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Original Research Article

Asymptomatic Bacteriuria due to MDR Organisms in Type 2 Diabetes Mellitus Patients

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ABSTRACT

Background & Objectives: Diabetic subjects, especially women, show high prevalence of asymptomatic bacteriuria (ASB). The aetiology and the antibiotic resistance of uropathogens have been changing over the past years. The present study was undertaken to evaluate the prevalence of asymptomatic bacteriuria & occurrence of multidrug resistant (MDR) strains of bacteria in Type 2 Diabetes Mellitus patients.
Methods: 200 type 2 diabetic males and females (aged between 30-80 years) who attended Maharashtra Institute of Medical Education & Research & BSTR hospital, Pune, India were included in the study. Mid-stream urine samples were collected from patients aseptically into sterile wide mouth container examined microscopically & was cultured using standard techniques. Isolates were tested against separate antibiotics for gram negative and gram positive organisms by the disc diffusion method.
Results: Significant bacteriuria was observed in 52% of urine samples (50 females and 54 males). Bacteria isolated included *Escherichia coli* (31%), *Klebsiella pneumoniae* (13%), *Citrobacter koseri (*13%), *Pseudomonas aeruginosa* (10%), *Proteus vulgaris* (3%), *Acinetobacter baumannii* (2%) among GNB & *Staphylococcus aureus* (25%) among GPCs. MDR was observed in 67 (60.36%) of the total 111 isolates obtained. Highest occurrence of MDR was observed among Acinetobacter *baumannii* (100%) followed by *Pseudomonas aeruginosa* (80%).
Conclusion: The high prevalence of ASB and the multiple resistances of most isolates is a major concern which suggests the need to enhance sensitization against antibiotic abuse so as to curb the spread of multi resistant uropathogens in the study area.

Key words: Asymptomatic Bacteriuria, *Escherichia coli,* MDR, Midstream urine, Type 2 diabetes mellitus.

INTRODUCTION

The term bacteriuria means the presence of bacteria in urine and it is taken to be significant if 10^5 organisms per millilitre of a fresh "clean catch" urine specimen are present in any patient. [1] Asymptomatic bacteriuria (ASB) major risk factor for the development of UTI in pregnancy due to physiological changes. [2]

Diabetes mellitus (DM) is a worldwide health problem with an expected prevalence of 593 million by 2035. [3] Prevalence of asymptomatic bacteriuria (ASB) in women has been reported as in school children (67%), during pregnancy

(6% asymptomatic) and 10-12% among elderly women. [4] Urinary tract infection (UTI) is the most common infection among patients with DM & is responsible for considerable morbidity, particularly if it is unrecognized or untreated. [5,6] Risk factors for UTI among patients with & without DM have been identified e. g. Obesity, female sex & prostate syndrome in men. [7,8] Furthermore glycosuria, low immunity & bladder dysfunction which are associated with DM, are considered particular risk factors for UTI. [9,10] Development of asymptomatic UTI in diabetic women has been reported to be much more common than in non diabetic women, men & from diabetic outpatients with urinary tract infections. [10,11] Most bacterial aetiologic agents in asymptomatic bacteriuria have been reported to include *Klebsiella pneumoniae, Escherichia coli, Enterobacter sp., Streptococcus agalactiae, Enterococcus faecalis, Coagulase Negative Staphylococcus and Streptococcus pyrogens.* [1,2,12] *Escherichia coli* is most commonly isolated organism in both diabetic & non diabetic patients. [13,14] Untreated ASB predisposes the individual to recurrent UTI which can cause renal disease. Patients with diabetes mellitus have been reported to have increased rates of UTI infections. [15]

Diagnosis of ASB is the most important step in managing ASB & the most important point for diagnosis is microbiological tests. In this context, the number of specimens for culture is crucial. In guidelines, ASB in men is defined as the isolation of> = 10^5 cfu / ml of bacteria in a single clean catch voided urine specimen. On the other hand in women, two consecutive urine specimen is needed for accurate diagnosis. [16] However in present study, we could collect a single urine specimen from men & women both.

Therefore aim of present study was to study prevalence of asymptomatic bacteriuria along with spectrum of uropathogens with their antibiotic resistance profile & to study occurrence of MDR strains of bacteria in Type 2 Diabetes Mellitus patients.

MATERIALS & METHODS

A prospective study was conducted at MIMER medical college during the period of January 2016 to October 2016 after obtaining approval from institutional ethical committee. Two hundred consecutive samples from asymptomatic male & female patients with type 2 diabetes (aged between 30-80 years), who attended Maharashtra Institute of Medical Education & Research & BSTR hospital, Pune, India were included in the study. Patients with overt diabetic nephropathy or nephropathy from other causes & patients with symptoms of UTI like frequency, dysuria, urgency etc. were excluded. Also, patients on antimicrobials, NSAIDs & immunosuppressors in last 14 days of study were excluded by the study.

Early morning mid-stream urine samples were collected from patients aseptically into sterile wide mouth container and delivered to microbiology laboratory immediately which were further examined microscopically. Samples were then inoculated on, Mac Conkey agar using standard techniques (calibrated loop). For calculating Colony forming units (CFUs), blood agar was inoculated using calibrated loop by T method. The plates were incubated at 37°C aerobically for 24 h. Colony forming units were counted.

Asymptomatic bacteriuria was defined as the presence of 1,00,000 or more colony forming units per ml (CFU/ml) of urine. [17]

Isolates were identified using standard biochemical techniques. ABST of isolates was carried out on Muller Hinton Agar using commercially available antibiotic discs (Hi-media, Mumbai) by Kirby Bauer Disk diffusion Technique & interpreted according to CLSI guidelines. [18] Bacterial isolates were labeled as MDR by using CDC Criteria (Isolate nonsusceptible to atleast 1 agent in $\geq$ 3

antimicrobial categories) by Kirby Bauer Disk Diffusion Susceptibility Test.

RESULTS

➢ AGE AND SEX DISTRIBUTION OF PATIENTS & ASB

Out of 200 urine samples included in study, 104 samples were from male pts and 96 were from female patients.

Table 1: Age and sex distribution of diabetics with ASB

Age (Years)	Males (%)	Females (%)
30-39	6 (11.11)	9(18)
40-49	7(12.96)	8(16)
50-59	16(29.62)	10(20)
60-69	12(22.22)	12(24)
Above 70	13(24.07)	11(22)
Total	**54 (51.92)**	**50(48.07)**

ASB was highest in age group 50-59 years among males, while age group 60-69 years showed high occurrence of ASB among females.

There was not much difference in overall occurrence of ASB among males (51.92%) than females (48.07%).

Microbial growth was present in 65% (130/200) of total samples. Asymptomatic bacteriuria (ASB) was present in 52% (104/200) of total samples.

➢ SPECTRUM OF UROPATHOGENS ISOLATED

Table 2: Organisms isolated and percentage

Bacterial isolates	No. (%)
E. coli	34 (31)
Klebsiella pneumoniae	14 (13)
Citrobacter koseri	14 (13)
Pseudomonas aeruginosa	10 (10)
Proteus vulgaris	3(3)
Acinetobacter baumannii	2(2)
Staphylococcus aureus	28(25)

We isolated different organisms including gram negative bacilli & gram positive cocci. Of the total 104 samples which showed ASB, number of organisms isolated was 111. Out of 111, 77 (69.36%) were gram negative bacilli, 28 (25.22%) included gram positive cocci and 6 (5.40%) isolates were of *Candida albicans*.

E. coli was the most frequently isolated strain, in 31% of patients. *Klebsiella pneumoniae* and *Citrobacter koseri* was isolated in 13% of patients, *Pseudomonas aeruginosa* in 10%, *Proteus vulgaris* in 3%, *Acinetobacter baumannii* in approximately 2% and *Staphylococcus aureus* was isolated in 25% of patients.

➢ ABST PATTERN OF ISOLATES

Results showed that *E.coli* showed maximum sensitivity to Nitrofurantoin, while few *Klebsiella* and *Pseudomonas* isolates were sensitive to Piperacillin-tazobactam. Out of 10 *Pseudomonas* isolates, 3 were sensitive to Tobramycin & 2 were sensitive to Meropenem. *Acinetobacter baumannii* showed high resistance to almost all antibiotics. Among all antibiotics, majority of isolates were resistant to Ampicillin-sulbactum.

For gram positive cocci, we tested 12 different antibiotics. Among gram positive cocci, all were *Staphylococcus aureus.* Among these, 50% (14/28) showed sensitivity to Nitrofurantoin & 57.14% (16/28) showed sensitivity to Linezolid.

➢ OCCURRENCE OF MDR AMONG ISOLATES

Table 3: Number of MDR isolates obtained

Organism	Number of MDR / Total isolates (%)
Acinetobacter baumannii	2/2(100)
Pseudomonas aeruginosa	8 /10 (80)
Citrobacter koseri	10 /14(71.42)
Staphylococcus aureus	19/ 28(67.85)
Klebsiella pneumoniae	9/14 (64.28)
E.coli	18/ 34(52.94)
Proteus vulgaris	1/ 3(33.33)
Total	**67/111 (60.36)**

MDR was observed in 67 (60.36%) of the total 111 isolates obtained. Highest occurrence of MDR was observed among *Acinetobacter baumannii* (100%) followed by *Pseudomonas aeruginosa* (80%). *Citrobacter koseri* showed 71.42% MDR strains while *Staphylococcus aureus* & *Klebsiella* showed 67.85% & 64.28% of MDR strains respectively. Among total *E.coli* isolates 52.94% were MDR while a few (33.33%) of *Proteus vulgaris* strains were MDR.

DISCUSSION

The main findings of the present study were that the prevalence of asymptomatic bacteriuria among diabetic

patients was 52%. This result is concurrent with that of study conducted in Cameroon, which also showed high prevalence (35.2-58.3 %) of bacteriuria [19,20] On the contrary, the prevalence of ASB in this study is higher than that of some studies which recorded prevalence of 5.3-26% [21-24] & 10.4%. [25] Few studies have recorded prevalence of 36.2 % in diabetics. [26] Consequently, the issue of prevalence of ASB remains debatable. This inconsistency has been attributed to variations in sample size, geographical location, culture or screening method. [2]

Also high prevalence of UTI in this setting may be explained by poor glycemic control in our diabetic patients. Poor control of DM increases the risk of UTI by 24%. [27] Generally, compared with non-diabetic patients, diabetic patients have a higher incidence of UTI and asymptomatic bacteriuria. [28,29]

Diabetic patients are at increased risk of infection in general and, in particular, to UTI. [30] The susceptibility of diabetic patients to UTI could be explained by diminished neutrophil response, lower urinary cytokines, and leukocyte concentrations, which might facilitate the adhesion of microorganisms to uroepithelial cells. [28,31,32]

The current study showed that E. *coli* was the most common organism isolated from asymptomatic diabetic patients which is similar to other studies. [13-15] 2nd most common organism isolated in present study was *Staphylococcus aureus.* The predominance of bacteria other than E. coli in the urinary tract is increasingly being reported. Recent study in Nigeria has also reported *Staphylococcus aureus* to be the most common uropathogen in diabetics. [41] The high prevalence of Staphylococcus sp in ASB may be due to the fact that these organisms are mostly normal skin flora and can be introduced to the urinary tract during sexual intercourse. [43]

Most of the *Staphylococcus aureus* isolates in our study were resistant to Cotrimoxazole & Norfloxacin which are commonly used antimicrobials for treating UTIs. High resistance to cotrimoxazole may be due its frequent use in our study area to treat UTIs and other infectious diseases. *E.coli* was resistant mainly to ampicillin, ampicillin-sulbactum, and amoxicillin-clavulanic acid norfloxacin. This is somewhat in line with reports from Ethiopia, Libya, and Kenya. [25,13,33] Furthermore, this is in agreement with a recent report from Ethiopia, where over 60% of the isolated urinary *E. coli* was resistant to ampicillin. [25] However, increasing evidence shows an increase in strains of MDR *E. coli* in diabetic and non-diabetic. [34,35] Niranjan and Malini claim that DM *per se* is a risk factor for infection by MDR *E. coli.* [36] This report is contradicted by other studies. [37,38] In our study also the number of MDR *E. coli* strains was high among total *E.coli* isolates obtained (18 out of 34 isolates). Hence we got 52.94% of MDR *E.coli* isolates. In the present study, *E. coli* strains demonstrated multi-drug resistance especially to Ampicillin-sulbactam, Cefuroxime, Ampicillin, Piperacillin. Multi-drug resistance of *E. coli* is a common phenomenon as reported by other authors. [14,39,40]

Patients' geographical region, lifestyle and health care factors may possibly be related to MDR *E. coli*. [41] *K. pneumoniae* and *Citrobacter koseri* were the second most commonly isolated gram negative organisms, which is in agreement with a recent report from Nepal which also reported *K. pneumoniae* as second most common organism isolated. [42]

Though *Candida sp* was isolated in less number (5.40 %) of patients in our study, higher carriage rate of Candida sp has also been reported in other studies. [44]

In the present study, gram negative bacteria showed high resistance to gentamicin (77.92%) compared to gram positive cocci (57.14%). This difference in resistance may be due to the over-expression of efflux pumps in gram negative bacteria. [45] Nitrofurantoin resistance is

usually uncommon; the moderate resistance observed in this study may be due to the development of cross-resistance. Gram negative bacteria showed moderate to high resistance to both the second and third generation cephalosporins. This is commensurate to previous reports. [22,46]

CONCLUSION

The present study showed a high prevalence of ASB of 52% along with much higher percentage (60.36%) of MDR organisms causing ASB in diabetes mellitus patients. 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.

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