diabetic patients as well as the leading cause of urinary tract infection among the examined patients. This is a fact because of the accumulated sugar within the mention patients is always high and tt is generally assumed that, glucosuria is one of the most important reasons for the high prevalence of urinary tract infections in diabetics (Boyoko *et al*., 2002).

From the organisms isolated Gram positive bacteria with 82 (65.6 %) recorded the highest prevalence compared with Gram negative bacteria 35 (28.0 %), this indicated the level of pathogenicity associated with the diabetic patients.

In terms of pathogenic distribution, *P. aeruginosa* (24.3%) was the most commonly isolated pathogen with high percentage which is unusual to other findings that reported mainly lesser prevalence ranging from 12% to 22% (Bonadio *et al*., 2006).

This study shows that majority (65.6%) of diabetic patients had UTI, which is in good agreement to the finding that has been reported by Tahir N, showing prevalence of UTI to be 66.4% in diabetic population in Pakistan.

CONCLUSION

The result obtained in this study indicated that, most of the diabetic patients attending general hospital katsina, are having urinary tract infections (UTI) due to the number of number of UTI pathogens isolated from their various samples. The most prevalence UTI pathogen isolated in this study is *P. aeruginosa* and was believed to be the most commong pathogen causing UTI in patient attending general hospital katsina.

RECOMMENDATIONS

Based on the results of this study the following recommendations should be considered.

1. Screening and treating bacteriuria should be implemented in antenatal irrespective of educational background.
2. Continued surveillance of morbidity and mortality rates among uropathogens is needed to ensure appropriate recommendations for the treatment of these infections.
3. Further research is needed to validate the result with the implementation of possible ways of treating such infection.

REFERENCES

Acharya A, Gautam R, and Subedee L (2013) Uropathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal. *Nepal Med Col J*. (1): 30-33

Adeyeba OA, Adesiji YO, and Omosigho PO (2007) Bacterial urinary tract infections in patients with diabetes mellitus. *Int J Trop Med*. :89-92.

Bonadio M, Costarelli S, Morelli G, and Tartaglia T. (2006). The influence of diabetes mellitus on the spectrum of uropathogens and the antimicrobial resistance in elderly adult patients with urinary tract infections. *BMC Infect Dis* 6:54.

Bonadio M, Meini M, Spetaleri P, and Gilgi C. (2001). Current Microbiological and clinical aspects of urinary tract infections. *Eur J Uro* ;40: 439-45

Boyoko EJ, fihn SD, Scholes D, Chen CL, Normand EH, and Yarbo P. (2002). Diabetes and Risk of acute urinary tract infection among post-menopausal women. *Diabetes care* 25(10): 1778-83.

Caihong W G R. M, Jane W. S, Qunyuan Z, Kn C. Herbert W. V. and Indira U. (2012). Mysorekar "Atg16L1 deficiency confers protection from Uro pathogenic *Escherichia coli. Infection in vivo*" May 22, 2012.

Cheebrough, M, (2006). Medical Laboratory manual for tropical countries. 2nd ed. England:Butterworth- Heineman Ltd P 201

Cheesbrough, M, (2000). District Laboratory Practice in Tropica Countries. Cambridge United Press, U.K. Part 27: 105.

Cheesbrough, M. (2009). District Laboratory practice in tropical countries, second edition in New York in USA, Chapter 7, P 132-304.