Prevalence and Risk Factors of Asymptomatic Bacteriuria in Pregnancy at a Tertiary Care Hospital: A Cross-sectional Study

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ABSTRACT

Introduction: Asymptomatic bacteriuria in pregnancy is associated with adverse maternal and fetal outcomes. The prevalence of asymptomatic bacteriuria is relatively higher in under developed and developing countries and is influenced by risk factors such as contraceptive use, prior urinary tract infections and comorbidities. This study aimed to determine the prevalence of asymptomatic bacteriuria among pregnant women and assess its association with various risk factors.

Methods: This is a hospital-based, cross-sectional study conducted on pregnant women. Pregnant women without symptoms of urinary tract infection were included. Convenient sampling technique was used. Data was collected through a pre-designed interviewer and observation-based questionnaire. R Programming and Microsoft Excel were used to carry out the analysis.

Results: The prevalence of asymptomatic bacteriuria was 10 (2.56%). *Escherichia coli* was isolated in 5 (1.28%) and *Klebsiella pneumoniae* in 3 (0.77%) samples. Nitrofurantoin was sensitive among 10 (100%) isolates. Sexual frequency of three or more times per week was seen in 7 (9.59%) cases of asymptomatic bacteriuria.

Conclusions: The prevalence of a symptomatic bacteriuria in pregnancy at a tertiary care hospital was comparable to other studies.

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INTRODUCTION

Urinary tract infections (UTIs) are common infections that happen when bacteria, often from the skin or rectum, enter the urethra and infect the urinary tract.[1] Asymptomatic bacteriuria (ASB) signifies the presence of a significant quantity of bacteria ($\geq 10^5$ CFU/ml) in a urine specimen without any urinary signs and symptoms.[2] Physiological and hormonal changes in pregnancy, including ureteral dilatation, vesicoureteral reflux, incomplete bladder emptying, and increased urinary glucose and protein, enhance susceptibility to infections.[3-6] Pregnant women with ASB face adverse outcomes: over 50% develop symptomatic UTIs or pyelonephritis, along with increased risks of intrauterine growth restriction, pregnancy-induced hypertension, preeclampsia, preterm labor, low birth weight, and perinatal mortality.[3,7,8] A recent study in Nepal reported an ASB prevalence of 42% among pregnant women.[9] Identified risk factors include prior UTI, diabetes mellitus, low socioeconomic status, high sexual frequency, or late prenatal care presentation etc.[10,11] Despite this burden, limited research exists in Nepal. So, we conducted this study to assess the prevalence of Asymptomatic UTIs (AUTIs) along with the associated risk factors. The cross-sectional study is reported in line with the STROBE guideline.[12]

METHODS

This is a hospital-based, descriptive cross-sectional study conducted on pregnant women visiting the Obstetrics and Gynaecology Department at Shree Birendra Hospital, Kathmandu, Nepal. The study was conducted at the antenatal clinic from March 15, 2022 to September 15, 2022. The main objective of this study was to determine the prevalence of asymptomatic bacteriuria among pregnant women attending her regular ANC checkups as well as to study the correlation between past history of UTIs, Diabetes mellitus, kidney disease, hemoglobin level, daily water intake, pH of urine, sexual activity, use of contraception and asymptomatic bacteriuria. The ANC clinic runs biweekly and records an average of 160 patients

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visits per day. Since the same women attend multiple follow-up visits, these numbers represent total visits rather than unique individuals. Unique pregnant women visiting OPD for her regular ANC checkups having no UTI symptoms were included in our study. Inclusion criteria were pregnant women attending regular antenatal check-ups who provided consent. Exclusion criteria included unwillingness to participate, presence of UTI symptoms, antibiotic use within two weeks prior to presentation, known renal pathology, and use of immunosuppressants.

Non-probability Convenient sampling technique was used.

Sample size was calculated by using a single population proportion formula based on the previous study conducted in Nepal. Therefore, we calculated the sample size using Cochran's formula (at 95% CI, 5%margin of error) as follows:

$n = (Z_{\alpha/2})^2.(p.q)/e^2$; Where, $Z\alpha_{/2}$= standard normal coefficient for a significant level = 0.05(confidence interval at 95%) which is 1.96, p= is an anticipated proportion of the previous study, the study of p previous Prevalence of UTI in pregnant women in a tertiary care hospital was found to be: 42%[9], i.e. 0.42. q= 1-p; 0.58, e = margin of error, 5% Calculated sample size (n) = $(1.96^2 \times 0.42 \times 0.58) / 0.05^2$ = $(3.84 \times 0.42 \times 0.58)/ 0.05^2$ = 374. 16 ~ 375 Considering 5% as a non-response rate, the required sample size becomes 394. So, a total of 395 participants were taken for the study. However, during the data collection phase, some collected data were discarded due to incomplete records. Consequently, a total of 390 valid responses were included in the final analysis.

A single institute-based convenient sampling technique was used for data collection. An informed verbal and written consent was taken from the pregnant women visiting the outpatient department by researchers. Data obtained were filled in predesigned questionnaire. The midstream urine sample was collected in a wide-mouthed, screw capped, leak proof, sterile universal container. Proper instructions were given to pregnant women to clean the area and to void the first part of urine into the toilet, then to collect the 'midstream' urine in the container, and again to void again the last part into the toilet. When immediate processing was not possible, samples were refrigerated at 4°C for up to four hours whereas for a delay of more than four hours, a special container with 1.8% boric acid was used for preservation.

Semi-quantitative cultures were done on blood agar and MacConkey agar with a calibrated loop. Colony counts of 10^5/ml were considered significant. Counts between 10^4/ml and 10^5/ml were of doubtful significance. Counts less than this were considered insignificant. Identification of significant isolates was done by microbiological techniques as described in the Bergey's Manual of Systematic Bacteriology (2nd Edition) which involves morphological appearance of the colonies, staining reactions and biochemical properties. Antibiotic susceptibility test of the isolated organisms was done by modified Kirby-Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines.[13]

A pre-designed interviewer and observation-based questionnaire consisting of demographic profile, clinical history, and laboratory variables was used. The primary outcome variable was UTI status, determined by urine culture positivity. Independent variables included socio-demographic factors (age, education, occupation, family income, religion), history (previous UTI, diabetes mellitus kidney stone, frequency of sexual intercourse per week, daily water intake and contraceptive use). Laboratory variables included urine routine examination findings and urine culture with antimicrobial sensitivity. Ethical approval was taken from the Institutional Review Committee (IRC Reg. No. 571, Ref No. 245), Nepalese Army Institute of Health Sciences. Written informed consent was taken from participants. The privacy and anonymity of patient information were well maintained. Data were analyzed using Microsoft Excel 2016 and R programming version 4. For primary objective, descriptive statistics including frequency, mean, standard deviation and percentage were calculated. For bivariate analysis, univariate logistic regression was used to examine associations between risk factors and asymptomatic bacteriuria. Odds ratios with 95% confidence interval were calculated.

RESULTS

A total of 390 pregnant women were enrolled in the study. In this study, we found that the mean age of participants was 28.4±4.2 years. Among the participants, 374 (95.90%) were Hindu, 8 (2.05%) were Buddhist and 8 (2.05%) were Christian. Regarding education status, 251 (64.36%) had secondary level education, 100 (25.64%) had bachelor level education, 25 (6.41%) had basic education, 7 (1.79%) were literate and 7 (1.79%) were illiterate. 318 (81.54%) participants were in occupations other than army personnel with 266 (68.20%) of them having family income in between Nepalese Rupees 10,000 to 50,000 per month (Table 1).

Among them, growth on culture was seen only in 13 (3.33%) samples. *Staphylococcus saprophyticus* were isolated from 3 (0.77%) samples which were discarded due to being commensal organisms. Thus, making true prevalence of Asymptomatic UTI in pregnancy 10 (2.56%) (1.25% - 4.71%: 95% CI). *Escherichia coli* was isolated in 5 (1.28%) samples, *Klebsiella pneumoniae* in 3 (0.77%), *Proteus mirabilis* and *Pseudomonas aeruginosa* were isolated from 1 (0.26%) each sample. Additionally, *Staphylococcus saprophyticus* (a gram-positive coccus considered a commensal) was isolated in 3 (0.77%) samples and excluded from true ASB cases. Among the true pathogens, all isolates were gram-negative bacilli (Table 2).

All 5 *E. coli* isolates (100%) were sensitive to nitrofurantoin. Proteus mirabilis shows sensitivity to amoxiclav while *P. aeruginosa* shows sensitivity to Amoxiclav and nitrofurantoin among used drugs in pregnant women.

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Table 1. Socio-Demographic characteristic of study population (n=395).

Variables	n(%)
Religion	
Hindu	374(95.90)
Buddhist	8(2.05)
Christian	8(2.05)
Others	1(0.30)
Educational status	
Bachelor	100(25.64)
Secondary Level	251(64.36)
Basic level (1 to 8)	25(6.41)
Literate	7(1.79)
Illiterate	7(1.79)
Occupation	
Army Personnel	72(18.46)
Others	318(81.54)
Family income	
<10,000	2(0.52)
10,000 - 50,000	266(68.20)
>50,000	122(31.28)

Antibiotics such as fluoroquinolones (e.g. ciprofloxacin, ofloxacin), tetracyclines (e.g. doxycycline, tetracycline), cotrimoxazole, aminoglycosides (streptomycin) were excluded from our sensitivity and resistance data due to their potential risks to fetal development and teratogenic effects (Table 2).

The bivariate analysis showed the ODDs ratio for 0-2 vs 3-7 sex per week was 11.1 (95% CI 2.80 - 44.05). Women with higher sexual frequency had 7 out of 73 (9.59%) ASB compared to 3 out 317 (0.95%) among those with low frequency. Similarly, ODDs for 0-2 vs 3-6 liters of drinking water per day was 3.5 (0.89–13.75) (Table 3).

DISCUSSION

The study aimed to identify potential associations between asymptomatic bacteriuria and various risk factors in 390 pregnant women. Out of these, only 13 participants had positive urine culture growth with 3 samples having positive growth of commensal organisms' i.e., Staphylococcus saprophyticus. Thus, making true prevalence of asymptomatic UTI in pregnancy is 10 (2.56%).

Table 2. Antimicrobial sensitivity pattern of bacteria isolated from pregnant women with asymptomatic urinary tract infection (n=390).

Bacteria	Amoxiclav	Nitrofurantoin	Cefotaxime	Cefixime	Ampicillin	Ceftriaxone
E. coli (n=5)	S: 60%, R: 40%	S: 100%	S: 60%, R: 40%	S: 60%, R: 40%	S: 60%, R: 40%	S: 60%, R: 40%
K. pneumoniae (n=3)	S:100%	S:100%	S: 66.7%, R:33.3%	S: 66.7%, R: 33.3%	S: 100%	S: 66.7, R: 33.3%
P. mirabilis (n=1)	S: 100%	R: 100%	R: 100%	R: 100%	R: 100%	R: 100%
P. aeruginosa (n=1)	S: 100%	S: 100%	R:100%	R: 100%	R: 100%	R:100%

E. coli= Escherichia coli, K. pneumoniae= Klebsiella pneumoniae, P. mirabilis= Proteus mirabilis, P. aeruginosa = Pseudomonas aeruginosa, S= Sensitive, R=Resistant

Key variables assessed included contraceptive uses; history of urinary tract infections (UTIs), DM, kidney stones; urine pH, sex per week and daily water intake. The prevalence of asymptomatic bacteriuria in our study (2.56%) aligns with findings from Nicolle LE et.al and Patterson TF et. Al who reported prevalence between 2 to 7%.[15,16] This rate also aligns with study from meta-analysis in Colombo, Srilanka 3.6%[17] and in Ghana 5.5%.[2]

However, our prevalence is lower than rates typically observed in our regional studies as that of Yadav K et.al as 42%[9], Yadav LK and Yadav RL as 51.83% in Chitwan Medical college[18], Valentina Y and Srirangaraj S as 45%[19], Shrestha T and Baral G in Paropkar Maternity and Women's Hospital as 7.1%. In our study, *Escherichia coli* was the most commonly isolated organisms among cases of asymptomatic bacteriuria, followed by *Klebsiella pneumoniae*. These findings are consistent with Yadav K et.al[9], Shrestha T et. Al[20] and other earlier studies.[21-23] The reason behind Gram negative bacteria being dominant etiologic agent may be due to poor hygienic practices causing infection by fecal contamination. Also, increased level of amino acids and lactose during pregnancy may help in E. coli proliferation.[24] Nitrofurantoin and Amoxiclav showed higher susceptibility rates among the tested antibiotics against these isolates. These findings are in line with findings by Yadav LK and Yadav RL in 2018, Yadav K et. al, and Shrestha T et al. suggesting role of nitrofurantoin as an effective first line treatment in asymptomatic bacteriuria cases in pregnancy.[9,19,20]

Our study showed associations between sex per week and urinary tract infection in pregnant women which is also supported by similar study by Raeem Hatamleh et. Al.[25] This association may be due to the anatomical relationship of vagina and urethra making it vulnerable to UTI during sexual intercourse.[26]

A association was seen in between patients who had sex 0-2 times per week versus 3-7 times per week, however due to large CI the precision is low. Daily water intake ≥ 3L was not associated with ASB (OR=3.5; CI:0.89-13.75). Although the point estimate suggested increased odds, the wide CI crossing 1.0 indicates uncertainty, likely due to sall number of ASB cases. However, study by AM Scott et. al. shows increased fluid intake shows reduced risk for UTI.[27]

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Table 3. Bivariate analysis of urinary tract infection in pregnant female at antenatal clinic (n=390).

Variables	n(%)	n*(%)	OR (95% CI)	Interpretation
Use of Contraception				
Yes	2(3.77)	51 (96.22)	1.61(0.33-7.81)	No significant association*
No	8(2.37)	329(97.63)		
History of UTI				
Yes	1(1.43)	69(98.57)	0.5(0.06-4.02)	Very few events, association is unreliable
No	9(2.81)	311(97.19)		
History of DM				
Yes	0(0.0)	13(100)	NA	No event, association is unreliable
No	10(2.65)	367(97.35)		
History of Kidney stone				
Yes	0	13(100)	NA	No event, association is unreliable
No	10(2.65)	367(97.35)		
Ph of urine				
Acidic	8(2.25)	347(97.75)	2.63(0.54–12.89)	No significant association*
Alkaline	2(5.71)	33(94.29)		
Hb level (mg/dl)				
Less than 12	4(3.67)	105(96.3)	1.75(0.48–6.31)	No significant association*
12-16	6(2.13)	275(97.86)		
Sex per week				
0-2	3(0.95)	314(99.05)	11.1(2.80-44.05)	**Significant association however wide CI suggesting low precision
3-7	7(9.59)	66(90.41)		
Daily Water Intake (L/day)				
0-2	3(1.30)	228(98.70)	3.50(0.89–13.75)	*Not Significant association, with wide CI crossing 1.0
3-6	7(4.40)	152(95.60)		

*No significant association does not mean that there is no association but it was not observed in this study; ** Sample size is calculated for prevalence therefore the study is not sufficiently powered to test association; L/day=Liters per day; UTI=Urinary tract infection, DM=Diabetes Mellitus, n= growth seen, n*= no growth seen

The study is designed to find the prevalence of UTI in pregnant female presenting to ANC clinic, therefore, sample size is calculated for prevalence hence the study is not sufficiently powered to test association.

There are some limitations to consider. This study had a relatively small number of positive culture cases, which limit the statistical power to detect associations. Appropriate sample size calculation followed by bivariate and multivariate analyses should be done to find out risk factors. Additionally, our study relied on a single urine sample per participant, which may not capture transient cases of bacteriuria that could occur at different stages of pregnancy. As convenience-based sampling was used, the possibility of selection bias and volunteer bias can't be excluded.

CONCLUSION

In conclusion, only 2.56% prevalence of asymptomatic bacteriuria with associations between daily water intake and sex per week.

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Conflict of Interest: None.

Data Availability Statement: The data are available from the

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corresponding author upon reasonable request.

Ethical Considerations: Ethical issues (including plagiarism, data fabrication, and double publication) have been completely observed by the authors.

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