

PREVALENCE & ETIOLOGY OF ASYMPTOMATIC BACTERIURIA AMONG ANTENATAL FEMALES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN

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Abstract

Background: Asymptomatic bacteriuria (ASB) is not uncommon in pregnancy because of various anatomical, physiological and hormonal changes. If it is untreated, this can lead to symptomatic bacteriuria and may have complications for mother and fetus during delivery. This study aimed to detect the frequency and causative agents of ASB in pregnant females who attend a tertiary care hospital and to ascertain its antimicrobial susceptibility pattern. **Materials and methods:** The current study was done during a period of 6 months from June 2022 to December 2022 with a total of 175 antenatal females. 2 consecutive clean catch midstream urine were collected from the females attending the department of Obstetrics & Gynecology (both OPD & IPD). Colony count more than 10⁵CFU/ml were considered as significant and their pattern of antimicrobial susceptibility was noted. **Results:** Out of 175 participants, 12 (6.86%) showed significant growth. *Escherichia coli* (41.67%) was the highly prevalent organism followed by *Enterococcus spp.* (25%), *Staphylococcus aureus* (16.67%), *Klebsiella pneumoniae* (8.33%) and *Pseudomonas aeruginosa* (8.33%). The most effective antibiotic agents against bacterial organisms studied was Nitrofurantoin except *Pseudomonas aeruginosa*. This study indicated that 50% of the pathogens were multidrug resistant (MDR), among that 33.33% were ESBL producing enterobacteriaceae and 8.33% were carbapenem resistant enterobacteriaceae (CRE) **Conclusion:** This study found that 6.86% of the participants has asymptomatic bacteriuria. Early screening, diagnosis and treatment with correct antibiotics is necessary to prevent complications. Routine urine culture is mandatory among the pregnant females to avoid serious consequences for mother and baby.

Keywords: Asymptomatic Bacteriuria, Pregnancy, Urinary Tract Infection (UTI), Urine Culture.

INTRODUCTION

UTI occurs frequently in females of all age group because of shorter urethra and closer to anus and it is most common in pregnancy because of stasis of urine, decreased immunity, increased levels of progesterone which leads to the relaxation of smooth muscles and contamination with the fecal flora.^[1] The proliferation of commensal and harmful bacteria is encouraged by pregnant women's apparent decrease in immunity. Risk factors of asymptomatic bacteriuria includes advanced maternal age, diabetes, low socio-economic status, etc. Poor genital hygiene practices among pregnant women may also be a contributing factor, as they may find it difficult to adequately wipe their anus after defecating or clean their genitals after passing urine. Elevated pH in urine also encourages the bacterial growth. A further explanation could

be the elevated progesterone secretion secreted by the placenta, which relaxes smooth muscles and reduces ureteric peristalsis. This, in turn, increases bladder capacity and causes urine stasis, which can result in infection.^[2]

Based on the symptoms UTI are of two types, asymptomatic and symptomatic bacteriuria.^[3] Bacteriuria without any symptoms and evidence of urinary tract infections, but presence of significant count of 10^5 CFU/ml or more of organisms in 2 consecutive voided clean catch midstream urine (with the isolation of same organisms) is considered as asymptomatic bacteriuria.^[4]

The frequency of ASB varies with age in healthy females and ranges from 1% among school going girls to nearly 20% above 80 years of age. Since it has a controversial clinical significance, asymptomatic bacteriuria preceding UTI does not always needs treatment. Treatment is recommended if screening of bacteriuria has ill effects that can be prevented by antimicrobial therapy as in pregnancy. This happens in 2 to 15% of antenatal females. Asymptomatic bacteriuria leads to complications like pyelonephritis, spontaneous abortion, maternal sepsis and premature preterm rupture of membranes for mother and fetal growth restriction, premature delivery, under weight, neonatal sepsis for babies.^[5,6,7] In untreated cases, asymptomatic bacteriuria leads to acute pyelonephritis in twenty to thirty percent of pregnant females.^[8,9]

Several organisms are related with urinary tract infection. Of these, most common microorganisms isolated in pregnancy are *Escherichia coli* in nearly 80% of infections, followed by gram positive cocci like *Staphylococcus saprophyticus*, *Group B Streptococci*, *Enterococcus spp.* and other gram negative bacilli like *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Providencia spp.*, *Acinetobacter spp.*^[1,3]

MATERIALS AND METHODS

A six-month cross-sectional study was done in the Department of Microbiology at a tertiary care hospital from June 2022 to December 2022, Salem after getting approval from Institutional ethical committee (VMKVMC&H/IEC/22/57). After obtaining consent, 175 antenatal participants (age range: 18–35 years) who did not exhibit clinical signs of a UTI were added to the study. Women who had a history of UTI symptoms or antibiotic use two weeks previous to the study's could not enroll.

Procedure:

Antenatal women were instructed to clean their meatus with soap and water before collecting the samples. In a sterile, universal wide-mouthed container, urine samples collected midstream, cleanly catch of about 10 to 20 ml were collected under strict aseptic precautions. The samples were transported immediately to the microbiological lab for further processing. In case of delay, specimens were stored in the refrigerator at 4°C for a maximum of 24 hours. The samples were examined under microscope and cultured onto MacConkey agar & incubated overnight at 37°C.

A colony count of $>10^5$ colony forming units (CFU) per millilitre was considered as significant bacteriuria & included for further study. Urine with lower counts ($<10^5$ colonies) was considered as insignificant bacteriuria and excluded from further study. Further identification was carried out based on the shape of the colonies and

biochemical tests by using conventional microbiological techniques. Gram positive bacteria were detected by catalase test, slide coagulase test, tube coagulase test, mannitol fermentation, bile esculin test, Salt tolerance test, 40% bile salts & gram negative bacteria were detected by catalase test, oxidase test, indole test, urease test, citrate utilization test, triple sugar iron agar and mannitol motility medium.

In accordance with Clinical Standard Laboratory Institute guidelines, antimicrobial susceptibility testing has been conducted using the Kirby-Bauer disc diffusion method on Muller Hinton agar. The panel of antimicrobial drugs used for gram positive organisms were Penicillin (10units), Ampicillin (10µg), Cefoxitin (30µg), Gentamicin (10µg), High level Gentamicin (120µg), Amikacin (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Ofloxacin (5µg), Norfloxacin (10µg), Netilmicin (30µg), Nitrofurantoin (300µg), Doxycycline (30µg), Vancomycin (30µg), Teicoplanin (30µg) and Linezolid (30µg) & for Gram negative organisms were Ampicillin (10µg), Ceftazidime (30µg), Cefotaxime (30µg), Cefepime (30µg), Cefaperazone-sulbactam (75µg+30µg), Amoxicillin-clavulanic acid (20µg+10µg), Piperacillin-tazobactam (100µg+10µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Ofloxacin (5µg), Norfloxacin (10µg), Netilmicin (30µg), Nalidixic acid (30µg), Nitrofurantoin (300µg) [except *Pseudomonas aeruginosa*], Co-trimoxazole (25µg), Doxycycline (30µg), Imipenem (10µg) and Meropenem (10µg). [10]

RESULTS

A sum of 175 participants were involved in this study with the age ranges between 18 to 35 years. Out of 175 participants, 12 showed significant growth and were considered as asymptomatic bacteriuria with a prevalence of 6.86%. The predominant organism detected were *Escherichia coli* (41.67%) followed by *Enterococcus spp.*(25%), *Staphylococcus aureus* (16.67%), *Klebsiella pneumoniae* (8.33%), *Pseudomonas aeruginosa* (8.33%). The prevalence was greater with multigravida between the age group of 21 to 25 years and in the 3rd trimester.

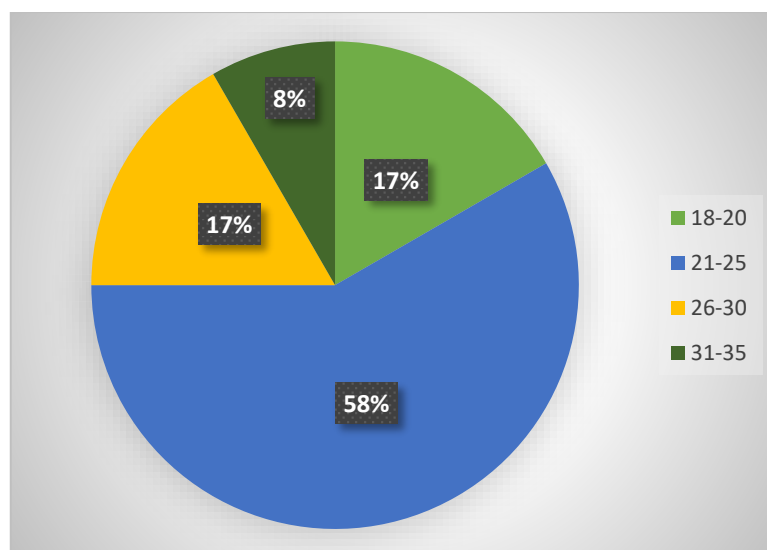


Figure 1: Age wise distribution of participants in years with Symptomatic bacteriuria

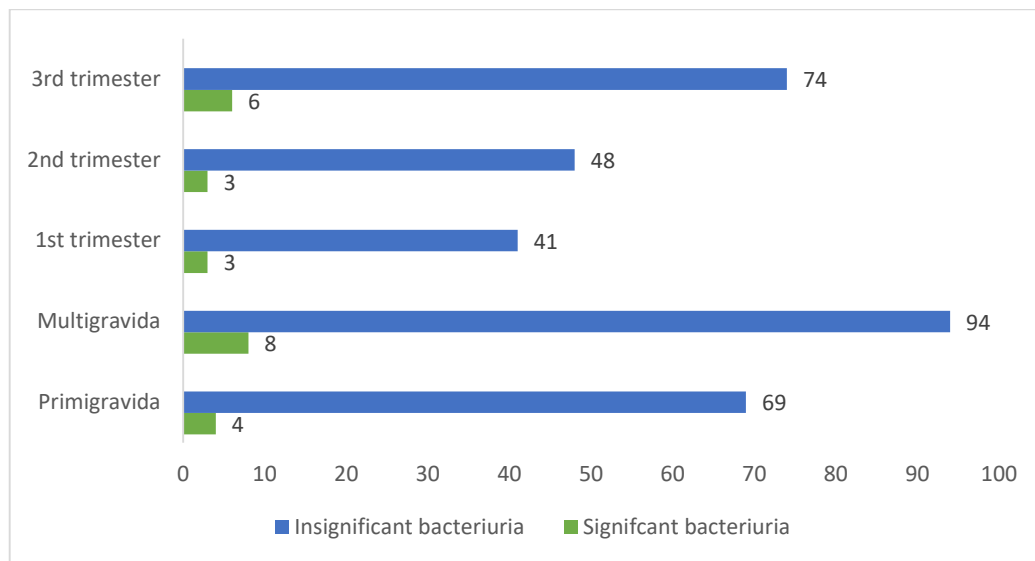


Table 1: Etiological profile of asymptomatic bacteriuria

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Organisms	Organisms isolated	No. of isolates (n=12)	Percentage
Gram Negative organisms	<i>E. coli</i>	5	41.67%
	<i>Klebsiella pneumoniae</i>	1	8.33%
	<i>Pseudomonas aeruginosa</i>	1	8.33%
Gram Positive organisms	<i>Enterococcus spp.</i>	3	25%
	<i>Staphylococcus aureus</i>	2	16.67%

Table 2: Antimicrobial susceptibility pattern of gram negative organisms

Drugs	<i>Escherichia coli</i> (n=5)	<i>Klebsiella pneumoniae</i> (n=1)	<i>Pseudomonas aeruginosa</i> (n=1)
Ampicillin	2	NA	NA
Cefotaxime	2	0	NA
Ceftazidime	2	0	1
Cefepime	2	0	1
Amoxicillin-clavulanate	4	0	NA
Cefaperazone-sulbactam	3	0	NA
Piperacillin-tazobactam	4	0	1
Gentamicin	4	1	NA
Amikacin	5	1	1
Ciprofloxacin	3	1	1
Levofloxacin	2	1	1
Norfloxacin	4	1	1
Ofloxacin	3	0	1
Netilmicin	4	0	1
Nalidixic acid	3	0	NA
Nitrofurantoin	5	1	NA
Cotrimoxazole	3	1	NA
Doxycycline	4	1	NA
Imipenem	4	0	1
Meropenem	3	0	1

Table 3: Antimicrobial susceptibility pattern of gram positive organisms

Drugs	<i>Enterococcus spp.</i> (n=3)	<i>Staphylococcus aureus</i> (n=2)
Ampicillin	3	0
Penicillin	2	0
Gentamicin	NA	2
High level gentamicin	2	NA
Amikacin	NA	2
Ciprofloxacin	0	1
Levofloxacin	1	0
Norfloxacin	0	1
Ofloxacin	1	2
Netilmicin	1	2
Nitrofurantoin	3	2
Doxycycline	2	2
Linezolid	3	1
Teicoplanin	2	2
Vancomycin	2	NA
Cefoxitin	NA	0

Table 4: Distribution of MDR pathogens

MDR pathogens	No. of isolates (Total isolates- 12)	%
E.coli	4	33.33%
Klebsiella pneumoniae	1	8.33%
MRSA	1	8.33%
Total	6	50%

DISCUSSION

Proper antenatal care plays a vital role in ensuring the health of both mother and baby. UTI is one of the globally prevalent disease because of lesser immunity and various physiological changes in pregnancy. Due to the absence of UTI symptoms, asymptomatic bacteriuria needs special attention. It is believed that multiple factors are liable for this change. Pregnancy itself is a relative immunocompromised state. Predisposition to infection is caused by ureteric dilatation brought on by gravid uterine compression. Enlargement of uterus also results in mechanical obstruction to the flow of urine.^[11] The frequency of ASB was found to be 6.86% in this study, which is consistent with the study done by Narayana Goruntala (6.4%).^[12] The prevalence of ASB varies from 4 to 29 % worldwide. The prevalence of ASB ranges from 5 to 18% in India.^[1,5,6,12,13,14] The rationale behind this change may be due to difficulty in maintaining the environmental and personal hygiene, socioeconomic status (most of the females lives in rural areas and low socio economic status) and stasis of urine which predisposes to urinary tract infection.^[15]

This study, which is comparable to the work published by Alghalibi et al., found that the age range of 21 to 25 years had a greater incidence of asymptomatic bacteriuria.^[16] In contrast to the current study, incidence was greater in the age ranges from 31 to 40 years by Muneeba Sadaf et al & in the study group of 26 to 35 years by B.Prasanna et al.^[9,14] The incidence was greater with multigravida in our study which is similar to the studies reported by V.Mallikarjun Rao et al, and Sujatha R.^[3,17] Incidence was

higher in multigravida in the early 20's, since early marriages are common in rural areas and women in rural areas have lack of awareness regarding maternal health and personal hygiene. Yamini Kura et al have stated that the incidence increased as the age progresses due to reduction of lactobacilli which facilitate the pathogens to easily adhere to the genital tract and also there will be decreased glycogen deposition. Higher incidence in multiparous females may be due to repeated exposure to stasis of urine because of gravid uterus.^[13]

A higher frequency of asymptomatic bacteriuria was seen in third trimester women in our study, that is comparable to research published by Naimshree Sonkar et al. and B. Prasanna et al.^[18,14] but in contrast to the present study, Sarojamma Chunchaiah et al and Muktikesh Dash et al had showed that incidence was greater in second trimester.^[19,20] Trimester did not affect the prevalence of asymptomatic bacteriuria, according to Paul Erhunmwunse Imade et al.^[21] Sujatha et al have showed higher incidence in first trimester due to hormonal changes in pregnancy.^[17] Because of physiological and anatomical changes brought on by growing gestational age, there is a higher prevalence in the third trimester. This causes urine stasis, which in turn leads to a greater colonization of harmful bacteria.

The most frequent organisms isolated in this study was gram negative organisms like *Escherichia coli* (41.67%) followed by *Klebsiella pneumoniae* (8.33%) and *Pseudomonas aeruginosa* (8.33%). This study finding is in concordance with the many studies conducted by Sudha Biradar k et al, V. Mallikarjun Rao et al.^[1,3] The gram positive organism isolated from this study was *Enterococcus spp.* (25%), following that *Staphylococcus aureus* (16.67%). Paul Erhunmwunse Imade et al has shown that *Staphylococcus aureus* was the second predominant organism isolated from asymptomatic bacteriuria in pregnancy.^[21] All enterobacteriaceae were 100% sensitive to Nitrofurantoin and Amikacin similar to the study reported by Yamini Kura et al and V. Mallikarjun Rao et al^[13,3]. More than 80% of gram negative isolates showed good sensitivity to Amoxicillin clavulanate, Netilmicin, Norfloxacin, Gentamicin, Doxycycline.^[22]

Antibiotic sensitivity pattern varies from one region to another and the multidrug resistant (MDR) pathogen are also varies in their extent and severity. Misuse or overuse of antimicrobial agents is the important cause of multidrug resistance.^[23,24] Among the Enterobacteriaceae isolated in this study, 3 *E.coli* were ESBL producers. Both the ESBL and non- ESBL producers were 100% sensitive to nitrofurantoin and amikacin as in Biset et al.^[25] A single isolate of *Klebsiella pneumoniae* was resistant to 3rd & 4th generation cephalosporins, beta-lactam/beta-lactam inhibitors and carbapenems. The prevalence ESBL producing enterobacteriaceae in this study was 33.33% and CRE was 8.33%. Bacterial isolates were considered to be MDR, when they exhibit resistance to atleast one drug in three or more classes of antimicrobial groups, in which they are usually susceptible. In this study, the prevalence of MDR pathogens were found to be 50%.^[26]

Gram positive organisms showed 100% sensitivity to Nitrofurantoin which is analogous with the study reported by Sarojamma Chunchaiah et al.^[19] Out of 2 *Staphylococcus aureus* isolates, both were *Methicillin resistant Staphylococcus aureus* (MRSA).

The most reliable and gold standard method for identifying bacteriuria in pregnant women who are asymptomatic is urine culture. Women who has significant bacteriuria have 20 to 50 fold increased chance of having pyelonephritis.^[27]

All the females who has significant bacteriuria must be treated with the appropriate antibiotics in the appropriate time according to their antibiotic sensitivity pattern. The chosen antibiotics should be always safer to mother and fetus with efficacy rate of >90% and with less resistance pattern.

FDA advices Ampicillin, Amoxycillin, Nitrofurantoin as the 1st line drugs for treating asymptomatic bacteriuria.^[19]

CONCLUSION

Significant bacteriuria was found in 6.86% of the antenatal females in our study. Women who experience asymptomatic bacteriuria have numerous detrimental effects for both the mom and the baby. Early screening and treatment with appropriate antibiotics during therapy aid in lowering the likelihood of complications. It is a cost effective interventions at health center for safer motherhood & newborn in developing countries like India.

Antimicrobial resistance is a result of the fact that many isolates nowadays are resistant to widely used antimicrobial drugs. In the present study, asymptomatic pregnant women had a significant prevalence of MDR pathogens.

Pregnant women ought to be evaluated for bacteriuria, and those who have a history of anomalies or recurring UTIs should be screened again throughout their pregnancy. Pregnant women should receive health education about personal and environmental hygiene in order to lower the risk of infection. This strategy will significantly reduce maternal and obstetric challenges related to pregnancy.

Limitation of the study:

The potential correlation between risk variables and asymptomatic bacteriuria in our investigation due to the small sample size of 175. Minimum inhibitory concentration (MIC) for Vancomycin was not checked for *Staphylococcus aureus*.

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Conflict of Interest:

The author declares that there is no conflict of interest regarding the publication of this study.

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