Volume : 5 | Issue : 2 | February 2016 • ISSN No 2277 - 8179

Research Paper

Bacteriological Profile and Antibiotic Susceptibility Pattern of Urinary Tract Infection in Diabetic Patients

Medical Science

KEYWORDS : UTI, Diabetes , Antimicrobial Susceptibility

Dr. Supriya Gambhir	PG Student (3rd year) , Dept. of Microbiology, Bharati Vidyapeeth deemed university medical college , Pune.
Dr .(Mrs) Bharati A.Dalal	Associate Professor , Dept. of Microbiology, Bharati Vidyapeeth deemed university medical college , Pune.
Dr . (Mrs) Meera Modak	Professor , Dept. of Microbiology, Bharati Vidyapeeth deemed university medical college , Pune.
Dr. K. K.Lahiri	Professor and H.O.D , Dept. of Microbiology, Bharati Vidyapeeth deemed university medical college, Pune.

ABSTRACT

Background : The risk of developing infections in diabetic patients is higher and the urinary tract is the most common site of infection. Our goal was to isolate and identify the organisms causing UTI in diabetes Type II patients and determine their antibiotic susceptibility pattern. Material and methods: The Cross sectional study was undertaken from Nov, 2013- Nov, 2014 . Data regarding the patients was recorded in a specific questionnaire. Identification of urinary isolates was done by microscopy, Culture methods, Biochemicals, Antibiotic Susceptibility test. Result: Of the 150 diabetic patients investigated, 84 samples showed significant growth. Of the total 95 isolates,the predominant organisms were :Escherichia coli - 46 (48.42 %) , followed by Klebsiella pneumoniae - 13 (13.68 %) , Pseudomonas aeruginosa - 12(12.63 %), Methicillin Resistant Staphylococcus aureus (MRSA) - 6 (6.32 %),e.t.c.We observed 70% resistant organisms to common antibiotics such as Ampicillin, Norfloxacin, Ceftriaxone, Ciprofloxacin and Levofloxacin. However, most were sensitive to Carbepenems and Piperacillin plus Tazobactam. Conclusion:This study will provide information for the selection of antimicrobial agents for treating such patients

INTRODUCTION:

Diabetes Mellitus is a complete metabolic syndrome caused by the lack of insulin resulting in inappropriate high blood glucose levels[1]The incidence of Diabetes Mellitus throughout the world is increasing strikingly and becoming a public health problem especially in developing countries.[1]

An association between Urinary Tract Infection and Diabetes Mellitus was noted in an autopsy series reported in 1940s[2] The urinary tract is the principal site of infection in diabetics [2] with increased risk of complications.

The mechanisms which potentially contribute to Urinary Tract Infection in diabetic patients are defects in local urinary cytokine secretions (IL-6,IL-8), increased adherence of microorganisms to uro-epithelial cells, partly due to changed and lowered Tamm-Horsfall protein, and granulocyte dysfunction possibly as a result of an abnormal intracellular calcium metabolism. On the other hand, hyperglycemia facilitates the colonization and growth of variety of organisms.[3,4] Also, the presence of diabetic cystopathy and microvascular disease in the kidney may play a role in the higher incidence of Urinary Tract Infection in the patients.[2,5]

Therefore, the successful management of diabetic patients suffering from Urinary Tract Infection depends on the identification of the type of organisms that cause the disease and the selection of effective antibiotic against them. The emergence of resistant bacterial strains in hospitals poses a continued challenge to treat and control spread of infections[5, 6] Although the infection seldom leads to complications, it can cause significant morbidity and mortality.[5]

There is a paucity of research addressing the etiologies, risk factors and management of Urinary Tract Infection in Diabetic patients in most developing countries[5] including INDIA. This study intends to address some of these issues. The focus will be on identifying type of bacteria and their antibiotic susceptibility pattern in diabetic patients with Urinary Tract Infection attending a tertiary care centre in Pune, India. Thus, the data in the study will provide information to the clinicians on the selection of antimicrobial agents for the treatment of these patients and will be helpful in developing Antibiotic Policy for the Type 2 Diabetic patients with Urinary Tract Infection.

AIM: To determine the bacteriological profile and antibiotic susceptibility pattern of urinary tract infection in diabetic patients attending a tertiary care centre in Pune.

MATERIAL AND METHODS :

The present study was carried out in the Department of Microbiology, Bharati Vidyapeeth hospital, Pune ,from November 2013 – Oct ober 2014. In this cross sectional type of study, total 150 clinically diagnosed cases of Diabetes Mellitus Type 2 were taken.

Cases were selected on the basis of the following criteria:

Inclusion Criteria:

All Indoor and Outdoor Adult(>18 years) Type 2 Diabetic patients attending tertiary care hospital.

Exclusion criteria:

Diabetic patients on antibiotic for the last two weeks

Pregnant women

Specimen Collection[2,3,5,7,8] Data regarding age, sex, type and duration of diabetes, signs and symptoms of urinary tract infection, blood sugar levels were recorded in the specific questionnaire form. Patients were also enquired regarding any past history of similar complaints and the duration of antibiotic treatment.

After the history, the patients were instructed to collect 'clean catch mid- stream' specimen

Processing of urine sample

1. Culture:[1,2,3,5,7,9,10]

Urine samples were inoculated on Blood agar plate and MacConkey agar plate by a semi-quantitative method of cultureusing a standard wire loop of internal diameter of 4 mm which carried0.01 ml of urine .The plates were incubated overnight at 37°C.

2.Direct microscopy :[1,2,5,7,9]

After the sample had been cultured, a drop of centrifuged urine was examined microscopically for the presence of leucocytes The criterion for significant pyuria was determined to be ≥ 10 pus cells / High power field.

3.Colony count [11]

This was established by counting the number of colonies and expressing the same in terms of Colony Forming Units per mL (CFU/mL)after multiplying with 100.

A diagnosis of Urinary Tract Infection was made if the urine cultures had $> 10^3$ to $> 10^5$colony forming units (CFUs)/mL of a single potential pathogen or two potential pathogens.

A pure culture of Staphylococcus aureus was considered to be significant regardless of the number of CFUs.

The pure culture of yeast in any number was also considered to be significant .

4.Identification of isolates:[1,2,7,9,10,12,13]

All bacterial isolates were identified by their colonial characteristics, gram stained appearances and a battery of standard biochemical tests.

5.Antimicrobial susceptibility[1,2,3,5,6,9,10,12]**:**

All aerobic bacterial isolates were subjected to Antimicrobial susceptibility by the Kirby Bauer'sdisc diffusion method on Muller-Hinton agar (Hi Media Pvt. Ltd, Mumbai, India)according to the CLSI guidelines .

Escherichia coli(ATCC®25922),*Staphylococcus aureus*(ATCC® 25923)and *Pseudomonas aeruginosa*(ATCC®27853)were used as reference strains.[14,15]

DATA ANALYSIS:

The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

Results were presented in a suitable tabular and graphical form.

RESULTS:

Out of the 150 urine samples from diabetic patients, growth was obtained in 84 cases. Therefore, the overall culture positivity i.e Prevalence in the cases under study was 56 %. Of the total 150 diabetic patients, 76 were male (50.67%) and 74 (49.33 %) were female patients.Of the 74 female diabetic patients studied , the urine sample of 45 (60.81 %)were culture positive Similarly, the culture positivity of 76 male diabetic patients was 39

(51.32 %). Total number of 150 cases were distributed between the range of 40 - 90 years. Though majority patients belonged to the age group of 61-70 years of age, the urine culture positivity was maximum in patients belonging to age group of more than 71 years old i.e 22 out of 30cases (73.33 %) patients showed significant growth in their urine sample , followed by patients of age group 40- 50 years with culture positivity of 68 % . Of the 150 diabetic patients, 90 (60 %) patients had RBS more than 200 mg/dL.Of these 90 patients, the urine sample of 58 (64.44%) were culture positive, i.e it showed significant growth in their urine sample .Similarly, 60 (40 %) diabetic patients had RBS less than 200 mg/dL. Of these 60 patients, the urine sample of 26 (43.33 %) were culture positive. Of the total 150 patients, 75 (50 %) patients were Asymptomatic. The urine sample of 34 (45.33%) of them were culture positive.Of the remaining 75 (50 %) Symptomatic patients, the urine sample of 50 patients (66.67%) were culture positive.Therefore, the patients having Asymptomatic UTI were 45.33 % while those with Symptomatic UTI were 66.67 %.Out of the total 95 organisms isolated, the most common - 77 (81.05%) were Gram Negative isolates, followed by 12 (12.63 %) Gram positive and 6(6.32%) *Candida species* isolates. Of the total 95 isolates, the predominant organisms are listed in Table 1 .Of the total 84 Culture positive cases, a single isolate was obtained in 73 (86.90%) cases and polymicrobial flora was seen in 11 (13.10 %) cases.

Table 1 : Distribution of organisms isolated in the cases under study

Organism isolated	Number	Percentage
Total GNB	77	81.05
Escherichia coli	46	48.42
Klebsiella pneumoniae	13	13.68
Pseudomonas aeruginosa	12	12.63
Citrobacter species	3	3.16
Acinetobacter baumanii	1	1.05
Proteus vulgaris	1	1.05
Serratia marcescens	1	1.05
Total GPC	12	12.63
MRSA	6	6.32
Enterococcus species	3	3.16
MSSA	2	2.11
CONS	1	1.05
Total Fungii	6	6.32
Non albicans candida	4	4.21
Candida albicans	2	2.11
Total isolates	95	100

Antimicrobial susceptibility

Gram Negative : The isolates of *Escherichia coli* showed high resistance to all Cephalosporins (91- 95 %) except Cefepime (82.61 %), followed by Amoxicillin- Clavulanic acid (84.78 %) and ciprofloxacin (82.61 %).Whereas least resistance was noted for Colistin, Polymyxin –B (0 %), followed by Imipenem (8.70 %), Nitrofurantoin, Amikacin (17.39 %) and Gentamycin(23.91 %).The isolates of *Klebsiella pneumoniae* showed a high rate of resistance to Piperacillin (76.92 %) followed by Cephalosporins (61- 69 %) which showed a moderate rate of resistance. The least resistance was shown towards Imipenem, Colistin, Polymyxin – B (0 %), Amikacin(38.46 %) and Cotrimoxazole (46.15%). The antimicrobial resistance pattern of other gram negative isolates is mentioned in Table 2. Gram Positive organism : *MRSA* isolates showed maximum resistance to Penicillin (100%), followed by Tetracycline and Norfloxacin (83.33%). Least resistance was seen to Chloramphenicol, Clindamycin, Gentamycin,Levofloxacin (33. 33%) and Nitrofurantoin (16.67 %)Whereas, *MSSA* isolates were 100 % resistant to Penicillin and Nitrofurantoin.All *MRSA* and *MSSA* isolates were 100 % sensitive to Linezolid, Vancomycin,Teicoplanin and Cotrimoxazole . [Table 3]

Table 2 : Percentage of Antibiotic Resistance pattern of Gram negative Isolates

ISOLATES ANTIBIOTICS	*E.coli (n=46)*	K.pneumoniae (n=13)	P.aeruginosa (n=12)	Citrobacter spp. (n=03)	A.baumannii (n=01)	P.vulgaris (n=01)	S.marcescens (n=01)
Piperacillin	80.43	76.92	91.67	100	100	0	100
Ampi- sulb	71.74	69.23	91.67	100	100	0	100
Amox-clav	84.78	69.23	100.00	100	100	0	100
Pip- tazo	43.48	61.54	41.67	33.33	100	0	100
Cephalothin	95.65	69.23	100.00	100	100	0	100
Cefuroxime	95.65	69.23	100.00	100	100	0	100
Ceftazidime	91.30	61.54	91.67	66.67	100	0	100
Cefepime	82.61	69.23	100.00	100	100	0	100
Imipenem	8.70	00.00	41.67	33.33	0	0	100
Cotrimox	56.52	46.15	83.33	66.67	100	0	0
Gentamycin	23.91	53.85	75.00	66.67	100	0	100
Amikacin	21.74	38.46	75.00	100	100	0	100
Norflox	78.26	61.54	83.33	100	100	0	100
Ciproflox	82.61	61.54	83.33	100	100	0	0
Nitrofurantoin	17.39	61.54	83.33	66.67	100	0	100
Colistin	0	0.00	8.33	33.33	0	100	100
Poly –B	0	0.00	8.33	33.33	0	100	100

Table 3 : Percentage of Antibiotic Resistance pattern of Gram positive Isolates among the cases under study

ISOLATES ANTIBIOTICS	MSSA (n=2)	MRSA (n = 6)	CONS (n =1)	*Enterococcus species (n =3)*
Penicillin	100	100	100	100
Cefoxitin	0	100	100	100
Vancomycin	0	0	0	0
Teicoplanin	0	0	0	0
Chloramphenicol	0	33.33	100	33.33
Clindamycin	0	33.33	100	100
Gentamycin	0	33.33	100	33.33
Tetracycline	0	83.33	100	33.33
Levofloxacin	0	33.33	100	100
Norfloxacin	0	83.33	100	100
Nitrofurantoin	100	16.67	100	66.67
Cotrimoxazole	0	0	0	100
Linezolid	0	0	0	0

DISCUSSION :

In the present study, out of 150 clinically diagnosed diabetic patients, prevalence of Urinary tract Infection was 56%.The study conducted by Jha P K *et al* (2014)[16] shows a prevalence rate of 54.76 % which is comparable with our study.Nevertheless the aforementioned studies have not specified / have included both Type 1 and Type 2 diabetic patients as the study subjects.The studies conducted by Janifer J *et al* (2009)[2] and Saber M H *et al* (2010)[3]have studied the prevalence of UTI in patients with Type 2 Diabetes Mellitus, which is similar to study conducted by us. In our study, Of the 150 diabetic patients, 90 (60 %) patients had RBS more than 200 mg/dL .Of these 90 patients, the urine sample of 58 (64.44%) were culture positive, i.e the samples showed significant growth.Similarly, 60 (40 %) diabetic patients had RBS less than 200 mg/dL. Of these 60 patients, the urine sample of 26 (43.33 %) were culture positiveIt is documented by Bettegowda *et al* (2014) [17] that poor glycaemic control increases the susceptibility of infections in patients withType 2 DiabetesMellitus.

As also studied by Fünfstück R*et al* (2012)[18], improper metabolic control acts as a general host factor enhancing the risk for urinary tract infection in diabetics.These studies support the finding observed in our study where the percentage of UTI was higher (64.44%) in patients with random blood sugar levels over 200 mg/dL than those patients having a blood sugar level less than 200 mg/dL (43.33 %).There is also a significant dimunition in the intracellular bactericidal activity of leucocytes with *Staphylococcus aureus* and *Escherichia coli* in patients with poorly controlled diabetes. [19]Therefore, regular blood glucose monitoring is recommended to aid in the day to day management of diabetes. [19] In the present study, 95 organisms were isolated from 150 patients. Among the 95 isolates,77 were Gram negative isolates,12 were Gram positive isolates and 6 were Candida species. Therefore, the Gram Negative organisms were the predominant isolates and *Escherichia coli* was the most frequent uropathogen isolated (48.42 %) amongst them.This corroborates the findings of Sharma V *et al* (2012)[20], Saber M H *et al*[3], Pargavi B *et al*[9]Prakash R *et al* (2015)[21],Janifer *et al* (2009) [2] who have also reported the Gram negative organisms to be the predominant isolates in the urinary samples of Diabetic patients. Candidial infections are asymptomatic , but may lead to Cystitis, Pyelonephritis, Renal abscesses, Fungal ball formationand renal candidiasis requires a more aggressive approach including irrigation of renal pelvis with antifungal drugs, oral or parenteral, and sometimes surgical intervention. Amphotericin B or Flucytosine is the treatment of choice. Initial antibiotic selection must account for a variety of host, microbiologic and pharmacological factors. Tailoring antimicrobial therapy based upon culture and sensitivity results will help reduce cost and minimize the emergence of resistance and morbidity.In our study, *E. coli* showed high resistance to Amoxicillin – Clavulanic acid(84.78 %), Cephalosporins (82 – 95 %), and Ciprofloxacin (82. 61 %), while low resistance was shown to Imipenem (8.70 %), Nitrofurantoin (17.39 %), Amikacin (21.74 %) and Gentamycin (23.29 %).The study by Jha P K *et al* (2014)[16] shows that *E.coli* had a high resistance to Cephalosporins and the least number of resistant strains were seen for Amikacin and Gentamycin, which is a similar finding to that seen in our study.In the study by *Shill M C et al* (2010) [1], *E. coli* showed high resistance with Ciprofloxacin, moderate resistance with Cephalosporins and least was seen towards Gentamycin , Amikacin and Nitrofurantoin as seen in our study.The

study by Sharma V *et al*(2012) [20] also support the results of our study. In their study, *E.coli*showed high resistance to Ciprofloxacin (71. 4%) and very less resistance was seen towards Amikacin(8.1%)In our study, *Klebsiella pneumoniae* showed moderate resistance to Amoxillin-Clavulanic acid (84.78%), Cephalosporins (61- 70 %) and Ciprofloxacin (61.54 %) and low resistance was shown to Imipenem (0%),Meropenem (15.38 %) and Amikacin (38.46 %).The study by Jha P K *et al* (2014)[16] shows that *Klebsiella pneumonia* had a high resistance to Cephalosporins and the least number of resistant strains were seen for Amikacin and Gentamycin, which is a similar finding to that seen in our study. In the study by Gizachew Yismaw *et al*(2012) [5], *S. aureus*was resistant to penicillin (100%) as.It is an established fact most *S. aureus*strains produce pencillinase and alternative penicillin binding proteins (PBP-2A) helps the organisms to become resistant to most beta lactam antibiotics .

In the study by Sharma V *et al* (2012)[20],*Staphylococcus aureus* was 100% sensitive to Linezolid which is a finding comparable to our study.

Chloramphenicol, Gentamycin and Tetracycline (33.33% each) and was 100% sensitive to Linezolid, Vancomycin and Teicoplanin. The high rates of antibiotic resistance observed in the present study may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogens. The knowledge of the usual causative organism in Urinary Tract Infection in diabetics and their antibiotic susceptibilities will allow clinicians to make informed choices.

CONCLUSION :

This study portrayed that the prevalence of Urinary tract infection among the diabetic patients was considerably high. As a complication of diabetes, UTI may be preventable with better glucose control and unnecessary use of antimicrobials. Gram negative organisms were the most common isolates from this group of patients; among which *Escherichia coli* was the principal urinary pathogen.

REFERENCE

1. Shill, M. (2010). Prevalence of Uropathogens in Diabetic Patients and Their Corresponding Resistance Pattern: Results of a Survey Conducted at Diagnostic Centers in Dhaka, Bangladesh. Oman Medical Journal. http://dx.doi.org/10.5001/omj.2010.82 | | 2. Viswanathan, V., Janifer, J., Geethalakshmi, S., & Satyavani, K. (2009). Prevalence of lower urinary tract infection in South Indian type 2 diabetic subjects. Indian J Nephrol, 19(3), 107. http://dx.doi.org/10.4103/0971-4065.57107 | | | 3. Saber, M., Barai, L., Haq, J., Alam Jilani, M., & Begum, J. (2011). The Pattern of Organism Causing Urinary Tract Infection in Diabetic and Non Diabetic Patients in Bangladesh. Bangladesh J Med Microbiol, 4(1). http://dx.doi.org/10.3329/bjmm.v4i1.8461 | | 4. HOEPELMAN, A. (2003). Pathogenesis and management of bacterial urinary tract infections in adult patients with diabetes mellitus. International Journal Of Antimicrobial Agents, 22, 35-43. http://dx.doi.org/10.1016/s0924-8579(03)00234-6 | | 5. Yismaw, G., Asrat, D., Woldeamanuel, Y., & Unakal, C. (2012). Urinary Tract Infection: Bacterial etiologies, drug resistance profile and associated risk factors in diabetic patients attending Gondhar university hospital. Gondar, Ethiopia. European Journal Of Experimental Biology, 2(4), 889 -898. Retrieved from http://European Journal of Experimental Biology | | 6. Singhai, M., Kumar, A., Jha, P., Goyal, R., & Rawat, V. (2012). Bacteriological and resistance profile in isolates from diabetic patients. North American Journal Of Medical Sciences, 4(11), 563. http://dx.doi.org/10.4103/1947-2714.103315 | | 7. Bonadio, M., Costarelli, S., Morelli, G., & Tartaglia, T. (2006). The influence of diabetes mellitus on the spectrum of uropathogens and the antimicrobial resistance in elderly adult patients with urinary tract infection. BMC Infectious Diseases, 6(54). http://dx.doi.org/10.1186/1471-2334-6-54 | | | 8. Uddin, T. (2009). Frequency of urinary tract infection in Diabetic females. KUST Medical Journal Medical Journal Medical Journal Medical, 1(2), 55-58. Retrieved from http://www.kmuj.kmu.edu.pk/article/viewFile/4665/3765 | | 9. Pargavi, B., Mekala, T., Selvi, A., & Moorthy, T. (2011). Prevalence of Urinary Tract infection in Diabetic patiens in Vandasvi,Tamilnadu,India. International Journal Of Biological Technology, 2(2), 42- 45. Retrieved from http://www.gayathripublishers.com/ijbt%20volume%20no%202%282%29/ijbtaug1511020207.pdf | | | 10. Collee, J., Fraser, A., Marmion, B., & Simmons, A. (2006). Mackie and McCartney Practical Medical microbiology. (14th ed.). Elsevier Health Sciences. | | 11. Forbes, B. (2007). Study guide for Bailey and Scott's diagnostic microbiology. St. Louis: Mosby Elsevier. | | | 12. Anathanarayan, & paniker,. (2013). Anathanarayan & Paniker's Textbook of Microbiology (9th ed., p. 672). Universities Press. | | 13. Cheesbrough, M. (2005). District laboratory practice in tropical countries. Cambridge: Cambridge University Press. | | 14. Bauer, A., Kirby, W., Sherris, J., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, 45(4), 493-496. | | 15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. (2011). Wayne, Pennsylvania. | | | | 16. Jha, P., Baral, R., & Khanal, B. (2014). International Journal of Biomedical Laboratory Science (IJBLS) 2014 Vo1. 3, No. 2:29-34 .Prevalence of Uropathogens in Diabetic Patients and Their Susceptibility Pattern at a Tertiary Care Center in Nepal-A Retrospective Stud. International Journal Of Biomedical Laboratory Science, 3(2), 29 - 34. Retrieved from http://www.ijbls.org/upfile/Issues/2015126102540.pdf | | 17. Betegowda, S., Iyengar, V., Gosain, V., Naik, V., Vuyyuru, S., & Bandreddi, G. (2014). Clinicalprofile and spectrum of Infections in Type 2 diabetes Mellitus patients: A retrospective study from Rural Tertiary Care hospital of south Karnataka, India. Scholars Journal Of Applied Medical Sciences, 2(6G), 3331-3336. Retrieved from http://saspublisher.com/wp-content/uploads/2014/11/SJAMS-26G3331-3336.pdf | | | 18. Fünfstück, R., Nicolle, L., Hanefeld, M., & Naber, K. (2012). Urinary tract infection in patients with diabetes mellitus. CN, 77(01), 40-48. http://dx.doi.org/10.5414/cn107216 | | 19. Nathan, D., Singer, D., Hurxthal, K., & Goodson, J. (1984). The Clinical Information Value of the Glycosylated Hemoglobin Assay. New England Journal Of Medicine, 310(6), 341-346. http://dx.doi.org/10.1056/nejm198402093100602 | | | 20. Sharma, V., Gupta, V., & Mittal, M. (2012). Prevalence Of Uropathogens In Diabetic Patients And Their Antimicrobial Susceptibility Pattern. National Journal Of Laboratory Medicine., 1(1), 26 - 28. Retrieved from http://njlm.net/articles/PDF/1942/7-4332.pdf | | 21. Pal, S., Sharma, N., Adekhandi, S., Juyal, D., Gaurav, V., & Prakash, R. (2014). Diversity of uropathogens and their resistogram in diabetic and non-diabetic patients in sub Himalayan region of Uttarakhand, India: A case control study. International Journal Of Medicine And Public Health, 4(1), 115. http://dx.doi.org/10.4103/2230-8598.127171 |