# Asymptomatic bacteriuria and associated host factors in Diabetic patients with special reference to UTI Chromagar

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## Abstract

**Introduction:** To study the prevalence of asymptomatic bacteriuria (ASB) in diabetics compared to non-diabetics and to determine the associated host factors responsible for ASB in diabetics. **Materials and Methods:** 250 diabetics and 100 controls were enrolled in the study and were screened for ASB using semi-quantitative culture methods and UTIchrom agar. The samples were screened for glucosuria, ketonuria and pyuria. **Results:** ASB was more common in diabetics compared to non-diabetics(12.8% v/s 6% ) and the increase was more in female diabetics when compared to female non-diabetics(16% v/s 6.15%) whereas male counterparts did not show much difference from the non-diabetics (6.09% v/s 5.71%). There was significant influence of age, duration of diabetes, glucosuria, ketonuria, proteinuria and pyuria on the incidence of ASB in diabetics thus proving them as possible risk factors for development of ASB. E.coli was the most common organism found in both diabetic as well as non diabetic cases of ASB (40.6% and 57.14% respectively). The next frequent organisms were Klebsiella and Staphylococcus. The sensitivity pattern was similar in both the population with ASB. Culture on UTI CHROMagar yielded quicker results when compared to standard culture methods. **Conclusion:** ASB is almost 3 times more common in female diabetics compared to female non-diabetics whereas there is not much difference among the male counterparts. The risk factors for ASB in diabetics included age, longer duration of diabetes, poor glycaemic control and renal dysfunction indicated by proteinuria. Culture on UTI CHROMagar is an easy and faster means of identification of organisms.

**Keywords:** Asymptomatic bacteriuria, Diabetics, Escherichia Coli, pyuria, UTI CHROMagar.
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## Introduction

Nearly 61.3 million Indians in their most productive years — (20 to 79 years) — are diabetic, a disease that exposes them to heart attack, stroke, amputations, ner.ve damage, blindness and kidney disease [1]. Diabetes and urologic diseases are very common health problems that markedly increase in prevalence and incidence with advancing age. Severe complications of urinary tract infections occur virtually only in patients with diabetes [2].

Patients with diabetes mellitus have a higher prevalence of asymptomatic bacteriuria (ASB) and incidence of urinary tract infections (UTIs) compared with patients without diabetes mellitus. In fact presence of ASB in patients with Type 2 diabetes acts as a predisposing

factor for subsequent development of a symptomatic UTI [3]. Complications of UTIs such as emphysematous cystitis, pyelonephritis and renal

papillary necrosis occur more commonly in subjects with Type 2 diabetes mellitus [4]. Several risk factors for asymptomatic bacteriuria in diabetics have been suggested (age, disease duration, quality of metabolic control of disease and status of diabetic complications) [4, 5].

The frequency of asymptomatic bacteriuria in patients with diabetes mellitus is 9-27% in females and 0.7-1% in males [6]. The increased frequency is probably secondary to autonomic neuropathy of the bladder. The static pool of urine due to bladder dysfunction and poor contractility serves as a favourable medium for bacterial growth. Even hyperglycemic urine promotes rapid

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bacterial growth and colonization. Diabetic patients with asymptomatic bacteriuria are more likely to have albuminuria and symptomatic UTIs [7].

Escherchia coli are the most common organism and are most likely to occur in healthy persons. A variety of organisms may be found including Enterobacteriaceae, Klebsiella pneumoniae, Proteus species, Enterococcus species and group B Streptococcus [8]. In men, Enterococcus species and gram-negative bacilli are common.

UTI CHROM agar medium is a chromogenic medium which is known to be a better medium for easy, accurate and early identification of organisms when compared to standard culture medium and is found to be less time-consuming [9,10].

In India, report on ASB in diabetics is scarce and its impact poorly documented. Consequently we decided to study on the frequency of asymptomatic bacteriura in clinically diagnosed patients with diabetes in comparison to non-diabetics and to verify whether there is association between bacteriuria and following suggested risk factors in diabetics – age, sex, duration of diabetes, metabolic control and proteinuria. Also the uropathogens responsible for bacteriuria and their antimicrobial susceptibility was evaluated.

## Materials and Methods

This is a comparative study conducted in the Department of Microbiology over a period of 6 months. Two hundred and fifty patients aged 30-80 years diagnosed with type 2 diabetes mellitus attending out-patient departments for routine check-ups and 100 age related controls were included in the study.

Exclusion criteria included history of UTI symptoms like dysuria, frequency and urgency, patients on urinary catheters or having renal ailments, pregnant women and patients who received antibiotics for the past 2 weeks. Approval to conduct the study was obtained from the ethical committee of the institution. The procedure and purpose of collecting samples for the study was explained to each subject in their own language, highlighting that their personal details and other specificities would not be revealed and that they would not incur any difficulties from participating in the study. Each subject was asked to sign an informed consent giving their permission to participate in the study.

The study group was interviewed and details on demographics (name, age, gender and address) and medical variables (diabetes type, duration and therapy, presence of diabetes related systemic diseases) were recorded in the proforma.

Clean-voided, mid-stream urine samples was collected in wide mouth sterile containers from both case and control groups. The samples were then transported to the microbiology laboratory for immediate processing (within 2 hours). The urine samples were processed according to the standard guidelines [11], culture put up first to prevent contamination and then used to test for urine sugar, ketones, albumin and wet mount examination.

Routine urine samples were cultured on blood agar and MacConkey agar by standard loop method for semi-quantitative culture [12]. The inoculated media was incubated aerobically at 37°C for 24 hrs. A diagnosis of asymptomatic bacteriuria in diabetics as well as non diabetics was made as per the 2005 IDSA guidelines [13]. In those cases with significant bacteriuria, the uropathogens were identified by biochemical reactions using standard methods [11]. Isolated strains were tested for antibiotic susceptibility testing by Kirby-Bauer's disc diffusion method following Clinical Laboratory Standards Institute (CLSI) guidelines [14].

The urine samples were also simultaneously inoculated on UTICHROMagar while inoculating on standard medium and incubated at 37°c for 24 hrs. The number and identification of different organisms grown on the UTICHROMagar medium were primarily assessed by colony colour and morphology as mentioned by the manufacturer.

Pyuria indicates inflammatory response in the urinary tract [11, 15] so the urine sample is examined by wet mount to detect the presence of pus cells. Glucosuria, ketonuria and proteinuria were evaluated using dip stick method.

**Data Analysis:** The collected data was statistically analyzed by using chi-square test ($x2$) and P value determined. T-values were calculated by student's 'T' test formula for means ± standard deviations of ages. P value of <0.05 was considered significant. The data collected was interpreted in tables.

## Results

Of the 250 diabetic cases enrolled in the study, 82 were males and 168 were females. The rate of ASB was 6% (5 of 82) among male diabetics and 16% (27 of 168) among female diabetics. There were 100 non-diabetic controls included in the study among which 2 out of 35 males and 4 out of 65 females had ASB accounting for 5.71% and 6.15% of incidence of ASB respectively.

It was found that the incidence of ASB was greater in diabetics (12.8%) compared to non diabetics (6%) [P value < 0.05]. Also among diabetic cases, female diabetics (16%) had a higher frequency of ASB compared to male diabetics (6.09%). There was almost 3 times increased frequency of ASB in female diabetics (16%) as compared to female non-diabetics (6.15%) [P value=0.02] whereas there was only 0.38% increase in male diabetics (6.09%) compared to male non-diabetics (5.71%) which is not significant [P value=0.48].

**Table 1:** Distribution of ASB with respect to age in diabetic cases

| Age of the patient | Sample Size | Asb Positives | Percentage |
|---|---|---|---|
| 30-40yrs | 26 | 2 | 7.7% |
| 40-50yrs | 68 | 9 | 13.2% |
| 50-60yrs | 73 | 8 | 10.9% |
| 60-70yrs | 66 | 9 | 13.6% |
| 70-80yrs | 17 | 4 | 23.5% |

**Table 2:** Distribution of ASB with respect to duration of diabetes

| Duration of diabetes | Sample size | Asb Positives | Percentage |
|---|---|---|---|
| <5yrs | 122 | 10 | 8.19% |
| 6-10yrs | 84 | 14 | 16.67% |
| 11-15yrs | 29 | 4 | 13.8% |
| 16-20yrs | 12 | 4 | 33.33% |
| >20yrs | 3 | 0 | * |

* > 20 yrs duration, the sample size was very less to correlate the findings.

**Table 3:** Distribution of ASB with respect to various risk factors

| Risk Factors | ASB + | ASB - | P Value |
|---|---|---|---|
| AGE:<br><55.36±11.02yrs<br>>55.03±11.02yrs | 12<br>20 | 120<br>98 | 0.034 |
| Duration Of Diabetes<br><7.15±5.46yrs<br>>7.15±5.46yrs | 22<br>10 | 106<br>112 | 0.017 |
| Glucosuria | 27 | 143 | 0.015 |
| Ketonuria | 22 | 114 | 0.041 |
| Proteinuria | 32 | 134 | 0.0000033 |
| Pyuria | 27 | 99 | 0.000013 |

**Culture:** Out of 32 diabetic cases wherein ASB was found, the most common organism responsible for ASB in diabetics was found to be E.coli (40.6%) followed by klebsiella (31.25%), staphylococcus aureus (25%) and citrobacter (3.125%).

E.coli was the main causative organism of ASB in non-diabetics as well (57.14%). Others included klebsiella (21.43%) and staphylococcus (21.43%).

The mean age of the patients with diabetes was 55.36±11.02 yrs and that of the controls was 53.12±9.31 yrs. As age increased susceptibility to ASB also increased (Table / Fig-1). Also as the duration of diabetes increased it was found that the incidence of ASB also increased (Table/Fig- 2). ASB was found to be associated with the following risk factors: 84.38% of cases with ASB had glucosuria as against 15.62% of ASB cases without glucosuria [p value=.015 and odds ratio=2.83]. Similarly 68.75% of ASB positive had ketonuria as against 31.25% of ASB cases without ketonuria [p value =.041 and odds ratio=1.8441]. Almost all the ASB positive cases were noted to have significant proteinuria [p value=.0000033]. 21.4% cases with ASB had pyuria against 4% cases with ASB but without pyuria showing a strong correlation between the two. (P value <0.05) [Table/Fig- 3]

The antimicrobials showed similar resistance pattern in both case and control groups. The resistance pattern of the organisms from the case group is illustrated in Table/fig-4.

**Table 4:** Antimicrobial susceptibility pattern of isolated organisms

| Organism | Total Samples | Cac | Cx | C | Cip | Cd | Cot | E | Pit | Lz | Va | Net | Gen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E.coli | 13 | 10 | 13 | 13 | 9 | - | 6 | - | 13 | - | - | 11 | 12 |
| Klebsiella | 10 | 9 | 10 | 10 | 6 | - | 6 | - | 10 | - | - | 10 | 10 |
| Citrobacter | 1 | 1 | 0 | 1 | 0 | - | 0 | - | 1 | - | - | 1 | 1 |
| Staphylococcus | 8 | - | 7 | 6 | 4 | 4 | 5 | 2 | - | 8 | 8 | 8 | 8 |

Cac- Ceftazidime /Clavulanic acid, Cx- Cefoxitin, C- Chloramphenicol, Cip- Ciprofloxacin, Cd-Clindamycin, Cot-Cotrimoxazole, E- Erythromycin, Pit- Piperacillin/Tazobactam, Lz-Linezolid, Va- Vancomycin, Net- Netilmicin, Ak-Amikacin

The organisms inoculated on UTI CHROMagar were identified within 24hrs of incubation with the following characteristic features:

**E.coli -** pink translucent colonies
**Klebsiella -** dark blue colonies
**Citrobacter -** magenta coloured colonies
**Staphylococcus -** tiny, cream- white convex colonies

## Discussion

In this study we found that the frequency of asymptomatic bacteriuria is more in diabetics (12.8%) compared to non-diabetics (6%). There was almost 3 times increased frequency of ASB in diabetic women compared to non-diabetic women (16% v/s 6%) but the frequency did not show much difference in male counterparts (5.71% v/s 6.09%). This was in concordance with other studies wherein in ASB was found to be higher in female diabetics [4, 16].

The incidence of ASB increased as the duration of diabetes increased with p value <0.05 thus signifying its influence. E. Geerlings, Ronald P,et al also found similar results in regards to influence of duration of diabetes with respect to age[3,17]. In this study we found a significant association between glucosuria, ketonuria and ASB indicating that poorer the diabetic control greater the incidence of ASB. Proteinuria was

present in all the patients with ASB indicating renal dysfunction in these individuals which may progress to further complications. 21.4% cases with ASB had pyuria against 4% cases with ASB but without pyuria showing a strong correlation between the two (P value <0.05). This is supported by a study done by Kelestimur F, Unal A,et al with 110 patients with diabetes, a significant association between bacteriuria , pyuria and HbA1c levels was found [18].

The most common organisms found in both case and control samples with significant bacteriuria were similar and their antimicrobial sensitivity was also similar signifying that both case and control had similar risk factors for developing antimicrobial resistance when they were enrolled in the study. Bonadio M, Mienni also proved E.coli to be the most common organism cultured from urine samples of UTI patients with

diabetes and found that the sensitivity pattern of the organisms cultured was similar to the pattern obtained from urine samples of UTI patients without diabetes [2].

The study showed that the frequency of E.coli was higher in non-diabetics compared to diabetics [57% v/s 40%] which is consistent with the result of other studies conducted by Lye WC and E Geerlings(17,19).

The sensitivity pattern revealed that the organisms were sensitive to most of the commonly used antibiotics with increased resistance noted to cotrimoxazole and ciprofloxacin among gram negative organisms. Staphylococcus showed some degree of resistance to ciprofloxacin, erythromycin,cotrimoxazole and clindamycin. Organisms from control group also showed similar pattern of resistance.

All the positive cultures picked up on standard medium were also picked up on UTICHROMagar and was faster and much easier as there was no need for sub-culture or other biochemical reactions for identification of organisms. Several other studies reported similar conclusion [9, 10, 20].

Hence it was demonstrated that UTICHROMagar could be used as a primary culture medium for identification of organisms as it is easier, faster and also reliable in comparison to conventional methods as the need for subculture and other confirmatory tests is overcome.

## Conclusion

we conclude that asymptomatic bacteriuria is higher is diabetic women and the risk factors increasing the incidence of ASB in diabetic patients included increased age, longer duration of diabetes, poor diabetes control indicated by glucosuria and ketonuria, macroalbuminuria and presence of pyuria. UTICHROMagar medium is an easy and faster means of identification of organisms in ASB.

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