Research Article

Asymptomatic bacteriuria among antenatal women attending a tertiary care hospital in Kanchipuram: evaluation of screening tests and antibiotic susceptibility pattern

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ABSTRACT

Background: Asymptomatic bacteriuria (ASB) occurring in pregnant women can lead onto complications like acute pyelonephritis, hypertensive disease of pregnancy, premature delivery and intrauterine growth retardation if untreated.
Methods: The present study aims to estimate the occurrence of asymptomatic bacteriuria in antenatal women and to study the antibiotic susceptibility pattern of the isolates. The Gram staining, pus cell count and culture was performed for 120 urine samples. Antibiotic susceptibility testing was done by Kirby Baeur disk diffusion method. MRSA (Methicillin resistant *Staphylococcus aureus*) and ESBL (Extended spectrum Beta Lactamases) producers were identified by Standard guidelines. The sensitivity, specificity, negative predictive values and positive predictive values of Gram staining and pus cell count was calculated.
Results: Out of the 120 samples 14 (11.66%) were positive for asymptomatic bacteriuria. The Gram staining showed specificity and negative predictive value of 95.2% and 98.1% respectively. Pus cell count showed a specificity and negative predictive value of 96.29% and 98.11% respectively. Escherichia coli were the predominant species isolated 5 (35.7%). Among the gram negative bacteria, amikacin and nitrofurantoin showed a susceptibility of 90% and 80% each. All the *staphylococcus aureus* isolates showed 100% sensitivity for nitrofurantoin. Two *Klebsiella spp* and one Escherichia coli isolate were identified as ESBL producers. Among the *S. aureus* isolates 3 were identified as Methicillin resistant (MRSA).
Conclusions: Urine culture should be performed for all pregnant women irrespective of the symptoms and should be treated promptly to prevent the complications arising out of ASB.

Keywords: Asymptomatic bacteriuria, Antenatal women, Antibiotic susceptibility pattern, MRSA, ESBL

INTRODUCTION

Urinary tract infections are a common health problem and are more common in women compared to men.[1] The altered physiological, anatomical and hormonal changes during Pregnancy makes the antenatal mother more prone for Urinary tract infection. Such infections can be either symptomatic or asymptomatic. The term asymptomatic bacteriuria (ASB) is used when a bacterial count of the same species over 10^5/ ml in midstream clean catch specimen of urine is obtained on two occasions without symptoms of urinary tract infection.[2] This is very

common during pregnancy (3-8%) with peak incidence between 9-17 weeks of gestation.[3]

India a developing country with a large number of women in the child bearing age group and most of them are from low socioeconomic status, owing to the lack of education and awareness of Asymptomatic bacteriuria, they tend to neglect minor symptoms and ultimately face complications like premature births, low birth weight and increased perinatal mortality. Pregnant women with Asymptomatic bacteriuria are more likely to develop acute pyelonephritis in later pregnancy, postpartum urinary tract infection, hypertensive disease of pregnancy, anaemia, chronic renal failure.[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 clinic in order to prevent mother and child from any form of complication that may arise due to infection.[5]

Hence the present study is undertaken to estimate the occurrence of asymptomatic bacteriuria among Antenatal mothers, to screen the urine for pus cell count, presence of bacteria by gram staining, to isolate, identify the bacterial aetiology, to study the antibiotic susceptibility pattern and to detect the presence of drug resistant isolates such as MRSA (Methicillin Resistant *Staphylococcus aureus*) and ESBL (Extended spectrum beta lactamases).

METHODS

This observational study was carried out in the Department of Microbiology for a period of two months (June- July 2015). One hundred and twenty Antenatal women were included in the study. The study was approved by the institutional ethical committee and informed consent was obtained from all the participants. One hundred and twenty antenatal women without signs and symptoms of urinary tract infection were included in this study. Patients with signs and symptoms of urinary tract infection, structural and functional abnormality of the urinary tract and women on antibiotic treatment for the past one week were excluded from the study.

Clean catch mid-stream urine sample was collected in a sterile universal container and transported within half an hour to the laboratory and processed without delay. Screening for bacteriuria was done by performing Gram staining of uncentrifuged urine. Presence of ≥ 1 bacteria per oil immersion field in 20 fields correlates with significant bacteriuria of $\geq 10^5$CFU/mL of urine.[2] The pus cell count was estimated with uncentrifuged urine using a neubar chamber. Pus cell count of ≥ 10 cells per microliter of urine is considered as significant.[2]

Urine culture was done with 0.001 ml of well-mixed urine delivered by a sterile calibrated wire loop of 4mm diameter and plated on Mac conkey agar and blood agar which were incubated aerobically at 35-37° C for 24

Hours. Repeat culture was ordered for contaminated specimens. Each significant isolate was identified by colony morphology, gram staining and biochemical reactions according to standard procedure.

Kirby Bauer disc diffusion method was used to test the antibiotic susceptibility of the Bacterial isolates for ampicillin, amoxicillin, amikacin, ciprofloxacin, nitrofurantoin, cefotaxime, ceftazidime, cotrimoxazole, vancomycin, piperacillin tazobactum as per Standard microbiological technique.[6] MRSA isolates were identified by cefoxitin disc diffusion method .ESBL producers were screened and confirmed by resistance to at least two third generation cephalosporins.[6]
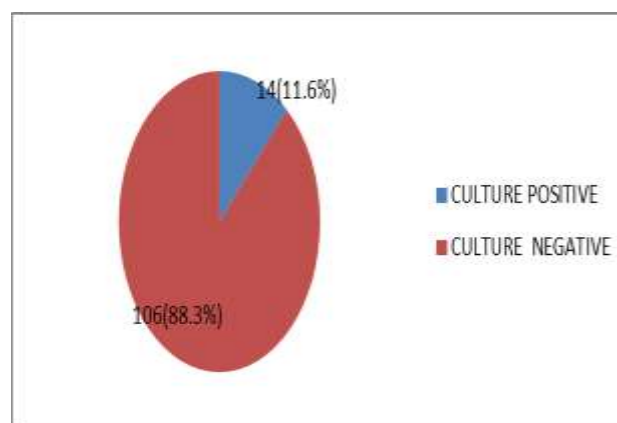
Statistical analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of the screening tests (Gram staining and the pus cell count) were compared with standard loop method of culture.

RESULTS

A total of 120 antenatal women without any signs and symptoms of urinary tract infection were included in the study. Predominant of the patients 110 (91.6%) patients were in the age group of 21- 30 years. Out of the 120 patients 14 (11.66%) were culture positive for Asymptomatic Bacteriuria and 8 (57.1%) of them were in their second trimester of pregnancy (Table 1) (Figure 1).

Table 1: Trimester wise distribution of the cases and the culture positivity percentage.

Trimester	Total number of cases	Number positive for asymptomatic bacteriuria
First trimester	11 (9.1%)	2 (14.28%)
Second trimester	102 (85%)	8 (57.1%)
Third trimester	7 (5.8%)	4 (28.57%)
Total	120	14

Figure 1: Distribution of number of cases.

The sensitivity, specificity, negative and positive predictive values of Gram staining and Pus cell count are depicted in Table 2. The Gram staining showed a specificity and negative predictive value of 95.2% and 98.1% respectively. Pus cell count showed a specificity and negative predictive value of 96.29% and 98.11% respectively.

Table 2: Sensitivity, specificity and predictive values of gram staining and pus cell count.

S.No.	Screening tests	Sensitivity	Specificity	Positive predictive value	Negative predictive value
1	Gram staining	84.6%	95.2%	98.1%	70.5%
2	Pus cell count	83.3%	96.29%	71.4%	98.11%

Table 3: Distribution of bacterial isolates (N= 14).

S.No.	Bacterial isolates	Percentage
1	*Escherichia coli*	5 (35.7%)
2	*Staphylococcus aureus*	4 (28.5%)
3	*Citrobacter species*	3 (21.4%)
4	*Klebsiella pneumonia*	2 (14.28%)
	Total	14

Escherichia coli was the predominant species isolated 5 (35.7%) followed by *Staphylococcus aureus* 4(28.57%), *Citrobacter spp.* 3 (21.4%) and *Klebsiella spp.* 2 (14.28%) (Table 3).

The Antibiotic susceptibility pattern of the isolates is as depicted in table 4. Out of the 10 Gram negative isolates 9(90%) were sensitive to Amikacin. Ciprofloxacin and nitrofurantoin had a susceptibility of 8 (80%). All the isolates were sensitive to co-trimoxazole, Imipenem and pipericiilin-tazobactum. Ampicillin and amoxicillin showed a sensitivity of 3 (30%) and 5 (50%) respectively. Among the *S. aureus* 03 isolates were susceptible to Amoxicillin. All the four isolates were susceptible to amikacin, ciprofloxacin, nitrofurantoin, cefotaxime, ceftazidime cotrimoxazole and vancomycin.

Among Escherichia coli 100% sensitivity was seen for cotrimoxazole, imipenem, pipericillin tazobactum and 60% sensitivity was seen for cefotaxime and ceftazidime and 40% for nitrofurantoin. Among the gram negative isolates 2 *Klebsiella spp* and 1 *E. coli* isolate were identified as ESBL producers by double disc diffusion method. Among the *S. aureus* isolates 3 were identified as methicillin resistant (MRSA) by cefoxitin disc diffusion method.

Table 4: Antibiotic susceptibility pattern of the isolates.

S.No.	Antibiotics	*Escherichia coli* N=5	*Citrobacter spp* N=3	*Klebsiella spp* N=2	Gram negative N=10	*Staphylococcus aureus* N=4
1	Ampicillin	1 (20%)	1 (33.33%)	1 (50%)	3 (30%)	3 (75%)
2	Amoxicillin	3 (60%)	2 (66.66%)	0	5 (50%)	3 (75%)
3	Amikacin	4 (40%)	3 (100%)	2 (100%)	9 (90%)	3 (75%)
4	Ciprofloxacin	4 (40%)	3 (100%)	1 (50%)	8 (80%)	3 (75%)
5	Nitrofurantoin	4 (40%)	3 (100%)	1 (50%)	8 (80%)	4 (100%)
6	Cefotaxim	3 (60%)	3 (100%)	1 (50%)	7 (70%)	3 (75%)
7	Ceftazifdime	3 (60%)	3 (100%)	1 (50%)	7 (70%)	4 (100%)
8	Cotrimoxazole	5 (100%)	3 (100%)	2 (100%)	10 (100%)	4 (100%)
9	Vancomycin	-	-	-	-	4 (100%)
10	Imipenem	5 (100%)	3 (100%)	2 (100%)	10 (100%)	-
11	Pipericillin tazobactum	5 (100%)	3 (100%)	2 (100%)	10 (100%)	4 (100%)

DISCUSSION

Urinary tract infection occurring during pregnancy should be considered with unique care for the purpose of both clinical and laboratory diagnosis and management. The main problem with asymptomatic bacteriuria in pregnancy is that the patients are asymptomatic and unless they are screened it can remain discreet leading on to grave complications for both the mother and the fetus.

The predominant age group in our study was between 21-30 years (91.6%) of age. Among the pregnant women with culture positivity for ASB 9 (7.5%) were in the age group of 21-30 years similar to Roy et al.[7] Lavanya et al

has reported that the incidence of ASB was high in less than 20 years of age which was in contrast to our study.

In this study Asymptomatic bacteriuria was detected in 14 (11.6%) of pregnant women which was similar to Anne et al where the prevalence was 13%.[8] Various studies in India have reported the prevalence ranging from 6% to 22%.[2,9,10] Reports from across the world has documented a prevalence of as high as 40-45%.[11,12]

As per the present study higher incidence of ASB was seen in the second trimester of pregnancy 8 (57.1%) which was similar to Nath et al and Roy et al.[7,13] Yasodhara et al has reported higher incidence of ASB in the first trimester of pregnancy in contrast to our study.[14] Higher incidence in the second trimester of pregnancy could be due the anatomical changes occurring in the uterus, structural and functional changes in the urinary tract like dilatation of ureters favoring stasis of urine leading on to infection. Usually the bacteria that are responsible for ASB are those that are present in the gastrointestinal tract like *E. coli* that colonize the periurethtral region. The transition from being a colonizer to a pathogen responsible for causing infection might be due to various host factors like hormonal and anatomical changes occurring in pregnancy and enhanced expression of virulence factors in the bacteria.

In this study Gram staining of uncentrifuged urine had a sensitivity and specificity of 84.6% and 94.25%. The negative predictive and positive predictive values were 98.1% and 70.5% respectively. This finding was in Similar to a study by Almudena Burillo et al.[15] In the present study we observed a high specificity and negative predictive values for gram staining of urine signifying it as a useful screening test for bacteriuria in resource constraint situations where culture facilities are not available .Nevertheless the limitation could be that it requires trained personnel to screen and interpret the results and observer variation may also occur in interpreting the result. Studies have reported the sensitivity and specificity of Gram staining of urine varying from 80- 99%.[2,15] Our study have found a high specificity (96.29%), high NPV (98.11%) for Pus cell count similar to the study by Sushma thakre.[9]

In the present study Escherichia coli 5 (38.4%) was the most common bacteria isolated. Studies done by Jain et al, Senthinath et al and Sevki C have also showed that Escherichia coli was the commonest isolate.[16-18] The second predominant isolate was *S. aureus* 4 (28.5%) followed by *Citrobacter spp* 3 (21.4%) and *Kebsiella spp* 2 (14.28%).[5,19]

Among the Gram negative isolates Escherichia coli showed 4 (80%) sensitivity to amikacin, ciprofloxacin and nitrofurantoin. cefotaxime and ceftazidime showed a sensitivity of 60%. All the five isolates were sensitive to cotrimoxazole and imipenem similar to Sujatha et al.[20] Among the *S. aureus* 3 (75%) were sensitive to amikacin,

ciprofloxacin, nitrofurantoin, cefotaxime, ceftazidime, co-trimoxazole and vancomycin. Nitrofurantoin had overall sensitivity of 10 (71.4%). Cotrimoxazole showed a sensitivity of 100%.

Two *E. coli* isolates and one *Klebsiella Spp* were confirmed to be Extended Spectrum Beta lactamases producers by double disc method. Three *S. aureus* isolates were identified as MRSA. Emergence of Drug resistant bacteria like MRSA and ESBL causing ASB is of serious concern as treating such infections becomes difficult. The occurrence of such Antibiotic drug resistance could be due to the prior antibiotic usage and self-medication.

CONCLUSION

In this study urine samples from pregnant women were analyzed for the presence of asymptomatic bacteriuria. Though Gram staining and pus cell count can be used as a screening test both requires expertise for interpretation and cannot be used as a single test for identifying ASB. Urine culture is a gold standard method for identifying ASB.

Studies have reported that repeating urine cultures in each trimester improves detection rates of ASB.[21] Hence all pregnant women should be screened for ASB during each trimester. Urine culture should be performed for all pregnant women irrespective of the symptoms and if found positive should be treated according to the Antibiotic susceptibility pattern to prevent the complications arising out of ASB.

On the wake of emergence of multidrug resistant isolates the common bacteria causing the ASB and their Antibiotic sensitivity pattern in a community or region should be studied to aid clinicians in choosing a empirical drug for treatment. Early screening and detection of antenatal women with asymptomatic bacteriuria is of immense value in preventing complications like progressive deterioration of renal function and premature delivery thereby reducing neonatal mortality rates.

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