Original article

Low prevalence of asymptomatic bacteriuria (ASB) in pregnant women – is routine screening justified?

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Abstract:

Introduction: A common dilemma in clinical medicine is whether to treat asymptomatic patients who present with bacteria in their urine. Because of increasing antimicrobial resistance, it is important not to treat patients with ASB unless there is evidence of potential benefit. Pregnant women should be screened for ASB in the first trimester and treated, if positive as it is associated with risk of preterm birth and pyelonephritis, if left untreated. Treating ASB in patients with diabetes, older persons, patients with or without indwelling catheters, or patients with spinal cord injuries has not been found to improve outcomes. Thus, the present study was designed to study about prevalence and organisms isolated in ASB in pregnancy.

Methods: Urine samples were collected from 300 random pregnant women. The samples were cultured on CLED (Cysteine Lactose Electrolyte Deficient) medium using a semi quantitative calibrated wire loop technique. The plates were incubated at 37°C for 24 hours. Growth of one organism of $>10^5$ cfu/ml was considered as significant bacteriuria. Antibiotic susceptibility was done by using Kirby Bauer Disc Diffusion method.

Results: Of the 300 samples, 31 were positive for ASB. Of the 31 culture positive cases, 20 cases were multigravida (64.5%). Highest incidence was seen in women in their third trimester (11.96%) and who belonged to age group 35 years and above (66.66%). Escherichia coli was the most common organism isolated.

Conclusions: Prevalence of ASB in pregnant woman was 10.3 % in our study. Though the prevalence of ASB is less, but it increases with advancing age and also increases with rising trimester, so we recommend its routine screening.

Keywords: ASB, Bacteriuria, Prevalence, Significant Bacteriuria, Urine Culture, CLED

Introduction:

Asymptomatic Bacteriuria (ASB) is common, with varying prevalence by age, sex, sexual activity and the presence of genitourinary tract abnormalities. It can be defined as the “presence of actively multiplying bacteria within the urinary tract excluding the distal urethra” at a time when the patient has no urinary tract symptoms. ASB in women is defined by the 2005 Infectious Diseases Society of America (IDSA) guidelines, as when two consecutive clean-catch voided urine specimens with isolation of the same organism in quantitative counts of $\geq 10^5$ cfu/mL are detected.

IDSA guideline recommends screening for ASB in pregnant woman at least once in early pregnancy as pregnant women with ASB are at an increased risk for adverse outcomes, and these can be prevented with antimicrobial treatment, if given early. Thus, all pregnant women should be screened for bacteriuria and treated if test results are positive.
It’s well established that ASB has serious outcomes in pregnancy in the form of fetal and maternal morbidity. 25% of the affected women are likely to develop acute pyelonephritis in the third trimester of pregnancy, if left untreated. It also causes anemia, hypertensive disease, genitourinary cancer, urolithiasis and chronic renal failure. In fetus it can lead to premature birth, low birth weight babies and perinatal death if left untreated. It is important to identify and treat the infected group, as 40% of the ASB’s develop acute symptomatic UTI. In a meta-analysis of 19 studies, Romero et al reported that women with asymptomatic bacteriuria had a 54% higher risk of a low birth weight infant and twice the risk of a pre-term infant compared with non-bacteriuric women. The mechanism by which asymptomatic bacteriuria promotes preterm labor is not clear, but subclinical amnionitis or phospholipid A2 production by bacteria have been proposed. The incidence of these can be decreased by treating ASB during pregnancy promptly. The American college of obstetrics and gynecology recommends that a urine culture should be obtained at the first perinatal visit usually between 12 and 16 weeks. A repeat urine culture should be obtained during third trimester, because the urine of treated patients may not remain sterile for the entire pregnancy. Pregnant woman should be treated as and when bacteriuria is identified. It has been demonstrated through randomized trials that antimicrobial treatment of asymptomatic bacteriuria during pregnancy will decrease the risk of subsequent pyelonephritis from 20–35% to 1–4% and the risk of having a low birth weight baby from 15% to 5%. Keeping all this in mind, the present study was done to find out the overall prevalence of Asymptomatic Bacteriuria (ASB) in pregnancy, to isolate and identify the organisms and to study the antibiotic susceptibility patterns of isolates to treat the patient as soon as possible in order to prevent the complications arising from untreated ASB in pregnancy. Our aim was to study the Prevalence of Asymptomatic Bacteriuria in pregnant women, study the bacterial isolates in cases of Asymptomatic Bacteriuria in pregnant women and to study the antibiotic susceptibility patterns of bacterial isolates in the cases of Asymptomatic Bacteriuria in pregnancy.

Materials and methods
A prospective study was done in the Department of Microbiology in collaboration with Department of Obstetrics and Gynecology, Santosh Medical College, Ghaziabad, U.P from July 2012 to May 2013. A total of 300 urine samples were collected randomly from the pregnant women attending the obstetric OPD of Santosh Medical College, Ghaziabad U.P.

Inclusion criteria
All the patients included in the study had the following criteria
- Pregnant women , in any trimester
- No symptom of urinary tract infection

Exclusion criteria
- Cases with any symptoms of urinary tract infection( dysuria, frequency, urgency etc )
- If cases have used antibiotics during the preceding 2 weeks.
- Any type of vaginal bleeding
- Pregnancy induced Diabetes Mellitus/ Hypertension

Sample collection
The patient was asked to clean the periurethral area well with a mild detergent to avoid contamination. Once cleaning is completed, the patient was told to
retract the labial folds, begin to void and then collect a midstream urine sample of 30 ml in a 100 ml sterile urine container. The samples are immediately transported to the laboratory and were processed within one hour. In case of delay, the samples are refrigerated at 4°C.

Methodology

1. Urine Culture - Semi quantitative standard loop method

Quantitative culture is the gold standard for diagnosis of bacteriuria. The urine was mixed throughout before plating. A calibrated loop of 0.01 ml volume was inserted vertically into the urine to allow urine to adhere to the loop. The loop was touched to the center of the CLED (Cysteine Lactose Electrolyte Deficient) agar plate, from which the inoculum was spread in a line across the diameter of the plate. Without flaming, loop was drawn across the first inoculums streak numerous times to produce isolated colonies. The plated cultures were incubated at 37°C for 24 hours. The number of colonies grown were counted next morning and interpreted as CFU/ml of urine by multiplying the number of colonies grown by 100. Colony counts exceeding $10^5$ CFU/ml were taken as significant bacteriuria.

Interpretation

- Presence of more than $10^5$ CFU/ml indicates significant bacteriuria.
- Presence of less than $10^5$ CFU/ml indicates contamination from the urethra/vagina.

Cystine Lactose Electrolyte Deficient agar (CLED) - CLED Agar is used for the differentiation and enumeration of microorganisms in urine. Lactose fermentation in acid medium is detected when the pH indicator (bromothymol blue) turns from green to yellow. Cystine favors the growth of coliform bacteria, usually forming small colonies on other media. The electrolyte deficiency reduces invasion by Proteus. Lactose fermenting organisms are seen as yellow colonies and non-lactose fermenting organisms are seen as blue colonies.
TABLE 1 : Interpretation of urine cultures :

<table>
<thead>
<tr>
<th>RESULT</th>
<th>SPECIFIC SPECIMEN</th>
<th>TYPE/ASSOCIATED CLINICAL CONDITION</th>
<th>WORK UP</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥10⁶ CFU/ml of a single potential pathogen</td>
<td>CCMS urine/pyelonephritis, acute cystitis, asymptomatic bacteruria or catherized urine</td>
<td>Complete work up for the predominant organism, description of the other organisms.</td>
<td></td>
</tr>
<tr>
<td>≥10⁷ CFU/ml of a single potential pathogen</td>
<td>CCMS urine/symptomatic males or catherized urines or acute urethral syndrome</td>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>&gt;3 organism types with no predominating organism</td>
<td>CCMS urine or catherized urine</td>
<td>Possible contamination</td>
<td></td>
</tr>
<tr>
<td>Either 2 or 3 organism types with predominant growth of one organism type and &lt;10⁴ CFU/ml of the other organism types</td>
<td>CCMS urine</td>
<td>Complete workup for the predominant organism, description of the other organisms.</td>
<td></td>
</tr>
</tbody>
</table>

Complete work up – identification of the organism and antibiotic sensitivity pattern.

2. Identification of bacterial isolates was done on the basis of colony characteristics, microscopic appearance on gram staining, motility and biochemical reactions using standard microbiological laboratory criteria.

3. Antibiotic sensitivity testing\(^1\) was performed by Kirby -Bauer disc diffusion method under CLSI guidelines. Antibiotics used were Ampicillin (10µgm), Nitrofurantoin (300 µgm), Amikacin (30 µgm), Nalidixic acid (30 µgm), Norfloxacin (10µgm), Ciprofloxacin (5 µgm), Cefotaxime (30 µgm), Cefuroxime (30 µgm).

A. Screening for ESBL producers\(^1\) was also done by the following methods.

Disk-Diffusion method.- The Clinical and Laboratory Standards Institute (CLSI) has proposed disk-diffusion methods for screening for ESBL production by Klebsiella pneumoniae, K. oxytoca, Escherichia coli, P. aeruginosa and Proteus mirabilis. Laboratories using disk-diffusion methods for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. In this study cefotaxime, which is consistently susceptible to CTX-M; and ceftazidime, which is a consistently good substrate for TEM and SHV variants were used.

B. Phenotypic Confirmatory Tests for ESBL

Cephalosporin/clavulanate combination disk method was used. The CLSI advocates use of cefotaxime (30 µg) or ceftazidime (30 µg) disks with or without clavulanate (10 µg) for phenotypic confirmation of the presence of ESBLs in Klebsiella and Escherichia coli.
coli, P. mirabilis and Salmonella species. The CLSI recommends that the disk tests be performed with confluent growth on Mueller-Hinton agar. A difference of $\geq 5$ mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disks was taken to be phenotypic confirmation of ESBL production. Sensitivity and specificity for this method were first reported to be 96% and 100%, respectively.

**Results**

**TABLE 1 – Culture Positivity**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Number of cultures</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>246</td>
<td>82</td>
</tr>
<tr>
<td>$&lt;10^5$ cfu/ml</td>
<td>23</td>
<td>7.66</td>
</tr>
<tr>
<td>$&gt;10^5$ cfu/ml</td>
<td>31</td>
<td>10.3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>300</strong></td>
<td></td>
</tr>
</tbody>
</table>

Of the 300 samples, 246 (82%) of the samples had no growth. 31 (10.33%) samples had significant bacteruria i.e. growth more than $10^5$ cfu/ml while 23 samples had counts less than $10^5$ cfu/ml (contaminants)

**GRAPH 1 – Distribution of organisms**

- Gram Positive Cocci: 19.35%
- Gram Negative Bacilli: 80.64%
TABLE 2 – Number of organisms isolated

<table>
<thead>
<tr>
<th>Organism Isolated</th>
<th>No. of organisms</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>14</td>
<td>45.16</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>8</td>
<td>25.80</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>3</td>
<td>9.67</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>6.45</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>2</td>
<td>6.45</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>1</td>
<td>3.22</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>3.22</td>
</tr>
<tr>
<td><strong>Total =</strong></td>
<td><strong>31</strong></td>
<td></td>
</tr>
</tbody>
</table>

Of the 31 isolates, Escherichia coli (14) was the most common organism isolated (45.16%). Among gram positive organisms, Staphylococcus saprophyticus was the most common organism isolated.

GRAPH 2 Culture Positive Cases According to Age

Highest incidence of significant bacteriuria were present in women who belonged to age group 35 year and above (66.66%).
Of the 31 culture positive cases, maximum cases were multigravida (64.51%)

**Graph 4**

Of the 31 culture positive cases, Maximum cases 18 (56.08%) were found in women in their third trimester.
Of the 14 E.coli isolates, maximum isolates were sensitive to Nitrofurantoin (85.71%) followed by Ampicillin (71.42%). Maximum isolates were resistant to Ampicillin (78.57%) followed by Norfloxacin (71.42%). 57.14% strains of E.coli were resistant to Cefuroxime and 50% strains were resistant to Cefotaxime. Strains that were resistant to third generation cephalorphorins were screened for ESBL production. Of the 14 E.coli, 2 (14.28%) were ESBL producers. Klebsiella pneumoniae isolates were sensitive to Nitrofurantoin and Amikacin and were resistant to Cefuroxime and Cefotaxime. 37% of the isolates were ESBL producers. Staphylococcus saprophyticus isolates were sensitive to Nitrofurantoin. Maximum resistance was shown to Ciprofloxacin (100%) followed by Nalidixic acid and Norfloxacin (66.6% each). All isolates of Staphylococcus aureus were sensitive to Amikacin, 50% isolates were sensitive to Nitrofurantoin. All isolates were resistant to Nalidixic acid. It was observed that most common urinary tract pathogens in pregnant women can be treated with nitrofurantoin which is found to be the most effective drug. A uniformly high resistance to fluoroquinolones was exhibited by the major uropathogens.

**Discussion**

ASB during pregnancy is a common cause of serious maternal and perinatal morbidity; with appropriate screening and treatment, this morbidity can be limited. Hence in the present study, an attempt was made to evaluate semi quantitative urine culture by standard loop method in detection of ASB in antenatal cases, to know the frequency of ASB, isolate and identify the organisms, and study their antibiotic susceptibility patterns.

300 pregnant women were screened randomly and mid-stream urine samples were collected. Maximum women were in the age group of 21-25 yrs. and minimum were in age group 35 years and above. Most of them were multigravida (66%) followed by...
women who were primigravida (44%). Maximum numbers of women screened were in their third trimester (51%). Considering the fact that 51.0% of all the subjects attended OPD in the last trimester, which could be due to lack of opportunity or awareness of need for check-ups till some pressure symptoms develop in the second or third trimester which forces medical attention.

Earlier studies that were conducted reveal that the prevalence of bacteriuria during pregnancy ranges from 2 to 11%. This variation appears to be related to the socio-economic status of the group of women studied. In the present study, of the 300 pregnant women, 31 women were culture positive, giving the incidence rate of ASB as 10.3%. This finding was in correlation with the study done by Mitra P. et al\textsuperscript{15} and Priyadarsini Indira et al\textsuperscript{16}. However, Perera Jennifer et al in Sri Lanka reported a much lesser incidence (3.6%) than found in our study\textsuperscript{17}. Studies outside India showed a much higher incidence of ASB showing as high as 86.6%.\textsuperscript{18}

Of the 31 culture positive isolates, gram positive cocci accounted for 19.35% of the total isolates, the most frequent one being Staphylococcus saprophyticus. Earlier workers did not give any significance to Coagulase negative staphylococci isolated from the urine sample and considered them as contaminants. But in recent years, Coagulase Negative Staphylococci in significant number has been viewed as causative agent for urinary tract infection.

The dominant isolate in our study was Escherichia coli 14 (45.6%) followed by Klebsiella pneumoniae 8 (23.08%). However, Akinola B Ajayi\textsuperscript{18} et al reported Staphylococcus aureus to be the most common organism in their study. Priyadarsini Indira\textsuperscript{16} et al found CONS as the most predominant organism (41.66%). This variation may be due to the fact that ASB is a community acquired infection and hence, the organisms isolated in each study differ.

Contributing risk factors for developing bacteriuria during pregnancy are age, parity, trimester, sexual intercourse, diabetes mellitus, sickle cell disease, trait, anatomical abnormalities of the urinary tract, previous history of UTI, and socioeconomic status. In this study, three variables, age, parity, and trimester are evaluated. In the present study, highest incidence of significant bacteruria was present in women who belonged to age group 35 and above years (66.66%). Lowest incidence of significant bacteruria was present in women of age group 18-20 years (6.97%). Incidence of Significant bacteriuria has increased according to age group. Nicolle LE et al found that higher prevalence of ASB in patients with advancing maternal age is due to increasing incidence of co morbid conditions, which is associated with neurogenic bladder and increased residual urine volume or urinary reflux.

Maximum culture positive cases were present in multigravida women (64.51%) as compared to primigravida women (35.48%). This may be a consequence of increasing age and parity predisposing the pregnant women to bacteriuria, due to its effect on pelvic tissue support which gets lax with advancing age, thus providing easier access of organisms in the urethra to the bladder and upper urinary tract. Also, it is likely that multipara have had more manipulations of the urinary tract with the catheter, which is an important factor in causation of bacteriuria. Besides, each child-birth produces some trauma to the genito-urinary tract which may predispose them to bacterial invasion during subsequent pregnancy. This finding was similar to Lavanya SV\textsuperscript{19} et al however, a study by G nathet al\textsuperscript{20}
showed that significant bacteriuria was more in primigravida women than in multigravida.

Conclusion
Prevalence of ASB in Pregnant Women is low (10.3%) but its complications are severe. The adverse effects of undiagnosed asymptomatic bacteriuria on mother and child have made us to suggest routine urine culture screening for all pregnant women attending antenatal clinic for the presence of bacteriuria at their first prenatal visit, preferably in the first trimester in order to prevent mother and child from any form of complication that may arise due to infection. It is regrettable that there are no adequately powered clinical trials to establish the optimal duration of therapy for asymptomatic bacteriuria, nor recent studies comparing currently recommended antibiotics that include relevant maternal and infant outcomes. Furthermore, there is also a paucity of data from low- and middle-income countries. It is important that the research agenda does not ignore these important deficiencies in our knowledge of the management of asymptomatic bacteriuria in pregnancy.

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