RESEARCH
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MICROBIAL CHALLENGES IN MATERNITY: PREVALENCE OF ASYMPTOMATIC BACTERIURIA AMONG PREGNANT WOMEN

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Abstract
Background: Asymptomatic bacteriuria, the presence of bacteria in urine without symptoms, is more common in pregnant women due to physiological changes. There is controversy over the need for antibiotic therapy even though untreated instances might result in complications such as pyelonephritis. Though worries over antibiotic side effects and trial biases continue, screening suggestions are prompted by variations in prevalence throughout the world.
Material and Methods: A cross-sectional descriptive study was conducted among the pregnant women attending for antenatal care. Mid-stream urine sample was collected. This study included 216 women of reproductive age group who were pregnant. Simple random sampling method was used in selecting study participants based on inclusion and exclusion criteria. All samples were subjected to standard microbiological procedures.
Results: Significant bacteriuria was observed in 19 urine samples and accounted for 8.8% prevalence. Significant difference was observed in the prevalence of asymptomatic bacteriuria with respect to age, parity and trimester (p. 0.001). The highest proportion of asymptomatic bacteriuria was observed between the age group 18-25 (6.02%). In the present study, preponderance of ASB was observed in third trimester 14(4.48%). E.coli and Coagulase Negative Staphylococci were the common isolates observed. Majority of isolates were found to be susceptible to Amoxicillin-clavulanate.
Conclusions: Significant bacteriuria, which was detected in 8.8% of samples, revealed differences in the incidence of asymptomatic bacteriuria according to trimester, age, and parity. The management of asymptomatic bacteriuria should take age, parity, and trimester-specific factors into account as E.coli and Coagulase Negative Staphylococci were frequent isolates that were mostly susceptible to Amoxicillin-clavulanate.
KeyWords: Significant bacteriuria, E.coli, Pyuria

INTRODUCTION

Pregnant women undergo distinct metabolic changes that may influence their immune system, perhaps rendering them more vulnerable to certain infections. Pregnancy-related infections are an essential concern since they can seriously endanger the growing fetus's health as well as the mother's. Asymptomatic bacteriuria refers to the presence of bacteria in urine. It is a condition in which urine culture reveals a significant growth of pathogens that is greater than10^5 bacteria/ml, but without the patient showing symptoms of urinary tract infection (UTI)[1]. While ASB is uncommon in infants and young children, women face a higher risk than men, and the likelihood of ASB increases with age. It's important to note that the majority of individuals diagnosed with ASB do not progress to symptomatic UTIs and generally experience no adverse effects. But pregnant women undergo anatomical and physiological changes in the urinary tract, resulting in an increase in the risk of ASB[2].

Factors like hydroureter, renal pelvis dilation, glycosuria, and aminoaciduria, contribute to urine stasis and provide an optimal environment for the proliferation of diverse bacterial species[3]. In addition to the defective vesicoureteral reflux and ureteric valves, decreased urine osmolality caused by physiologic shift also promotes bacterial colonization and increases ascending infection.
There is much of fluctuation in the reported risk of pyelonephritis associated with untreated asymptomatic bacteriuria in pregnancy Pyelonephritis has been linked to disastrous fetal outcomes like low birth weight and premature delivery as well as maternal septicemia, renal dysfunction, and anaemia[5]. However, asymptomatic bacteriuria was not linked to premature birth, according to a recent research. Therefore, it is unclear if asymptomatic bacteriuria and complications during pregnancy are related[6].
According to reports, the prevalence of ASB ranges between 2–10% worldwide. But various studies show prevalence higher than this from different parts of the world[7,8]. In order to administer antibiotics when bacteriuria is found, screening for asymptomatic

bacteriuria is done. Screening recommendations for asymptomatic bacteriuria must take into account both the potential advantages and risks of antibiotic use during pregnancy. However, randomised controlled trials (RCTs) of antibiotic therapy for asymptomatic bacteriuria in pregnancy have come under scrutiny for inadequately disclosing antibiotic side effects and other forms of bias[9].

There are scarce data on the prevalence of ASB in the study region. The aim of this study was to determine the prevalence of ASB, its associated factors, and antimicrobial susceptibility profile of bacterial isolates among pregnant women attending Government General Hospital[7].

METHODOLOGY

A cross-sectional descriptive study was conducted among the pregnant women attending for antenatal care at Government general hospital, Kadapa. All the laboratory procedures were carried out in the department of Microbiology from June 2023 to August 2023. This study included 216 women of reproductive age group who were pregnant. Simple random sampling. method was used in selecting study participants based on inclusion and exclusion criteria.

Inclusion criteria: Pregnant women aged 18 years or more with singleton pregnancy attending Obstetrics OPD for antenatal care with no history of increased frequency of micturition, dysuria, loin pain or fever.

Exclusion criteria: Pregnant women with vaginal discharge or bleeding per vagina, pregnancy induced Diabetes Mellitus/ Hypertension, known congenital anomalies of the urinary tract and individuals who have received antibiotics in past two weeks prior to urine sample collection.

The participant's socio-demographic status, medical and obstetric information was obtained. A clean catch mid-stream specimen of urine was collected after giving appropriate instructions to the participants during sample collection and the specimens were sent for culture and sensitivity within two hours of collection. A wet mount preparation of loopful of well-mixed uncentrifuged urine was examined for pus cells, red blood cells, casts, parasites and fungi. Culture for aerobic microorganisms in urine was done by inoculation on MacConkey agar and Blood agar using standard loop method (Semiquantative method)[10].

The plates were read after 24 hrs of incubation at 37°C. They were incubated for another 24 hrs before a negative report was issued. A sample with single organism obtained in counts $>10^5$ colony forming units (cfu/ml) was taken as positive. Sensitivity testing was done using drugs safe in pregnancy namely Amoxycillin-clavulanate, Ampicilin, Cephalexin, Cefuroxime, Cefotaxim, Amikacin, Gentamicin and Nitrofurantoin.

Determination of Methicillin Resistant Staphylococcus aureus: Methicillin resistance was evaluated using cefoxitin disc (10µg) on Mueller-Hinton agar. An inhibition zone diameter of ≤ 21mm indicated Methicillin Resistant Staphylococcus aureus (MRSA).

Screening for potential ESBL producing isolate: Detection of Extended spectrum β-lactamases was performed using a combined disc test. A disk of Ceftazidime (30µg) and Ceftazidime + Clavulanic acid (30µg/10µg), were placed at appropriate distance (15mm apart) on a Muller-Hinton Agar (MHA) plate. An increase in the inhibition zone diameter of greater than 5 mm for a combination disc versus ceftazidime disc alone was an indication of ESBLs production[11].

Quality control strains: Quality control for the new batch was performed using E. coli (ATCC 25922) to check the quality of culture media and antibiotic discs. For ESBL and MRSA confirmatory test, K. pneumoniae ATCC 700603 (ESBLs positive) and E. coli ATCC 25922 (ESBLs negative) control strains, Methicillin sensitive S.aureus (MSSA) ATCC 25923, Methicillin resistant S.aureus (MRSA) ATCC 43300 were used to check the quality of the commercially purchased antibiotic discs.

Prior to entering the data, the precision and thoroughness of the data collecting form were verified. Results of tests for antibiotic susceptibility and culture were meticulously documented prior to being imported into SPSS programming (version 20).

Ethical considerations

Ethical approval was obtained from institutional ethics committee of the institution (IEC approval No.ACAD/E3B/2023). Written Informed consent was obtained from all participants. The laboratory results of participants were made available to the clinicians for management of the asymptomatic urinary tract infection.

RESULTS

A total of 216 urine samples were processed during the study period. Significant bacteriuria was observed in 19 urine samples and accounted for 8.8% prevalence. Significant difference was observed in the prevalence of asymptomatic bacteriuria with respect to age, parity and trimester (p-0.001) (Table 1). The highest proportion of asymptomatic bacteriuria was observed between the age group 18-25 (6.02%) followed by 26-35(2.8%). The highest proportion of Significant bacteriuria was observed among multiparous women 14(6.49%). In the present study, preponderance of ASB was observed in third trimester 14(4.48%) followed by first 3(1.39%) and second trimester 2(0.93%).

Table.1 Characteristics of the study population

Age in Years	Number(n=216)	ASB (%)
18-25	161	13(6.02%)
26-35	54	6(2.8%)
36-45	1	0(0%)
Parity		
Primi	79	5(2.31%)
Multi	137	14(6.49%)
Trimester		
First	55	3(1.39%)
Second	28	2(0.93%)
Third	133	14(4.48%)

In the present study, preponderance of E. coli 6(2.8%) and Coagulase negative Staphylococci 6(2.8%) was observed and grown in equal proportion followed by Klebsiella species 5(2.3%) and Staphylococcus aureus 2(0.9%).

Table2: Spectrum of bacterial isolates

Isolate	Number	Percentage
E. coli	6	2.8%
CONS	6	2.8%
Klebsiella species	5	2.3%
S. aureus	2	0.9%
No Growth	197	91.2%
Total	216	100%

In the present study, E.coli was found to be the potent inducer of pyuria among pregnant women. E.coli shows an association with higher levels of pyuria, with 1.9% falling in the 10-25 pus cells/ HPF range. Gram positive cocci and Klebsiella species were significantly less likely to induce pyuria. A total of 7(3.2%) urine samples showed 10-25 pus cells/HPF in spite of no growth on bacteriological media (Sterile pyuria). (Table.3)

Table. 3 Association of organism with Pyuria.

Organism	Pyuria						Total
	0-5	%	5-10	%	10-25	%	
E.Coli	0	0.0%	2	0.9%	4	1.9%	6 (2.8%)
CONS	4	1.9%	1	0.5%	1	0.5%	6 (2.8%)
Klebsiella	3	1.4%	1	0.5%	1	0.5%	5 (2.3%)
S. aureus	2	0.5%	0	0.0%	0	0.0%	2 (0.9%)
No Growth	177	81.9%	13	6.0%	7	3.2%	197 (91.2%)
Total	186	86.1%	17	7.9%	13	6.0%	216 (100%)
Chi Square test value			54.752	p value		0.0001 Sig	

In the present study, Augmentin was found to be the most effective antibiotic for all the isolates. The susceptibility of Augmentin ranged from 80-100%. Further, Nitrofurantoin showed good susceptibility against all the isolates except Klebsiella species (40%). Least susceptibility was observed with Ceftriaxone. All the isolates were found to be resistant to Ampicillin and Penicillin. In the present study, one strain was observed as ESBL producer and found to be resistant to all the antibiotics tested. No MRSA was detected in the present study.

Table.4 Susceptibility pattern of isolates

Antibiotic	E.coli (n=6)	CONS(n=6)	Klebsiella(n=5)	S.aureus(n=2)
Ampicillin	0(0%)	0(0%)	0(0%)	0(0%)
Erythromycin	-	3(50%)	-	0(0%)
Nitrofurantoin	5(83.33%)	4(66.67%)	2(40%)	2(100%)
Gentamycin	4(66.67%)	6(100%)	3(60%)	1(50%)
Augmentin	6(100%)	6(100%)	4(80%)	2(100%)
Ceftriaxone	3(50%)	3(50%)	2(40%)	1(50%)
Ceftazidime	4(66.67%)	2(40%)	0(0%)	0(0%)
Ciprofloxacin	4(66.67%)	3(50%)	3(60%)	2(100%)
Penicillin	0(0%)	0(0%)	0(0%)	0(0%)

DISCUSSION

In our study asymtomatic bacteriuria among pregnant women was 8.8%, which is similar to the study conducted by Mukherjee et al (8.4%)[12]. The prevalence of asymtomatic bacteriuria among pregnant women was lower inanother study conducted by Armugam et al (5%)[13]. Globally, the existence of ASB has been reported to be between 2–10%[14]. But studies from various parts of the world reported higher prevalence rates of ASB among pregnant women. The prevalence of ASB reported from Iraq (42.9%)[15], Nigeria (37.1%)[16], Saudi Arabia (32.1%)[17] and Adigrat, Ethiopia (21.2%)[18].

There was a significant difference in the prevalence of asymptomatic bacteriuria with respect to age group ($P < 0.0001$). In our study,asymptomatic bacteriuria was found to be predominant among the age group 18-25 years(6.02%) followed by 26-35 years(2.8%). In our study, no asymtomatic bacteriuria was observed among the antenatal women attending with advancedage group(36-45 years). This could be due to single participant in this particular age group. However, study previously conducted by Akinloye et al.[9] reported that advanced age group is one of the risk factors for aymtomatic bacteriuria.

The current study found a significant correlation ($p < 0.05$) between the gestational period and asymptomatic bacteriuria (ASB), with the condition being more common in the third trimester. Similar findings were also found in a research carried out in Ethiopia[2]. This association might be explained by the fact that urinary tract infections (UTI) or antibiotic-associated bladder infections are frequent in pregnant women. UTIs usually start around week six and peak between weeks twenty-two and twenty-four. The main cause of this phenomenonis thought to be urethral dilatation, which causes an increase in bladder volume, a decrease in bladder tone and a decrease in urethral tone, which in turn promotes the development of organisms in urine. It is important to remember that the period between 12 and 16 weeks of pregnancy would be ideal for ASB screening.

In our study the most prevalent organism observed was Escherichia coli (2.8%)and Coagulase negative Staphylococci(2.8%) followed by Klebsiella species (2.3%). Preponderance of Escherichia coli in our study agrees with previous studies[14,20]. This may be because most Escherichia coli strains prefer the conditions of urine stasis, which is frequent during pregnancy, and leads to urinary tract infections (UTIs). An additional factor can be the unhygienic habits of pregnant women, who might have trouble adequately cleaning their genital area after passing urine or after defecating. This is in contrast with the study

conducted by Akerele et al[21]. As per Akerele et al, Staphylococcus aureus(29.8%), Escherichia coli (29.1%), and Klebsilla pneumonia (21.5%) were the most isolated pathogens. As per the study conducted by Muharram et al, Klebsiella species(71.4%) was predominant followed by Escherichia coli (29.6%)[22]. Other studies have reported the presence of group B Streptococcus, Diphtheroids and Candida albicans[23].

In the present study, it was observed that commonly used antibiotics showed high degree of resistance which is comparable to the previous studies[2]. The high proportion of non-prescription medication usage and its easy availability over the counter might be the cause of the high prevalence of MDR seen in this investigation. It may also be connected to the high frequency of antimicrobial drug abuse, including self-medication, inappropriate usage, disregard for recommended treatment protocols, and insufficient or nonexistent antimicrobial drug resistance surveillance programs. Augmentin was found to be the most effective antibiotic for all the isolates. The susceptibility of Augmentinranged between 80-100%. The comparatively high cost and limited accessibility toAugmentin in contrast to other antimicrobial drugs may be the cause of the low degree of resistance seen with this medication. As a result, this antibiotic may be used as a substitute in the empirical management of UTIs.

The conventional worldwide protocol for verifying asymptomatic bacteriuria is the observation of significant growth in the culture of two successive urine specimens[24]. In the current study, a single sample collection was employed. Due to the inability to track patients, a second urine sample from the same patient could not be tested to confirm asymptomatic bacteriuria.

Conclusion: In our study, the overall prevalence of asymptomatic bacteriuria was found to be 8.8%.E. coli and Coagulase Negative Staphylococci were the common isolates observed. High degree of resistance was noticed towards commonly used antibiotics.Majority of isolates were found to be susceptible to Amoxicillin-clavulanate. Further investigation is required to examine the possible effects of asymptomatic bacteriuria (ASB) on maternal and newborn morbidity that may arise in the course of pregnancy. In these situations, determining whether repeat screens are clinically warranted is crucial.

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