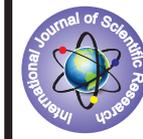


Bacteriological profile and antimicrobial susceptibility testing of isolates in urine of asymptomatic diabetic patients at tertiary care hospital



Microbiology

KEYWORDS: Asymptomatic bacteriuria, E.coli, Diabetes mellitus (DM).

DABHI C S	Resident Doctor, Department of Microbiology, B.J. Medical College, Ahmedabad, India
PATEL V J	Resident Doctor, Department of Microbiology, B.J. Medical College, Ahmedabad, India
KHANDELWAL N A	Professor, Department of Microbiology, B.J. Medical College, Ahmedabad, India
VEGAD M M	Professor & Head of Department of Microbiology, B.J. Medical College, Ahmedabad, India
SHRIVASTAV M S	Resident Doctor, Department of Microbiology, B.J. Medical College, Ahmedabad, India
SODHATAR K H	Resident Doctor, Department of Microbiology, B.J. Medical College, Ahmedabad, India

ABSTRACT

BACKGROUND & OBJECTIVES: Diabetes mellitus (DM) has been considered to be a predisposing factor for infection in urinary tract and commonly present as Asymptomatic bacteriuria. Since the pattern of bacterial resistance is constantly changing due to widespread use of antibiotics, the monitoring of the antimicrobial susceptibilities of the predominantly isolated organisms becomes more important. The current study is aimed at investigating the prevalence and aetiology of Asymptomatic Bacteriuria and antimicrobial resistant pattern of urinary isolates in diabetic patients. **METHODS:** A total of 53 patients who visits at diabetic clinics in civil hospital, Ahmedabad, Gujarat over the duration of four months (January 2016 to April 2016) were included in this study. Significant Asymptomatic bacteriuria was defined as urine culture of $> 10^5$ cfu/ml without symptoms of cystitis. Isolates were identified using standard biochemical methods. Antimicrobial susceptibility testing was done by the Kirby Bauer disk diffusion method using standard procedures. **RESULT:** The overall prevalence of Asymptomatic bacteriuria in this study was 37.7%. The most prevalent organisms isolated were E.coli (13.2%), followed by Enterococcus (13.2%), Staphylococcus hemolyticus (3.8%), Candida sp (3.8%) and Kleb. pneumoniae (1.8%). All the organisms showed maximum sensitivity to Nitrofurantion and highest resistance to Co-trimoxazole. **INTERPRETATION & CONCLUSION:** Gram-negative bacilli are the most frequent organisms involved. Women are at increased risk to develop UTI than men. The high prevalence of ASB and the multiple resistances of most isolates is a major concern that requires prompt action. Tight control of blood sugar in the diabetic patient is a desirable goal in the prevention.

Introduction

Diabetes mellitus (DM) has long been considered to be a predisposing factor for infection in urinary tract [1] and most common presentation occur as Asymptomatic bacteriuria (ASB) which is defined as the presence of at least 10^5 CFU/ml in 1 culture of clean-voided mid-stream urine specimen or obtained by urethral catheterisation. If not properly treated, these bacteriuria may lead to serious complications such as emphysematous pyelonephritis and renal papillary necrosis leading to increase frequency of hospitalization and cost. Most common organism isolated is E.coli followed by enterococcus spp., pseudomonas. Since the pattern of bacterial resistance is constantly changing due to widespread use of antibiotics, the monitoring of the antimicrobial susceptibilities of the predominantly isolated organisms becomes more important.^[2-3] Although Asymptomatic bacteriuria is a major concern in diabetics, there is little information on the occurrence of Asymptomatic bacteriuria among diabetics in our local setting. Thus, the current study is aimed at investigating the prevalence and aetiology of Asymptomatic Bacteriuria and antimicrobial resistant pattern of urinary isolates in diabetic patients.

Method and Materials

A total of 53 patients who visits at diabetic clinics in civil hospital, Ahmedabad, Gujarat over the duration of four months (January 2016 to April 2016) were included in this study. Data regarding age, sex, type & duration of diabetes, sign and symptoms of UTI were recorded in specific questionnaire forms. Criteria for inclusion were age > 18 years; history of diabetes, its duration. The criteria used for identification of diabetic patients

was by post prandial blood sugar ≥ 140 mg/dl as obtained from department of biochemistry. All clinic patients were listed according to their current treatment regimen, either diet alone, oral hypoglycemic agents or insulin. Acceptability for entry into the study was based on the following criteria: (1) minimum of three patient visits during the observation period at which times no clinical evidence of infection existed; (2) treatment by one regimen-diet alone or an oral hypoglycemic agent or insulin -for the entire study period. Exclusion criteria included pregnancy; signs and symptoms of UTI and antibiotic /antimycotic usage within one week. The study participants were educated on how to collect a "clean-catch" midstream urine specimen and how to avoid contamination. They were advised to wash their hands prior to collection and labia separation, especially in females. Urine samples were collected into sterile containers, placed in a cool box (4°C) and transported to the laboratory within 2 hours. The samples were then stored in a refrigerator and analysed within 8 hours of collection. Each sample was separated into two parts under sterile conditions; one part for urinalysis and the other for culture. Wet preparation of centrifuged urine was observed using 40 times objective lens to detect blood, pus and other cells. A calibrated 1 mm wire loop was used to inoculate uncentrifuged urine into MacConkey agar and blood agar, plates were incubated aerobically at 37°C for 24 hours. Significant Asymptomatic bacteriuria was defined as urine culture of $> 10^5$ cfu/ml without symptoms of cystitis.^[4] Isolates were identified using standard biochemical methods.^[5] Antimicrobial susceptibility testing was done by the Kirby Bauer disk diffusion method using standard procedures.^[6] Disks of commonly used drugs against yeasts, Gram positive and negative organisms were

selected. These included; nalidixic acid, nitrofurantoin, ceftriaxone, cefuroxime, doxycycline, ciprofloxacin, chloramphenicol, gentamicin, cefuroxime cotrimoxazole, vancomycin, doxycycline, oxacillin, erythromycin, augmentin, nystatin, ketoconazole, miconazole, flucytosine and fluconazole. The plates were incubated aerobically at 37°C for 18 hours after which the zones of inhibition were measured in milliliters and recorded. Data for antimicrobial resistance was analysed using CLSI guidelines 2016.

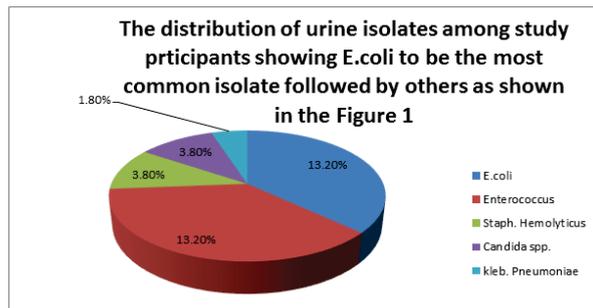
Result

Of the 53 participants enrolled into the study majority of participants were in the age group 50 years and above while the least age range was less than 40 years. The overall prevalence of asymptomatic bacteriuria in this study was 37.7%. Table 1 shows the distribution of asymptomatic bacteriuria with respect to sex. There was no significant difference in the distribution of ASB with age among the study participants. However, women demonstrated a higher prevalence than men in diabetic groups.

Table 1

	Total No.	Positive cases	% prevalence rate
Male	18	5	27.7%
Female	35	13	37.1%

The distribution of urine isolates among study participants showing E.coli to be the most common isolate followed by others as shown in the Figure 1



All the organisms showed maximum sensitivity to Nitrofurantion and highest resistance to Co-trimoxazole with variable resistance to other most commonly used drugs as shown in the table 2.

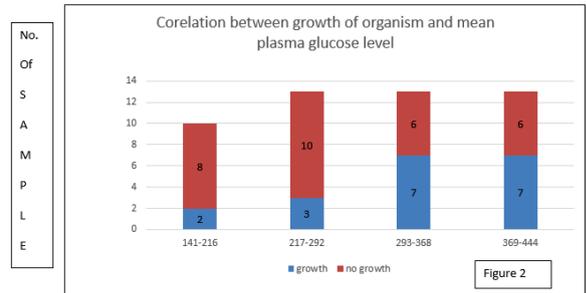
Table 2

	E.Coli	Enterococcus	S. hemolyticus	Kleb. Pneumonia
Ciprofloxacin	85.7%	0.0%	0.0%	57.1%
Levofloxacin	42.9%	0.0%	0.0%	42.9%
Co-trimoxazole	57.1%	42.8%	57.1%	42.8%
Nitrofurantion	14.2%	0.0%	0.0%	0.0%
Tetracycline	28.5%	28.5%	0.0%	57.6%
Gentamicin	42.8%	14.2%	0.0%	28.5%
Amikacin	14.2%	14.2%	0.0%	28.5%
Ampicillin	28.5%	0.0%	0.0%	0.0%
Cefuroxime	42.9%	0.0%	0.0%	0.0%

Antifungal drug	Candida spp. Resistance %
Itraconazole	0.0
Miconazole	50%
Fluconazole	50%
Ketoconazole	50%
Nystatin	50%
Amphoterecin B	0.0

Figure 2 shows a striking direct correlation between the overall prevalence of infection and the mean plasma glucose levels in these patients (representing three or more post prandial blood

glucose determinations taken at times when no evidence of infection existed). It is interesting to note that in this population there were no statistically significant correlations observed between the frequency of infection and age of onset, duration of diabetes and complications or associated diseases such as hypertension.



Our own chart review data show a significant correlation between frequency of infection and plasma glucose levels. These new data, demonstrate an increase in infection morbidity and a decrease in host defense capacity with poor control of plasma glucose levels. Therefore, we believe it is reasonable to suggest that tight control of plasma glucose to levels at or approaching those of non diabetic subjects, when feasible, could reduce the frequency of infection in diabetic patients. Percentage of growth However, as shown in figure 3, we also observed that patients on insulin and on combination therapy of insulin and hypoglycaemic drugs showed increase isolation of bacteriuria as compared to those on oral hypoglycaemics.

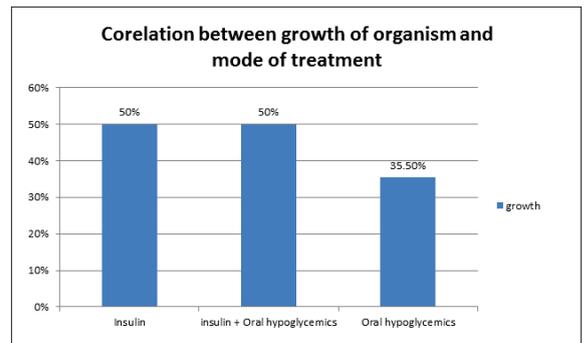


Figure 3

DISCUSSION

Urine is the most common specimen received in our laboratory. The association of diabetes mellitus and urinary tract infections is increasingly being reported. Asymptomatic bacteriuria is common among diabetic patients and has been identified as a risk factor for acquiring symptomatic UTIs especially in diabetic women. UTIs are more severe in diabetic patients involving life-threatening complications such as emphysematous pyelonephritis and renal papillary necrosis¹³. especially if glycaemic control is poor. That is why In this study, we have tried to determine whether there are differences in the bacteriologic patterns isolated from urine and in the antibiotic sensitivity patterns of the pathogens concerned in diabetic patients.

The mechanisms which potentially contribute to UTI in these patients are defects in the local urinary cytokine secretions(IL-8, IL-6), increased adherence of the microorganisms to the uroepithelial cells, partly due to a changed and lowered Tamm Horsfall protein, incomplete emptying of bladder due to autonomic neuropathy and granulocyte dysfunction, possibly as a result of an abnormal intracellular calcium metabolism.^[6,9] On the other hand, hyperglycemia facilitates the colonization and growth of variety of organism.^[10]

It has shown in several studies that women are at increased risk to develop UTI then men^[7] due to shorter urethra and close proximity to anus. Majority of the culture positive patients in our study were also female (37.1%).

Gram-negative bacilli are the most frequent organisms involved. In addition, gram-negative bacteremia is a life-threatening condition to which diabetic patients are more susceptible than non diabetic subjects, particularly following urethral catheterization or other genitourinary instrumentation^[11].

A large spectrum of organism were isolated with E.coli being most common isolate, an organism that may not be handled in normal fashion by host defenses in the diabetic patient^[12]. Since diabetic patients most commonly present as asymptomatic bacteriuria Antibiotic sensitivity were described to assess the resistance pattern in urinary isolates to commonly used antibiotics.

The present study revealed an overall **prevalence of ASB of 37.7% in diabetics**. This result is concurrent with that of other studies which recorded prevalence of 36.2% in diabetics^[4]. **Higher carriage rate of Candida sp** has also been reported in diabetics and this is **attributed to the presence of excess glucose** in diabetics that may encourage the growth of Candida sp.^[5] High resistance to cotrimoxazole may be due its frequent use in our study area to treat UTIs and other infectious diseases.

In the present study, Gram negative bacteria showed high resistance to gentamicin (59.3%) compared to Gram positive cocci (11.8%). **This difference in resistance may be due to the over-expression of efflux pumps in Gram negative bacteria.**^[6] Nitrofurantoin resistance is usually uncommon; the moderate resistance observed in this study may be due to the development of cross-resistance. In the present study, E. coli strains demonstrated multi-drug resistance especially to gentamicin, ciprofloxacin, cefuroxime and co-trimoxazole. Multi-drug resistance of E. coli is a common phenomenon as reported by other authors.^[17,18,19]

The high prevalence of ASB and the multiple resistances of most isolates is a major concern that requires prompt action. Consequently, there is the need to enhance sensitization against antibiotic abuse so as to curb the spread of multi resistant uropathogens in the study area.

The data suggest to us that tight control of blood sugar in the diabetic patient is a desirable goal in the prevention of certain infections (candidal vaginitis, for example) and to ensure maintenance of normal host defence mechanisms determining resistance and response to infection.

Acknowledgement

We would like to thank all the diabetic patients and healthy volunteers who consented to take part in this study. Our sincere gratitude also goes to the staff of Department of microbiology and Department of biochemistry, B J Medical College Ahmedabad, where the study was conducted.

REFERENCES

- Bonadio M, Costarelli S, Morelli G, Tartaglia T. The influence of diabetes mellitus on the spectrum of uropathogens and the antimicrobial resistance in elderly adult patients with urinary tract infection. BMC Infect. Dis. 2006; 6:54.
- Boyko EJ(2005) Risk of urinary tract infection and asymptomatic bacteriuria among diabetic and nondiabetic post menopausal women. Am J epidemiol. 161:557-64
- Bonadio M, Boldrini E, Forrotti G, Matteucci E, Vigna A, et al. (2004) Asymptomatic bacteriuria in women with diabetes: influence of metabolic control. Clin Infect Dis 38: e41-45
- Das RN, Chandrashekhar TS, Joshi HS, Gurung M, Shrestha N, Shivananda PG. Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal. Singapore Med J 2006; 47(4): 281-5.
- Colle JG, Miles RS, Watt B. Tests for the identification of bacteria. In : Mackie and MacCartney Practical Microbiology, 14th edn. New York: Churchill Livingstone Inc 1996; pp: 131- 49
- Bauer AW, Kirby WMM, Sherris JC, Tierch M. Antibiotic susceptibility testing by a standardized simple disk method. Am J Clin Pathol 1966; 45:493-9
- Raco MVO, Barez MYC. Profile of community acquired urinary tract infections in

- Davao city. Phil J.Microb. Infect Dis. 1998; 28 (2): 62-6.
- Sahib AKY. Study of ciprofloxacin resistant Escherichia coli (CREC) in type 2 diabetic patients with symptomatic urinary tract infections. Iraq J Comm Med 2008; 21(1): 58-63.
- Baqi R, Aziz M, Rasool G. Urinary tract infection in diabetic patients and biofilm formation uropathogens. Infect.Dis. of Pakistan 2008; 17(1): 7-9.
- Hasan MK, Nazimuddin K, Ahmed AKMS, Sarker RSC, Haque M, Musa AKM. Differences in a bacteriological and antibiotic sensitivity patterns in UTI among hospitalized diabetic and non diabetic patients. J Medicine 2007; 8: 10-3.
- Schneerson SS: Diabetes and infection. NY State J Med 1962; 62: 676.
- Aruyan S, Halasz NA: The incidence of post operative gram-negative shock in diabetes. Am J Med Sci 1967; 254: 808.
- (Geerlings SE, Stolk RP, M. Camps MJL, Netten PM, Hoekstra JB, Bouter PK, et al. Asymptomatic bacteriuria may be considered a complication in women with diabetes. Diabetes Care 2000b; 23: 744-749.)
- Ophori EA, Imade P, Johnny EJ. Asymptomatic bacteriuria in patients with diabetes. J Bacteriol Research 2010; 2 (2): 14-17.
- Al-Attas SA, Amro SO. Candidal colonization, strain diversity and antifungal susceptibility among adult diabetic patients. Annals of Saudi Medicine 2010; 30 (2): 101-108.
- Li XZ, Livermore DM, Nikaido H. Role of efflux pump(s) in intrinsic resistance of Pseudomonas aeruginosa resistance to tetracycline, chloramphenicol, and norfloxacin. Antimicrobial Agents and Chemotherapy 1994; 38: 1732-1741
- Bonadio M, Costarelli S, Morelli G, Tartaglia T. 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 2006; 6 (54): 1-7
- Ophori EA, Imade P, Johnny EJ. Asymptomatic bacteriuria in patients with diabetes. J Bacteriol Research 2010; 2 (2): 14-17.
- Hamdan HZ, Ziad AHM, Ali SK, and Adam I. Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. Annals Clin Microbiol Antimicrob 2011; 10(2).