ORIGINAL ARTICLE

The enemy in shadows – asymptomatic bacteriuria in pregnancy: a prospective, observational study

Short title: Asymptomatic bacteriuria in pregnancy

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ABSTRACT

Background. One of the most prevalent infections encountered in clinical practice is urinary tract infection (UTI). Females are particularly vulnerable to UTI due to the short length of the urethra and proximity to the warm, wet anal canal and vagina. A urinary tract infection might be symptomatic or asymptomatic. Asymptomatic bacteriuria is defined as the presence of bacteria in significant numbers, i.e., 10^5 bacteria per ml, within the urinary system despite the absence of evident symptoms. Because of the various morphological and physiological changes that occur during pregnancy, pregnant women are at a significant risk of having asymptomatic bacteriuria.

Materials and Methods. A prospective study was carried out among asymptomatic antenatal cases. Mid-stream clean catch urine sample was collected, semi-quantitative culture and antibiotic sensitivity was performed for the isolated organisms.

Results. Out of the 264 urine samples collected, 30 showed significant growth of organisms after overnight aerobic incubation at 37 ˚C (Asymptomatic Bacteriuria) with primigravida mothers at an increased risk. *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Staphylococcus saprophyticus* were the common isolates. The gram-negative bacilli showed maximum resistance to Cefoperazone and least resistance to Nitrofurantoin. The gram-positive isolates were Cefoxitin resistant.

Conclusions. The Gold standard for detection of asymptomatic bacteriuria in antenatal mothers is urine culture at first prenatal visit or between 12 and 16 weeks of gestation.

Without proper diagnosis and treatment, there is a high chance of developing symptomatic UTI, including pyelonephritis leading to preterm deliveries and perinatal complications.

Key words

Escherichia coli; pregnancy; urinary tract infection.

INTRODUCTION

Asymptomatic bacteriuria is defined as persistently and actively multiplying bacteria in significant numbers i.e., 10^5 colony forming units per ml within the urinary tract without any obvious symptoms [1].

Pregnant females are two times more commonly affected than age matched non-pregnant females due to different morphological & physiological changes occurring during pregnancy. The apparent reduction in immunity of pregnant women appears to encourage the growth of both commensal and non-commensal microorganisms [2]. In addition to reflux of urine from the bladder back up the ureters and to the renal pelvis (owing to relaxation of the vesico-ureteric junction), there is urinary stasis caused by the progesterone impact in pregnancy, which causes ureteric smooth muscle relaxation. Urine concentration is lowered during pregnancy due to the physiological increase in plasma volume. Seventy percent of pregnant women experience glycosuria, which is a perfect environment for bacteria to grow in the urine [2-5].

The Gold standard investigation for detection of asymptomatic bacteriuria is urine culture. Therefore, urine culture at first prenatal visit or between 12 and 16 weeks of gestation should be considered as a screening test of choice [5, 6]. In the absence of standard bacteriuria screening, a presumptive diagnosis is determined based on a history of previous pregnancies with preterm delivery between the 16th and 36th week of gestation. The risk of asymptomatic bacteriuria is thought to be increased in pregnant women who have had more than one preterm delivery [7, 8].

Research from developed nations has reported that the frequency of asymptomatic bacteriuria during pregnancy ranges from 2 to 11%, however findings from India indicate a greater incidence of 5 to 17%. Research conducted in impoverished and underdeveloped nations, including those in Africa, revealed a frequency as high as 86.6%. *Escherichia coli* is the most frequently found bacteria responsible (80–85%), followed by *Klebsiella*, coagulase-negative *Staphylococcus, Pseudomonas*, and *Proteus* species [3, 9-13].

This study highlights the importance of unveiling this hidden enemy which due to lack of sufficient research is missed during routine antenatal check-ups and thereby adversely affects the outcome of many pregnancies.

MATERIALS AND METHODS

A prospective study was carried out in the Department of Microbiology in collaboration with the Department of Gynaecology and Obstetrics at a tertiary medical college in Eastern India, in accordance with ethical standards. Antenatal mothers, attending Obstetrics clinic, asymptomatic as per the UTI Symptom Assessment Questionnaire (UTISA, Developed by MAPI Research Trust
PROVIDE™, France, 2005), were included in the study [14]. (Table 1)

Antenatal mothers who show any of the signs and symptoms of UTI as per the UTISA questionnaire (dysuria, frequency, urgency, gross haematuria, incomplete voiding, lower back and/or abdominal/suprapubic discomfort) were excluded.

Following confirmation that the women's UTISA questionnaire score was zero, each participant signed a consent form, informing them about the details of the study. Then, they were instructed on how to get a midstream urine sample using the clean catch technique. Using a forward-to-back motion, two or three gauze pads saturated with soapy water were used to clean the periurethral area and perineum. This was followed by a rinse with sterile water. In order to clear the urethra of bacteria, the labia were held apart during voiding and the first few milliliters of urine were transferred into a bedpan or toilet bowl. The urine's midstream portion was then collected and placed in a sterile wide-mouthed container with a screw-capped lid that fits tightly.

Fresh urine samples collected using the aforementioned method were tested for pyuria, culture and sensitivity. After gently mixing the uncentrifuged urine sample, 0.05 ml was placed in the center of a microscope slide. Immediately after, a 22 x 22 mm cover slip was put to prevent bubbles from being trapped. One leucocyte for every seven high power fields corresponded to 10^4 leucocytes per milliliter, provided the area of a High power field (HPF) was 0.15 mm^2 and the depth under the cover slip was 0.1 mm.

The semi-quantitative culture was performed by inoculating urine on Blood agar, MacConkey agar & CLED media with a 0.001ml calibrated loop within 2 hours of collection of the sample. All plates were incubated overnight at 37^0 C under aerobic condition to obtain accurate colony count. A colony count of 100 indicated 10^5 CFU /ml organisms, and was considered significant [15]. Thereafter, all the isolates were subjected to gram staining and routine biochemical tests for identification, followed by antibiotic sensitivity done by modified Kirby Bauer Disc Diffusion method as recommended by CLSI guidelines [16], on Mueller-Hinton agar (MHA) media. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were used as quality control strains.

RESULTS

Out of the 264 urine samples collected from asymptomatic antenatal mothers, 30 showed significant growth of organisms after overnight aerobic incubation (asymptomatic bacteriuria) which accounted to 11.4% whereas 5 samples showed growth of contaminants. Out of the 30 antenatal women, 21(8%) were in the 21-30 years age group, making it the most common age group affected. 7 (2.6%) were in the 18-20 years age group whereas only 2 (0.8%) were in the 31- 40 years age group. 18 cases (60%) were Primigravida while the remaining 4 (12%) were 1st Para, 2 (8%) were 2nd Para and 6 (20%) Multipara. 8 antenatal mothers (26.8%) were in the 1st Trimester, 10 (32.8%) in the 2nd Trimester and 12 (40.4%) were in the 3rd Trimester. The study thus found that primigravida mothers were at an increased risk of developing asymptomatic bacteriuria.

17 (56.7%) cases had a past history of UTI whereas 13(43.3%) had no significant past history. Among the remaining 234 subjects, 62 (26.5%) had a past history of UTI while 172 (73.5%) had no such history.

In this study, maximum culture positive cases of asymptomatic bacteriuria (47%) belonged to the upper lower socio-economic class. The distribution of cases according to the Modified Kuppuswamy's Socioeconomic Status Scale is shown in Table 2 [17].

Presence of more than 1 pus cell per 7 high power fields (significant pyuria) in wet mount of uncentrifuged urine samples was compared with the urine culture results. All 30 culture

positive cases showed presence of significant pyuria (100%). Of the remaining 234 samples, only 18(7.7%) showed presence of significant pyuria while 216 (92.3%) were reported negative.

7(22%) isolates were gram positive cocci and 23 (78%) were gram negative bacilli. *Escherichia coli* (40%) was the most common isolate, followed by *Klebsiella pneumoniae* (36%), *Staphylococcus aureus* (15%), *Staphylococcus saprophyticus* (7%) and *Proteus mirabilis* (2%). (Table 3)

In this study, the gram-negative bacilli showed maximum resistance to Cefoperazone and Amikacin. 58.3% of *Escherichia coli* isolates and all the *Klebsiella pneumoniae* isolates (100%) were resistant to Cefoperazone. *Escherichia coli* showed least resistance (8.3%) to both Ceftriaxone and Piperacillin-tazobactam, followed by Nitrofurantoin (16.7%) and Imipenem (16.7%). *Klebsiella pneumoniae* showed least resistance to Nitrofurantoin (20%) followed by Levofloxacin (30%). *Proteus mirabilis* showed resistance to Ceftriaxone and Cefoperazone while being sensitive to Nitrofurantoin, Cotrimoxazole, Amikacin, Imipenem and Piperacillin-tazobactam. Therefore, overall least resistance was seen to Nitrofurantoin (17.4%) followed by Piperacillin- tazobactam (21.7%), Levofloxacin and Imipenem (both at 26.1%). Isolates of *Klebsiella pneumoniae* showed a higher degree of resistance to antibiotics as compared to the other gram-negative Bacilli. (Table 4)

Staphylococcus aureus and *Staphylococcus saprophyticus* isolates were both found to be Cefoxitin resistant (indicating Methicillin Resistance). The *Staphylococcus aureus* isolates were 100% sensitive to Vancomycin, Linezolid, Ciprofloxacin and Nitrofurantoin. *Staphylococcus saprophyticus* showed 100% sensitivity to Vancomycin and Linezolid while being 50% sensitive to Clindamycin, Erythromycin, Ciprofloxacin and Nitrofurantoin. Gram positive organisms showed least resistance to Vancomycin (0%) and Linezolid (0%), followed by Nitrofurantoin (14.3%) and Ciprofloxacin (14.3%). (Table 5)

DISCUSSION

In this study, out of the 264 urine samples collected from asymptomatic antenatal mothers, the prevalence of asymptomatic bacteriuria is 11.4%. Studies by Sevki Celen *et al*., Andrew W Robertson *et al.* and Kasinathan A *et al.* showed the prevalence of asymptomatic bacteriuria to be 8.4%, 8.5%, and 12.6% respectively [18-20].

In this study, the age group of 21–30 years old accounted for the majority of instances of asymptomatic bacteriuria (8%) followed by 18–20 years old (2.6%) and 31–40 years old (0.8%). The outcome is similar to that of a study conducted by Khaled A. Almehdawi *et al*. [21], in which the age range of 25–30 years old had the highest prevalence (8.33%). Additionally, Kerure S. B. *et al*. discovered that the age group of 26–30 years old had the highest prevalence of asymptomatic bacteriuria (4.6%) [22]. R. Sujatha *et al*. found similar results, with a highest prevalence of 5.33% for women in the 21–30 age range for asymptomatic bacteriuria [23].18 out of the 30 cases (60%) in this study were Primigravida and the remaining 12 (40%) were of higher parity, 4 (12%) in 1st Para, 2 (8%) in 2nd Para and 6 (20%) were Multipara. S V Lavanya *et al*. similarly reported in her study that 66.6% of her cases were Primigravida and 33.4% were multiparous [13]. This was also seen in the studies conducted by Kerure S B *et al.* and Bose AM *et al*. where asymptomatic bacteriuria was more common in Primigravida with the prevalence being 56% and 53.85% respectively [22, 24]. The present study had 8 (26.8%) cases in the 1st Trimester, 10 (32.8%) in the 2nd Trimester and 12 cases (40.4%) were in the 3rd Trimester, marking 3rd trimester as the time of maximum occurrence of asymptomatic bacteriuria. This is comparable to the results obtained by Gayathree *et al*. where the highest prevalence of asymptomatic bacteriuria was

seen in the 3rd trimester (61.7%) [25]. Oli *et al.* and Patel *et al.* also reported 72.30 % and 68.18% of cases in the third trimester [26, 27]. The fact that the majority of the women were sent from primary health centers to tertiary care hospitals for their initial visit during the third trimester may help to explain the finding of an increased number of cases of asymptomatic bacteriuria in the third trimester [28].

In this study, the maximum number (47%) of cases of asymptomatic bacteriuria belonged to the upper lower socio-economic class according to the Kuppuswamy's Socioeconomic Status Scale followed by 30% cases in the Lower class. This is similar to the findings of the study conducted by Kasinathan A *et al.* where 45.5% cases belonged to the upper lower class and 31.8% cases in the lower class. Similar trend was also seen in the study conducted by S V Lavanya *et al*. who found 66.6% cases in the Upper lower class [20, 16].

Out of 30 culture positive cases of asymptomatic bacteriuria in this study, 17 (56.7%) had a past history of UTI. A similar significant association was seen in the study by Manasi Patnaik *et al*., where 43.8% of the patients diagnosed with asymptomatic bacteriuria had a past history of UTI [29]. Rohin U V *et al.* also discovered that past history of UTI was a significant risk factor for asymptomatic bacteriuria in pregnancy [28].

Presence of significant pyuria was seen in all 30 culture positive cases of asymptomatic bacteriuria in this study (100%) while 92.3% of culture negative cases had no pyuria. T. Jeyaseelan Senthinath *et al*. also found 100% correlation between significant pyuria and culture positivity while 78.1% of culture negative cases reported absence of pyuria [9].

In this study, out of the 30 organisms isolated, gram negative bacilli were more predominantly isolated (78%) than the gram positive cocci (22%). Predominance of gram negative bacilli was similarly reported by Jeyaseelan Senthinath *et al*., Kasinathan A *et al*., Kerure S B *et al.* and Madhu Udawat *et al.* at 85%, 82%, 89% and 79.4%, respectively [9, 20, 22, 30]. *Escherichia coli* was the most commonly isolated organism in this study (40%) followed by *Klebsiella pneumoniae* (36%), and *Staphylococcus aureus* (15%). Kasinathan A *et al.* also found the commonest bacterial isolate to be *Escherichia coli* (63.63%) followed by *Klebsiella pneumoniae* (18.18%) and *Staphylococcus aureus* (13.64%) [20]. Similar trends have been reported in other studies by Jeyaseelan Senthinath *et al*., Kerure S B *et al.*, Chandel LR *et al.*, Delzell JE *et al.*, Mac Nair RD *et al.*, and Nithyalakshmi J *et al.* [9, 22, 31-34]. In this study, the gram-negative bacilli showed least resistance to Nitrofurantoin (17.4%) followed by Piperacillin- tazobactam (21.7%). A similar observation was made by S V Lavanya *et al.* who noted that Nitrofurantoin was one of the highly sensitive drugs amongst the gram-negative bacilli [13]. Kerure S B *et al*. also reported that the isolates were most sensitive to Nitrofurantoin and Imipenem [22]. Another study by Ojide CK *et al*. mentioned that Escherichia coli showed 76.9% sensitivity to Nitrofurantoin [35]. Antibiotic susceptibility patterns similar to the present study were reported in the studies done by Paul Erhunmwunse Imade *et al*. and Kasinathan A *et al*. and [3, 20]. Amongst the gram positive cocci isolated in this study, maximum sensitivity was seen to Vancomycin (100%), Linezolid (100%) followed by Nitrofurantoin (85.7%) and Ciprofloxacin (85.7%). Sensitivity to Cefuroxime was comparable between this study (80%) and the study conducted by R. Sujatha *et al.* (86%) [23]. Madhu Udawat *et al.* mentioned in her study that the isolates were 100% sensitive to Vancomycin and 80% to Nitrofurantoin [30]. The consensus document by American College of Obstetricians and Gynecologists suggests that the patients should be given a five-to-seven-day course of antibiotics as per the culture report only in cases where the colony count is 10^5 CFU/ml or higher. Studies indicate that this regime has a high cure rate with decreased incidence of pyelonephritis in cases of asymptomatic bacteriuria [36, 37].

CONCLUSION

The Gold standard for detection of asymptomatic bacteriuria in antenatal mothers is urine culture at first prenatal visit or between 12 and 16 weeks of gestation. Without proper diagnosis and treatment, there is a high chance of developing symptomatic UTI, including pyelonephritis leading to preterm deliveries and perinatal complications.

This research highlights the necessity of screening expectant mothers for asymptomatic bacteriuria and routinely checking uropathogens for antibiogram patterns because the information not only lowers the prevalence but also aids in rationalizing the use of antibiotics to prevent the emergence of resistance.

Compliance with Ethical Standards

Author contributions

S.D.G., R.D.R: Data curation, Investigation, formal analysis, writing – original draft, writing – review & editing. M.B., S.K.: Conceptualization, methodology, supervision.

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Study registration

Not applicable.

Disclosure of interests

The authors declare that they have no conflict of interests.

Ethical approval

Study was approved by Institutional Ethics Committee of R.G. Kar Medical College, West Bengal, India.

Informed consent

All participants were provided with necessary explanations about the objectives and each enrolled patient gave informed consent to allow data collection and analysis for research purposes prior to the start of the study.

Data sharing

Data are available under reasonable request to the corresponding author due to privacy/ethical restrictions.

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Table 1. UTI severity assessment questionnaire (UTISA)

Please indicate whether you have had the following symptoms/problems in the past 24 hours and how severe they were?				**Symptoms**	**If you have experienced these symptoms/problems in the past 24 hours, please indicate how bothersome they were?**			
Did not have	**Mild**	**Moderate**	**Severe**		**Not at All**	**A little**	**Moderately**	**A lot**
0	**1**	**2**	**3**	**Frequency of urination (going to the toilet very often)**	**0**	**1**	**2**	**3**
0	**1**	**2**	**3**	**Urgency of urination (a strong and uncontrollable urge to pass urine)**	**0**	**1**	**2**	**3**
0	**1**	**2**	**3**	**Pain or burning when passing urine**	**0**	**1**	**2**	**3**
0	**1**	**2**	**3**	**Not being able to empty your bladder completely/ passing only small amount of urine**	**0**	**1**	**2**	**3**
0	**1**	**2**	**3**	**Pain or uncomfortable pressure in the lower abdomen/ pelvic area caused by your urinary tract infection**	**0**	**1**	**2**	**3**
0	**1**	**2**	**3**	**Low back pain caused by your urinary tract infection**	**0**	**1**	**2**	**3**
0	**1**	**2**	**3**	**Blood in urine**	**0**	**1**	**2**	**3**

Table 2. Socio-economic distribution of cases

Socioeconomic class	Frequency of cases	Percentage of cases (n=30)
Upper class	0	0
Upper middle class	1	4%
Lower middle class	6	19%
Upper lower class	14	47%
Lower class	9	30%
Total	**30**	**100%**

Table 3: Distribution of organisms isolated in cases of asymptomatic bacteriuria.

Organism	Frequency of cases	Percentage of cases (n=30)
Escherichia coli	12	40%
Klebsiella pneumoniae	10	36%
Staphylococcus aureus	5	15%
Staphylococcus saprophyticus	2	7%
Proteus mirabilis	1	2%
Total	**30**	**100%**

Table 4: Antibiotic resistance patterns of gram negative organisms

Antibiotic	*Escherichia coli* (n=12) No. (%)	*Klebsiella pneumoniae* (n=10) No. (%)	*Proteus mirabilis* (n=1) No. (%)	Total isolates (n=23) No. (%)
Nitrofurantoin (NIT)	2 (16.7%)	2 (20%)	0 (0%)	4 (17.4%)
Cotrimoxazole (COT)	3 (25%)	6 (60%)	0 (0%)	9 (39.1%)
Ceftriaxone (CTR)	1 (8.3%)	5 (50%)	1 (100%)	7(30.4%)
Cefoperazone (CPZ)	7 (58.3%)	10 (100%)	1 (100%)	18(78.2%)
Amikacin (AK)	7 (58.3%)	10 (100%)	0 (0%)	17 (73.9%)
Levofloxacin (LE)	3 (25%)	3 (30%)	0 (0%)	6 (26.1%)

Imipenem (IPM)	2 (16.7%)	4 (40%)	0 (0%)	6 (26.1%)
Piperacillin-Tazobactam (PIT)	1 (8.3%)	4 (40%)	0 (0%)	5 (21.7%)

Table 5: Antibiotic resistance pattern of gram positive organisms

Antibiotic	***Staphylococcus aureus* (n=5)** **No. (%)**	***Staphylococcus saprophyticus* (n=2)** **No. (%)**	**Total isolates (n=7)** **No. (%)**
Nitrofurantoin (NIT)	0 (0%)	1 (50%)	1(14.3%)
Vancomycin (VA)	0 (0%)	0 (0%)	0 (0%)
Linezolid (LZ)	0 (0%)	0 (0%)	0 (0%)
Clindamycin (CD)	4 (80%)	1 (50%)	5 (71.4%)
Roxithromycin (RO)	4 (80%)	1 (50%)	5 (71.4%)
Ciprofloxacin (CIP)	0 (0%)	1 (50%)	1(14.3%)
Cefoxitin (CX)	1 (20%)	2 (100%)	3 (42.8%)
Ticarcillin-clavulanate (TCC)	1 (20%)	2 (100%)	3 (42.8%)
Cefuroxime (CXM)	1 (20%)	2 (100%)	3 (42.8%)