ASYMPTOMATIC BACTERIURIA AMONG PREGNANT WOMEN ATTENDING A TERTIARY CARE HOSPITAL IN WESTERN NEPAL: A CROSS-SECTIONAL PROSPECTIVE STUDY

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ABSTRACT

INTRODUCTION

Urinary tract of female undergoes tremendous changes during pregnancy which increases their risk of acquiring infection. Asymptomatic bacteriuria is a common entity among pregnant women which refers to significant bacteriuria (>10^5 bacteria per ml of urine) without any typical symptoms of urinary tract infection. Asymptomatic bacteriuria can lead to various maternal and fetal complications if not detected and treated on time.

MATERIAL AND METHODS

Total 280 urine samples were collected in sterile universal containers from pregnant women not showing typical symptoms of urinary tract infection at the time of sample collection. Urine samples were inoculated in both MacConkey agar and Blood agar by semi quantitative culture method. Culture plates were reported after 24 hours of incubation at 37^0C. Bacteria isolated were subjected to antibacterial susceptibility testing by modified Kirby-Bauer disc diffusion method.

RESULTS

Out of 280 urine cultures, 213 samples were sterile. Significant bacteriuria was seen in 25 cases (8.9%) followed by insignificant bacteriuria (20, 7.14%) and contamination (17, 6.10%). Highest number of cultures positive were in age group 21-30 years (19, 9.1%). Out of 25 cases of significant bacteriuria, 60% were primigravida and 40% were multigravida. The most common organism isolated was *Escherichia coli* (10, 40%) followed by *Klebsiella pneumoniae* (5, 20%).

CONCLUSION

Screening of all pregnant women for asymptomatic bacteriuria is essential during their antenatal checkup. *Escherichia coli* is the commonest organism that cause asymptomatic bacteriuria. Appropriate antibiotic therapy is absolutely necessary for positive cases on the basis of antimicrobial susceptibility test result to prevent unwanted obstetric complications.

KEYWORDS
Asymptomatic bacteriuria, *Escherichia coli,* Pregnant women, Urinary tract infection.

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INTRODUCTION

Bacteriuria is the presence of bacteria in urine which can be either symptomatic or asymptomatic. There is continuous multiplication and activity of bacteria in urinary tract even in asymptomatic cases of bacteriuria. Presence of $\geq 10^5$ colony forming unit (CFU) or bacteria per ml of freshly passed mid-stream clean-catch urine with no typical symptoms (urgency, burning micturition, dysuria, foul smell and altered color of urine) of urinary tract infection is known as asymptomatic bacteriuria (ASB).[1] ASB is common among pregnant women and often associated with adverse maternal and fetal outcome such as acute pyelonephritis, preterm labor and low birth weight fetus.[2-4]

Urinary tract of women undergoes profound physiological and anatomical changes during pregnancy that contribute to the higher risk of infection.[5] Dilatation of ureters and renal calyces occurs as progesterone causes relaxation of smooth muscles. In addition, when gravid uterus compresses ureters and urinary bladder, vesicoureteral reflux may occur. All these factors collectively contribute to increasing the risk of urinary tract infection in pregnancy. Female urethra being short and in close proximity to anus, gastrointestinal flora easily get access to urinary tract and cause infection. Thus, pregnant women are at high risk of both symptomatic and asymptomatic urinary tract infection. Moreover, as patients do not show symptoms in ASB, cases do not seek medical advice and remain undiagnosed. Regular screening of pregnant women is essential to detect ASB. Previous studies have demonstrated factors like low socioeconomic status, high parity and informal settlement as important risk factors for asymptomatic bacteriuria.[2,3]

ASB is the most significant factor predisposing pregnant women to symptomatic bacteriuria and eventually pyelonephritis. Culture of freshly passed mid-stream clean-catch urine is the most sensitive and standard method to detect ASB in pregnant women. Cases of ASB with significant colony count (10^5 CFU/ml of urine) need treatment with antibiotic to prevent further complications. Precise antibiotic treatment on the basis of antibacterial susceptibility report may be associated with a reduction in the cases of preterm and low birth weight newborns. Antibiotic therapy can also reduce persistent bacteriuria at the time of delivery.[1]

The high prevalence of ASB among pregnant women and its probable harmful consequences to mother and baby demands timely screening and treatment of the positive cases. However, the actual scenario of ASB during pregnancy in different parts of Nepal is not known which is evidenced by the paucity of the literature on this topic. This study takes an opportunity to investigate ASB in pregnant women in Western Nepal in regards to its prevalence, demographic profile of the participants, the common microbiological isolates responsible for, their antibiotic profile and drug resistance pattern.

MATERIAL AND METHODS

This study was a cross-sectional prospective study conducted in a tertiary care hospital in Western part of Nepal with the help of preformed questionnaires and laboratory analysis of urine samples obtained from pregnant women visiting outpatient department of Gynecology for routine antenatal checkup. Total 280 urine samples were collected in sterile universal containers from pregnant women who showed no any apparent signs of urinary tract infection at the time of sample collection. The study was conducted for a time period of six months from June to December 2019.

Pregnant women were interviewed using the questionnaires in the Performa before collection of urine. Samples were sent to Microbiology Laboratory immediately after collection. Urine samples were inoculated in both MacConkey agar and Blood agar by semi quantitative culture method. Culture plates were reported after 24 hours of incubation at 37^0C. Both culture plates were observed for confluent growth or significant bacteriuria. Significant bacteriuria was considered when colony count of the bacteria grown in culture plate was $\geq 10^5$ CFU per ml of urine. Colony count less than that was considered as insignificant bacteriuria.When more than three types of bacteria were observed in culture plate and when commensals of urinary tract were noted, such cases were reported as contamination. Pure colonies from significant bacteriuria cases were subjected to Gram staining. Subsequently, suitable biochemical tests were performed and isolates were subjected to antibacterial susceptibility testing by modified Kirby-Bauer disc diffusion method in Mueller Hinton agar (MHA). The antibiotic disc selected for Gram negative bacteria were ampicillin (10 µg), amoxiclav (20/10 µg), amikacin (30 µg), cotrimoxazole (1.25/23.75 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cefuroxime (30 µg) and imipenem (10 µg).

For *Pseudomonas aeruginosa*, additional antibiotics such as piperacillin (100 µg), carbenicillin (100 µg), ceftazidime (30 µg), colistin (10 µg) and polymyxin B (300 units) were selected. Antibiotics such as penicillin G (10 units) and vancomycin (30 µg) were added for Gram positive bacteria. All these antibiotics were selected and their susceptibility pattern were reported as per the guidelines of CLSI (2018).[6] Antibiotic discs were bought from HiMedia diagnostic laboratory, Mumbai, India. For antibacterial susceptibility testing, antibiotic discs were placed on MHA maintaining 25 mm distance between two discs, incubated at 37^0C for 18-24 hours. Next morning, zone of inhibition was measured for

each antibiotic and results were interpreted according to CLSI guidelines as sensitive or resistant. Obtained data were entered in Microsoft excel software first and then transferred to SPSS 21 version for statistical analysis.

RESULTS

Total 280 samples were collected from pregnant women visiting Gynecology outpatient department for regular antenatal checkup with no any typical symptoms of urinary tract infection. There was no growth in 218 samples out of 280 (77.85%) urine culture. Significant bacteriuria was seen in 25 cases (8.9%) followed by insignificant bacteriuria (20, 7.14%) and contamination (17, 6.10%) [Table 1].

Table 1. Results of urine culture of pregnant women

Culture result	Number of cases	Percentage
Significant bacteriuria	25	8.90%
Insignificant bacteriuria	20	7.14%
Contamination	17	6.10%
Sterile	218	77.85%
Total	280	100%

Highest number of cultures positive cases were observed in age group 21-30 years (76%, 19/25). Out of 25 cases of significant bacteriuria, 60% were primigravida and 40% were multigravida [Table 2].

Table 2. Distribution of culture positive cases with respect to age and parity

Age group	Culture positive (n/%)	Culture negative (n/%)
<20 years	0 (0%)	13 (5.1%)
21-30 years	19 (76%)	190 (74.5%)
31-40 years	6 (24%)	46 (18.0%)
>40 years	0 (0%)	6 (2.4%)
Parity		
Primigravida	15 (60%)	163 (63.9%)
Multigravida	10 (40%)	92 (36.1%)
Total	25 (100%)	255 (100%)

The most common organism isolated was *Escherichia coli* (10, 40%) followed by *Klebsiella pneumoniae* (5, 20%) [Table 3]. Gram positive bacteria were also isolated but comparatively less in number than Gram negative bacteria. *Staphylococcus aureus* (3, 12%) was the commonest organism isolated among Gram positive bacteria.

Table 3. Distribution of bacterial isolates in culture positive cases

Bacterial isolates	Number of cases	Percentage
Escherichia coli	10	40%
Klebsiella pneumoniae	5	20%
Pseudomonas aeruginosa	3	12%
Staphylococcus aureus	3	12%
Enterococcus spp	2	8%
Proteus mirabilis	1	4%
Streptococcus agalactiae	1	4%
Total	25	100%

Both *Escherichia coli* and *Klebsiella pneumoniae* were 100% sensitive to imipenem and 100% resistant to ampicillin. *Escherichia coli* were highly sensitive to ciprofloxacin (90%), nitrofurantoin (90%), amikacin (80%), ceftriaxone (70%), amoxiclav (60%) and norfloxacin (60%) whereas less sensitive to cotrimoxazole (50%) and cefuroxime (40%). Similarly, *Klebsiella pneumoniae* showed high sensitivity to amikacin (100%), norfloxacin (100%), nitrofurantoin (100%), ciprofloxacin (100%), ceftriaxone (100%), cotrimoxazole (80%), amoxiclav (60%) and cefuroxime (60%). Among Gram positive bacteria, *Staphylococcus aureus* was the commonest isolate which was 100% sensitive to amikacin, ciprofloxacin, nitrofurantoin and vancomycin. Sensitivity to cotrimoxazole and ceftriaxone was 66.6% whereas sensitivity to norfloxacin, cefuroxime and penicillin was 33.3% [Table 4].

Table 4. Antimicrobial susceptibility pattern of the isolated organisms

Antibiotics	*E. coli* n (%)	*K. pneumoniae* n (%)	*P. aeruginosa* n (%)	*P. mirabilis* n (%)	*S. aureus* n (%)	*Enterococcus* spp n (%)	*S. agalactiae* n (%)
AMP	0 (0%)	0 (0%)	0 (0%)	1 (100%)	ND	ND	ND
AMC	6 (60%)	3 (60%)	1 (33.3%)	1 (100%)	ND	ND	ND
AK	8 (80%)	5 (100%)	3 (100%)	1 (100%)	3 (100%)	2 (100%)	1 (100%)
NX	6 (60%)	5 (100%)	3 (100%)	1 (100%)	1 (33.3%)	0(0%)	1 (100%)
CIP	9 (90%)	5 (100%)	3 (100%)	1 (100%)	3 (100%)	2 (100%)	1 (100%)
NIT	9 (90%)	5 (100%)	2 (66.6%)	1 (100%)	3 (100%)	2 (100%)	1 (100%)
COT	5 (50%)	4 (80%)	3 (100%)	1 (100%)	2 (66.6%)	2 (100%)	1 (100%)
CTR	7 (70%)	5 (100%)	3 (100%)	1 (100%)	2 (66.6%)	2 (100%)	1 (100%)
CAZ	ND	ND	3 (100%)	ND	ND	ND	ND
PI	ND	ND	2 (66.6%)	ND	ND	ND	ND
CB	ND	ND	2 (66.6%)	ND	ND	ND	ND
CL	ND	ND	3 (100%)	ND	ND	ND	ND
PB	ND	ND	3 (100%)	ND	ND	ND	ND
CFM	4 (40%)	3 (60%)	1 (33.3%)	0 (100)	1 (33.3%)	0 (0%)	1 (100)
IPM	10(100%)	5 (100%)	3 (100%)	1 (100%)	ND	ND	ND
P	ND	ND	ND	ND	1 (33.3%)	1 (50%)	1 (100%)
VA	ND	ND	ND	ND	3 (100%)	2 (100%)	1 (100%)

*AMP-Ampicillin (10 µg), AMC-Amoxiclav (20/10 µg), AK-Amikacin ((30 µg), NX-Norfloxacin (10 µg), CIP-Ciprofloxacin (5 µg), NIT-Nitrofurantoin (300 µg), COT-Cotrimoxazole (1.25/23.75 µg), CTR-Ceftriaxone (30 µg), CAZ-Ceftazidime (30 µg), PI-Piperacillin (100 µg), CB-Carbenicillin (100 µg), CL-Colistin (10 µg), PB-Polymyxin B (300 units), CFM-Cefuroxime (30 µg), IPM-Imipenem (10 µg), P-PenicillinG(10units), VA-Vancomycin(30µg)

DISCUSSION

Pregnant women with asymptomatic bacteriuria have increased risk to suffer from symptomatic urinary tract

infection and various obstetric complications later.[7] Timely screening and treating ASB with appropriate antibiotic has become a new protocol of a standard care of pregnant women. Along with other routine investigations during regular antenatal visits, culture of urine is essential for the pregnant women even when they do not have urinary problems.

Asymptomatic cases of significant bacteriuria more than 1,00,000 bacteria/ml of urine was only considered as asymptomatic bacteriuria (ASB) among pregnant women in this study. The rate of ASB detected by this study was 8.9% which is higher than 7.3% reported by a study in India and lower than 10% reported from Egypt.[8,9] Some studies reported higher prevalence rate of ASB up to 45.3%.[10] Within Nepal, Neupane et al reported 26% of ASB from Chitwan.[11] These huge variations in prevalence of ASB from place to place may be associated with environmental conditions, socioeconomic status, personal hygiene, education level, number of pregnancies, maternal age, religious beliefs and sexual behavior of the study population.

Highest number of cultures positive cases were in age group 21-30 years (76%, 19/25). Out of 25 cases of significant bacteriuria, 60% were primigravida and 40% were multigravida. These findings are consistent with the results of a study by Sujatha R *et al* where as another study in Yemen reported a highest prevalence of ASB among 35-39 years of pregnant women.[8,12] Women in different countries prefer different part of their reproductive age to start family. In this study, maximum pregnant women (74.6%, 209/280) were in the age group 21-30 years and maximum ASB was also seen in the same group.

The most common organism isolated was *Escherichia coli* (10, 40%) followed by *Klebsiella pneumoniae* (5, 20%) in this study. *Escherichia coli* is the normal flora of gastrointestinal tract which can easily get access to urinary tract because of their close proximity in female. In pregnant women also, *Escherichia coli* is the predominant organism to cause ASB which is evidenced by many studies.[8,13-16] *Escherichia coli* was 100% sensitive to imipenem and 100% resistant to ampicillin. *Escherichia coli* were highly sensitive to ciprofloxacin, nitrofurantoin, amikacin, ceftriaxone, amoxiclav and norfloxacin whereas less sensitive to cotrimoxazole and cefuroxime. Only 24 % of the causative organisms of ASB were Gram positive bacteria. The commonest organism among Gram positive bacteria was *Staphylococcus aureus*. Some studies reported Gram positive bacteria as the commonest cause of ASB.[17,18] A study in Iran reported 16.8% of coagulase-negative staphylococci as a cause of ASB.[17] Other studies also reported coagulase-negative staphylococci as an important cause of ASB.[18]

Common organisms causing ASB such as *Escherichia coli* and *Klebsiella pneumoniae* showed high sensitivity to amikacin, norfloxacin, nitrofurantoin, ciprofloxacin, ceftriaxone and imipenem. Same results were observed in various other studies.[8,10] A study recommended Fosfomycin as an important drug to treat ASB in pregnant women considering its high sensitivity, convenient route of administration and safe nature.[7] Microorganisms are evolving day by day and becoming resistant to commonly used drugs. Irrational use of antibiotics that includes availability of the drugs over the counter without the prescription by physician and not considering culture and sensitivity reports are the major factors for the emergence of drug resistant strains of bacteria.[19,20] The knowledge of the common pathogens that cause ASB in a particular population and their antimicrobial susceptibility pattern is irreplaceable for reducing maternal and fetal morbidity and mortality by ASB. In addition, teaching pregnant women to maintain personal hygiene and cleanliness of area around urogenital organ and anus helps to reduce the contamination of urinary tract by gastrointestinal flora.

LIMITATION

Sample size and duration of this study were less. Extending the duration of the study and increasing the sample size will add value to this study. Number of microorganisms isolated from urine cultures were few. So, the antimicrobial susceptibility test report of each bacterium may not echo with the antibiogram of the bacteria in general.

CONCLUSION

Asymptomatic bacteriuria is common among pregnant women. Periodic screening of all pregnant women by urine culture for asymptomatic bacteriuria is essential along with other routine laboratory investigations during their antenatal checkup. Asymptomatic bacteriuria can progress to symptomatic cases and lead to maternal and fetal complications in pregnancy. Appropriate antibiotic therapy is absolutely necessary for asymptomatic bacteriuria to prevent these consequences.

CONFLICT OF INTEREST

None

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