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Original Article

PREVALENCE OF ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN ATTENDING TERTIARY CARE HOSPITAL-A CROSS-SECTIONAL STUDY

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ABSTRACT

Objective: Urinary tract infection (UTI) is one of the most common bacterial infections during pregnancy due to anatomical changes and physiological adaptations during pregnancy. Asymptomatic bacteriuria is the significant presence of bacteria in the urine of an individual without symptoms. Untreated asymptomatic bacteriuria (ASB) in pregnancy predisposes to symptomatic UTI in 25% of infected women. Screening of antenatal women help in early diagnosis and treatment of ASB and thus to prevent maternal and fetal morbidity and mortality. The present study was carried out to determine the prevalence of UTI in pregnant women and to study the bacteriological profile and antimicrobial sensitivity patterns of uropathogens.

Methods: A Cross-sectional study was conducted for a period of six months and midstream urine specimens were collected from 480 pregnant females and were processed by standard protocols. All subjects were clinically identified to have no signs and symptoms of UTI. Antibiotic susceptibility testing was done as per CLSI guidelines.

Results: Prevalence rate of asymptomatic bacteriuria was seen 10% in pregnant women. Majority of the culture-positive patients belonged to the age group of 26-30 y (31.25%). 70.84% were Gram-negative isolates and 29.16% were Gram-positive organisms. The commonest pathogen isolated was Escherichia coli (33.33%). In the present study, Extended Spectrum Beta-Lactamase (ESBL) production was seen in (20.58%) isolates, and Metallo Beta-Lactamase (MBL) production was seen in (17.64%) isolates.

Conclusion: This study reveals the importance of screening of pregnant women for UTI. Emerging multi-drug resistance seen in uropathogens emphasizes the need to rationalize use of antibiotics, which eventually prevent development of resistant strains.

Keywords: Asymptomatic bacteriuria, Pregnant, Urinary tract infection, Uropathogens

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INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common infectious diseases that we encounter in hospital settings. UTI in pregnancy can result in serious life-threatening complications if left untreated [1]. Asymptomatic bacteriuria is one of the clinical manifestations of UTI. It is defined as persistently and actively multiplying bacteria in significant numbers (more than 10,000 per milliliter) within the urinary tract without any obvious symptoms [2]. Predisposing determinants of high prevalence of UTI in pregnancy include hormone-induced ureteral dilatation, urinary stasis, reduced immune function, and presence of vesicoureteral reflux [2]. Maternal complication includes overt pyelonephritis in 25%–40% of patients as pregnancy advances among those with asymptomatic bacteriuria, and in 1%–2% in those without asymptomatic bacteriuria [3]. The fetus is at risk of prematurity, low birth weight, intrauterine growth restriction, and fetal death [4]. The adverse effects of undiagnosed asymptomatic bacteriuria on mother and child have made researchers to suggest routine culture screening for all pregnant women attending antenatal checkups to prevent mother and child from any form of complication that may arise due to infection [4].

It is universally accepted that UTI can only be ascertained based on microscopy and microbial culture. The dipstick/dip-slide method used in many centers serves only as a screening method but culture is needed for the final diagnosis. When bacteria are detected on screening, it should be treated [5, 6]. In general, only the Penicillin and Cephalosporin can be regarded as safe throughout the gestation. A 3-day course of an oral agent in either class should be employed for screening bacteriuria. Although Escherichia coli and other gram-negative rods are associated with pyelonephritis during pregnancy, other organisms may be important in other adverse pregnancy outcomes [7]. A continuous research studies are carried on the prevalence of urinary tract pathogens in asymptomatic UTI in pregnant women and their antimicrobial sensitivity and resistant patterns. Such studies would further help in laying down antibiotic policies, to prevent development of multidrug resistant pathogens. Aim of the study is to determine the prevalence of asymptomatic bacteriuria, its etiological agents and their antibacterial susceptibility pattern among the pregnant women attending the Tertiary Care Hospital.

METHODS AND MATERIALS

Study design

The cross-sectional study was conducted in Department of Microbiology, Andhra Medical College, and Visakhapatnam for a period of six months from October 2023 to March 2024. 480 pregnant women attending out-patient department of King George Hospital, Visakhapatnam were enrolled for the study during the time period. Total 480 urine samples were collected from the subjects enrolled for the study.

Pregnant women with varying gestational periods from 18-45 y without any symptoms of UTI were included in the study. Pregnant women with known congenital anomalies of urinary tract, Diabetes or Gestational diabetes, signs and symptoms of UTI, pyrexia, history of antibiotic therapy for any reason within 72 h of specimen collection were excluded from the study. Information regarding age, gravida, trimester, date of last menstruation period, associated risk factors, and expected delivery date were documented. The study was approved by Institutional Ethics Committee.

Statistical analysis

Data analysis was done using the statistical software SPSS v 23. Percentages were calculated for qualitative variables. Statistical analysis was performed by the chi-square ($\chi 2$) test. P-value of<0.05 was deemed statistically significant.

Methodology

Specimen collection

The specimen is collected preferably at 1st ante-natal visit as per ACOG (American College of Obstetrics and Gynecology) guidelines. Clean-catch midstream urine (MSU) samples received in leak proof wide-mouthed sterile screw-capped container from the participants and transported immediately to Microbiological laboratory for isolation of microbiological isolates [8].

Processing of samples

Initially, samples were examined microscopically. Semi-quantitative culture of urine samples was done on blood and MacConkey agar by standard calibrated bacteriological loop technique [9]. Culture results were reported based on standard Kass criteria [10]. Bacterial pathogens were identified by gram reactions, motility, and biochemical characteristics as per standard microbiological techniques [11]. Growth was interpreted as significant in Gram-negative bacteria if colony count was>= to 105 colony forming unit per milliliter (CFU/ml) of urine and if the colony count is below 10 5 considered as insignificant growth [12]. In Gram-positive bacteria colony count of 102 CFU/ml of urine was considered significant [13]. All bacterial isolates showing significant growth were identified by standard biochemical methods [14]. More than one type of colonies on culture plate was considered as contamination and repeat sample was requested. The antibiotic sensitivity testing (AST) of the isolates was be done by Kirby Bauer method according to CLSI guidelines [15, 16]. MRSA was detected using Cefoxitin 30µg disc. ESBL production and MBL production in Gram-negative bacteria was detected by using Potentiated Disc Diffusion test (PDT). The resistance patterns were further determined by E-test by interpreting Minimum inhibitory concentration (MIC) values (mcg/ml) [17].

RESULTS

A total of 480 urine specimens from pregnant women were screened for asymptomatic bacteriuria. 72 (15%) urine specimens yielded bacterial growth. Out of which 48 (10%) samples showed significant growth. Majority of urine specimens yielded significant bacterial growth belonged to the age of 26-30 y 15 (31.25%) followed by 21-25 y (25%), 15-20 y (20.83%),>35 y (12.51%) and 31-35 y (10.41%) table 1.

Table 1: Age-wise distribution of asymptomatic bacteriuria

Age (in years)	No. of Samples	No. of positive samples	% of positive samples
15-20 y	76	10	20.83%
21-25 y	120	12	25%
26-30 y	168	15	31.25%
31-35 y	72	5	10.41%
> 35 y	44	6	12.51%
Total	480	48	10%

Significant bacterial growth was found in the specimens collected during three trimesters. Predominantly growth was seen from the urine specimens collected during second 19 (39.58%) and third trimester 17 (35.41%). least isolation rate was seen in first trimester 12(25%) (table 2)

Table 2: Trimester-wise distribution of asymptomatic bacteriuria

Trimester	No. of Samples	No. of positive samples	% of positive samples
1st trimester	260	12	25%
2nd trimester	140	19	39.58%
3rd trimester	80	17	35.42%
Total	480	48	10%

In the present study, amongst all the bacterial isolates, Escherichia coli 16 (33.33%) was the predominant isolate followed by Klebsiella species 10 (20.83%), Staphylococcus aureus, 5 (10.41%) Enterococcus species 5 (10.41%), Coagulase Negative Staphylococci 4 (8.33%), Proteus species 4 (8.33%), Pseudomonas aeruginosa 2 (4.16%) and Acinetobacter baumanii 2 (4.16%).

Table 3: Distribution of bacterial isolates in asymptomatic bacteriuria

Organism	Total number of isolates	% of Isolates
Escherichia coli	16	33.33%
Klebsiella species	10	20.83%
Staphylococcus aureus	5	10.41%
Enterococcus species	5	10.41%
Coagulase Negative Staphylococci	4	8.33%
Proteus species	4	8.33%
Pseudomonas aeruginosa	2	4.16%
Acinetobacter baumanii	2	4.16%
Total	48	10%

The total isolates obtained were 48 (10%) out of total 480 samples. Gram-positive isolates were 14 (29.16%), gram-negative isolates were 34 (70.84%), respectively.

Among the Gram-positive cocci, Staphylococcus aureus was 100% sensitive to Teicoplanin followed by linezolid (100%), Vancomycin (80%), levofloxacin (80%), Azithromycin (80%), Cefoxitin (60%), Amoxicillin and Clavulanate (40%), Ceftriaxone (40%) and Norfloxacin (40%). Coagulase Negative Staphylococci were 100% sensitive to linezolid, Teicoplanin followed by Vancomycin (75%), Azithromycin and levofloxacin (75%), Ceftriaxone, Norfloxacin and Cefoxitin (50%), Amoxicillin+Clavulanate (25%). and Enterococcus faecalis showed 100% sensitive to Vancomycin and linezolid.

Out of total 5 Staphylococcus aureus isolates, 2(40%) were Methicillin Resistant Staphylococcus aureus (MRSA) and 3(60%) were Methicillin Sensitive Staphylococcus aureus (MSSA).

Table 4: Antibiotic susceptibility pattern of gram-positive cocci (n = 14)

Organism	NX	AMC	CX	CTR	TEI	LE	AZM	VA	LZ
Staphylococcus aureus (n=5)	2 (40%)	3 (60%)	3 (60%)	2 (40%)	5 (100%)	4 (80%)	4 (80%)	4 (80%)	5 (100%)
Coagulase negative Staphylococci (n=4)	2 (50%)	1 (25%)	2 (50%)	2 (50%)	4 (100%)	3 (75%)	3 (75%)	3 (75%)	4 (100%)
Enterococci species (n=5)	3 (60%)	4 (80%)	4 (80%)	4 (80%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)

Among the Gram-negative isolates varied antimicrobial susceptibility pattern has been noted. Escherichia coli showed 100% sensitivity to Piperacillin+Tazobactam, followed by Ceftazidime+Clavulanic acid (87.5%), levofloxacin (87.5%), Meropenem (75%), Ceftazidime (75%), Nitrofurantoin (75%), Amikacin (62.5%), Amoxyclav (56%), and Ampicillin (31.2%). Among the Klebsiella pneumoniae isolates 100% showed sensitivity to Piperacillin+Tazobactam and Ceftazidime+Clavulanic acid. 90% sensitivity to levofloxacin, 80% sensitivity to Meropenem, Nitrofurantoin and Amikacin and 75% sensitivity to Ceftazidime. 50% sensitivity to and Amoxyclav and least sensitive to Ampicillin. Proteus mirabilis isolates were 100% sensitive to Piperacillin+Tazobactam, Ceftazidime+Clavulanic acid, Amikacin, levofloxacin and Meropenem and 75% sensitivity to Ceftazidime and Nitrofurantoin. 50% sensitivity to Amoxyclav, and least sensitive to Ampicillin (25%). Pseudomonas aeruginosa showed 100% sensitivity to Piperacillin+tazobactam, Ceftazidime+clavulanic acid, Meropenem and levofloxacin. 50% sensitivity to Ceftazidime, Amikacin and Nitrofurantoin. Acinetobacter baumannii showed 100% sensitivity to Piperacillin+Tazobactam, levofloxacin and Meropenem. Whereas 100% resistance has been reported for Ampicillin, Amoxyclav, Ceftazidime, Ceftazidime+clavulanic acid, Amikacin and Nitrofurantoin

In the present study, out of total 34 Gram-negative isolates, Extended Spectrum Beta-Lactamase (ESBL) production was seen in 7 (20.58%) isolates and Metallo Beta-Lactamase (MBL) production was seen in 6 (17.64%) isolates.

Table 5: Antibiotic susceptibility pattern of gram-negative bacilli (n = 34)

Organism	AMP	AMC	PIT	CAZ	CAC	MRP	AK	LE	NIT
Escherichia coli (16)	5 (31.2%)	9 (56%)	16 (100%)	12 (75%)	14 (87.5%)	12 (75%)	10 (62.5%)	14 (87.5%)	12 (75%)
Klebsiella species (10)	3 (30%)	5 (50%)	10 (100%)	7 (70%)	10 (100%)	8 (80%)	8 (80%)	9 (90%)	8 (80%)
Proteus species (4)	1 (25%)	2 (50%)	4 (100%)	3 (75%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	3 (75%)
Pseudomonas aeruginosa (2)	0	0	2 (100%)	1 (50%)	2 (100%)	2 (100%)	1 (50%)	2 (100%)	1 (50%)
Acinetobacter baumanii (2)	0	0	2 (100%)	0	0	2 (100%)	0	2 (100%)	1 (50%)

DISCUSSION

Urinary tract infections are most common infections in females, especially in pregnancy. ASB in pregnancy is known to cause grave obstetric complications. Hence it is very important to screen all pregnant women for bacteriuria in every trimester and administer appropriate treatment to prevent perinatal and maternal complications [18, 19]. Incidence of ASB in our study was 10%. Different Indian and international studies showed incidence range of ASB as 2 to 50%. Difference in incidence in studies across the world and in same country is due to differences in geographical location, social behavior, level of education, study population and sample size of participants. The age, gravida and trimester of participants did not have any statistical significance on ASB in present study. The reason for age distribution not having significant association with ASB in present study may be due to the factor that majority of antenatal women enrolled in this study belonged to 20-30 y than those between 31-45 y age group. The parity and gestational age not having significant association with ASB in present study correlated with previous researchers [20-22] In present study both Gram-negative and Gram-positive bacteria were predominantly responsible for ASB during pregnancy. The most common Gram-negative Uropathogen was Escherichia coli (33.33%). Different studies done by Patel *et al.* (33.33%) [23], Harish Babu *et al.* (33.33%) [24], Gopalakrishnan R. *et al.* (32.6%) [25], Ali *et al.* (31.04%) [26], and Bose *et al.* (26.92%) [27], showed Escherichia coli as most common uropathogen.

In the present study, the prevalence of asymptomatic bacteriuria was found to be 10%. This is in agreement with the studies conducted by Harish Babu *et al.* (10%) [24], Alemuet (10.4%) [28], Kehinde *et al.* (10.7%) [29] and Kalagara P *et al.* (10.9%) [30]. According to Turpin *et al.* [31] the prevalence of asymptomatic bacteriuria was found to be 7.3% and another study conducted by Gayathree *et al.* [32] showed 6.2% of asymptomatic bacteriuria among pregnant women. Least prevalence was shown Sheiner *et al.* [21] only 2.5% ASB among pregnant women.

Few studies conducted previously showed high prevalence of asymptomatic bacteriuria among pregnant women. According to the study conducted by Akerele *et al.* [33], the prevalence rate of asymptomatic bacteriuria was 86.6% among pregnant women. As per Patel *et al.* [23], prevalence rate of asymptomatic bacteriuria among pregnant women was 13.2%. These varying results may have been due to differences in the areas being studied, in the social habits of the communities being studied and in the socio-economic statuses, standards of personal hygiene and education levels of the patients being studied.

Asymptomatic bacteriuria was predominantly found between the age group of 26-30 y (31.25%) followed by 21-25 y (25%), which correlated with Reshma Gopalakrishnan *et al.* [25]. As per the study conducted by Shirazi *et al.* [34] prevalence was 13.8% in age group less than 21 y compared to 3% in age group over 30 y. As per Alghalibi *et al.* [35] prevalence of ASB was high in pregnant women whose age ranged between 21-25 y of age. But in the study conducted by Turpin *et al.* [31] higher prevalence of ASB in pregnant women was ranged between 35-39 y of age. This high incidence of ASB in the young reproductive age group is due to early pregnancy and multiparity in our country, particularly in the rural areas.

In our study, prevalence rate asymptomatic bacteriuria during second and third trimester was 39.58% and 35.42%, respectively. Prevalence rate of ASB among urine specimens collected during first trimester showed only 25%, which correlated with study of Abbas N *et al.* [36], Mukherjee K *et al.* [37], Verma A *et al.* [38] and R Sandhiya *et al.* [39] who reported lowest asymptomatic bacteriuria respectively 23.81%, 18.51%, 25% and 22.73% in the first trimester which correlates with our findings. According to the study conducted by Patel P *et al.* [23] asymptomatic bacteriuria was found to be high in 3rd trimester (68.18%) compared to 2nd trimester (22.78%) and in the 1st trimester (10.6%) of pregnancy. Similar studies conducted by Prasanna *et al.* [40] and Abbas *et al.* [36] majority of the women with ASB were in 3rd trimester (49% and 50%, respectively). The incidence of ASB is more pronounced in the third trimester, may be due to the changes related to advancing gestational age. This leads to stasis of urine and encourage bacterial multiplication.

In the present study, Escherichia coli was the predominant bacteria isolated and accounted for 33.33% followed by Klebsiella species

(20.83%), Staphylococcus aureus (10.41%), Enterococcus species (10.41%), Coagulase negative Staphylocooci (8.33%), Proteus species (8.33%), Pseudomonas aeruginosa (4.16%) and Acinetobacter baumanii (4.16%) respectively. Patel P *et al.* (33.33%) [23], Reshma Gopalakrishnan *et al.* (32.6%) [25] Prasanna N *et al.* (54.55%) [40] and Imade PE *et al.* (27.1%) [41] Have also showed that Escherichia coli as the commonest isolate.

Antimicrobial susceptibility of uropathogens differ from region to region and even from hospital to hospital due to the emergence of resistant strains caused by the indiscriminate use of antibiotics. In our study majority of bacteria were resistant commonly used antibiotic ampicillin. Resistance to ampicillin was exhibited by majority of strains of E. coli in European countries and Canada averaged 29·8%, but was as high as 53·9% in Spain. In our study E. coli was sensitive to Piperacillin+Tazobactam, levofloxacin, Ceftazidime+Clavulanic acid, Ceftazidime, Meropenem, Nitrofurantoin and Amikacin but resistant to Ampicillin. Gram positive cocci was sensitive to linezolid, Vancomycin, Azithromycin, levofloxacin, Ceftazidime+clavulanic acid, Cefoxitin and Amoxyclav but resistant to Ceftriaxone and Cefoxitin.

In the present study Nitrofurantoin was found to be the most effective antibiotic among commonly used antimicrobials against uropathogens, which correlated with studies of Kalagara *et al.* [30], Reshmi Gopalakrishnan *et al.* 25] and Harish Babu *et al.* [24]. But certain limitations restrict the usage of nitrofurantoin. Fluroquinolones and Cephalosporins are the mainstay of treatment of UTI in Antenatal women due to less side effects.

CONCLUSION

In our study, prevalence of asymptomatic bacteriuria among pregnant women was 10%. Escherichia coli was the predominant pathogen isolated. Nitrofurantoin was found to be the most effective drug among commonly used antibiotics to treat urinary tract infections but is restricted in antenatal women due to its limitations. Fluroquinolones and Cephalosporins are used in pregnant women for UTI due to less side effects. Every pregnant woman in each trimester should have a urine culture done and detected cases should be treated according to the antibiotic susceptibility test. In present study both gram negative and gram-positive bacteria were predominantly responsible for ASB. Culture plays a pivotal role as choice of antibiotic varies with the uropathogen isolated. Increasing resistance to commonly used antibiotics shows selective pressure due to their increased prescription. Antibiotic susceptibility testing will aid policy makers to determine the antibiotics to be used for ASB thereby reducing the perinatal and maternal morbidity and mortality.

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AUTHORS CONTRIBUTIONS

The authors made a substantial and contribution to the work and approved it for publication.

CONFLICT OF INTERESTS

Declared none

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