Prevalence, bacteriological profile and antimicrobial resistance pattern of aerobic bacterial isolates from antenatal mothers with asymptomatic bacteriuria

Shalima S[1], Kalaivani R[2,*], Seethesh G[3], Seetha KS[4]

[1]Student, [2]Associate Professor, [4]Professor & HOD, Dept. of Microbiology, [3]Professor & HOD, Dept. of Obstetrics & Gynecology, MGM College & Research Institute, Pondicherry

***Corresponding Author:**
Email: kalaimicro21@gmail.com

Abstract

Introduction: Urinary tract infections (UTI) in pregnancy are more common among antenatal women because of the physiological and morphological changes that occur during pregnancy. UTI may progress from asymptomatic to symptomatic bacteriuria as the pregnancy progresses. Asymptomatic bacteriuria (ASB) if not diagnosed earlier and treated properly, may lead to pyelonephritis, postpartum UTI, hypertensive disorders, prematurity, low birth weight babies and pre-natal death, etc.

Materials and Method: The present investigation was based on a total samples from 637 antenatal mothers with asymptomatic bacteriuria during August 2015 to October 2015. Their mid-stream clean catch urine was collected and was screened for asymptomatic bacteriuria by semi quantitative culture method. Isolates were identified and their antibiotic susceptibility testing was recorded.

Results: Out of 637 asymptomatic bacteriuria suspected antenatal women, 268 got reported as sterile, and the remaining 315 samples showed significant growth of single type of aerobic bacterial growth. Majority (66%) of these isolates were isolated from antenatal mother with age group between 21-35 years. Significant numbers of positive urine cultures were from antenatal women with third trimester of pregnancy with primigravidae. The proportion of asymptomatic bacteriuria in our study group was 49.5%. Gram negative bacilli were the predominant pathogen isolated, of which 56% were *E.coli* followed by others. All our Gram positive and Gram negative isolates showed very significant amount of resistance towards all the available group of drugs.

Conclusion: Though asymptomatic bacteriuria is more common during pregnancy, it needs early diagnosis with appropriate and adequate management. Thus the morbidity and mortality can be kept under control.

Keywords: Asymptomatic bacteriuria, Antenatal UTI, Significant bacteriuria, UTI in pregnancy.

Introduction

Significant bacteriuria is defined as, the presence of $\geq 10^5$ colony forming units (CFU)/ml of bacteria in the urine sample of an individual with or without symptoms.[1,2] Majority of Urinary Tract Infection (UTI) may be of symptomatic. Asymptomatic bacteriuria (ASB) will be more common among antenatal women due to various factors. Approximately 2-7% of pregnancies may develop asymptomatic bacteriuria. Of which about 30-49% might further progress to acute pyelonephritis and neonatal complications.[3] Maternal complications such as pyelonephritis, hypertension, preeclampsia, anemia, amnionitis and endometritis are more common.[4] Further rarely it may progress to renal scarring and renal failure also.[5] Neonatal complications include premature rupture, preterm deliveries, low-birth weight (LBW), recurrent abortions, Intrauterine Growth Retardation (IUGR), polyhydramnios, oligohydramnios, etc.[6]

The commonest etiological agents responsible for asymptomatic bacteriuria were *Escherichia coli (E.coli), Proteus* sp., *Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus* sp., etc.[7] These pathogens have already acquired various resistance mechanisms against almost all available groups of drugs as a result of inappropriate and indiscriminate use of various antimicrobial agents in the community and in the hospital settings. Thus, it created an additional burden to the patients and clinicians. To overcome various medical and obstetric complications caused by asymptomatic bacteriuria among antenatal women and neonates, our study was aimed to know the distribution of various pathogenic bacterial agents and its antibiotic resistance pattern.

Materials and Method

A prospective study was conducted at department of Microbiology in a tertiary care hospital, Pondicherry from August 2015– October 2015 involving all pregnant women with asymptomatic bacteriuria attending the antenatal clinics during the study period. Pregnant women who were on antibiotic treatment two weeks prior to their initial visit, those with a history of UTI symptoms (e.g., dysuria, frequency and urgency, etc.), pregnant women who exhibited clinical signs and symptoms of urinary tract infection (UTI), those who were at 38 weeks of gestation or more, pregnancy induced Diabetes Mellitus, Hypertension, known congenital anomalies of the urinary tract were excluded.

Sample Collection: Antenatal mothers fulfilling our criteria's were properly educated to collect clean catch midstream urine into a sterile wide-mouthed, screw capped container (approximately 10-20ml). All the samples were properly labeled, transported and

immediately processed onto appropriate culture media, using standard loop calibrated to hold 0.01 ml of urine onto Blood agar and Cysteine Lactose Electrolyte deficient (CLED) agar. Inoculated plates were incubated at 37 degree C aerobically for overnight incubation.

Colony count was done to check for significant bacteriuria ($\geq 10^5$CFU/ml) by semi quantitative method. All the significant isolates were identified by colony characters, Gram's staining, IMViC tests. Antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method.[9,10] Quality control was done with the following ATCC control strains; *E. coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923.[8,9]

All antibiotics discs were obtained from Hi Media laboratory, India. List of antibiotics used were, Cotrimoxazole (SXT)25/23.75µg, Ceftazidime (CAZ)30µg, Cefotaxime (CTX)30µg, Gentamicin (GEN)10µg, Amikacin (AMK)30µg, Imipenem (IPM)10µg, Vancomycin (VAN)30µg, Polymixin B (POL)300µg, Colistin (COL)10µg, Norfloxacin(NX)10µg, Nitrofurantoin(NF)300µg, Nalidixic acid(NA)30µg, Cefoperazone sulbactam(CFS)30/15µg, Ampicillin (AMP)10µg, Ciprofloxacin(CIP)5µg, Cefoxitin(CFX)30µg, Linezolid(LNZ)30µg, Teicoplanin(TEC)30µg, Erythromycin(ERY)15µg, Penicillin(PEN)10µg, Tetracycline(TCY)30µg.

Results

A total of 637 asymptomatic antenatal mothers urine sample were included in this study, over a period of three months. Of which, 54 samples were rejected as mixed growth, 268 got reported as sterile. And the remaining 315 samples showed significant growth of single type of pathogenic bacteria (Graph 1).

Majority of the isolates were isolated from antenatal mother with age group between 21-35 years of age (66%), followed by age group <20 years (24%) and >36 years (10 %) (Table 1). Based on the gestational age, significant numbers of positive cultures were predominantly isolated from antenatal women with third trimester of pregnancy, followed by first and second trimesters. Of which, 62 % of them were with primigravidae and the remaining 38% were with multigravida (Table 2).

Graph 1: Distribution of Urine sample received (n=637)

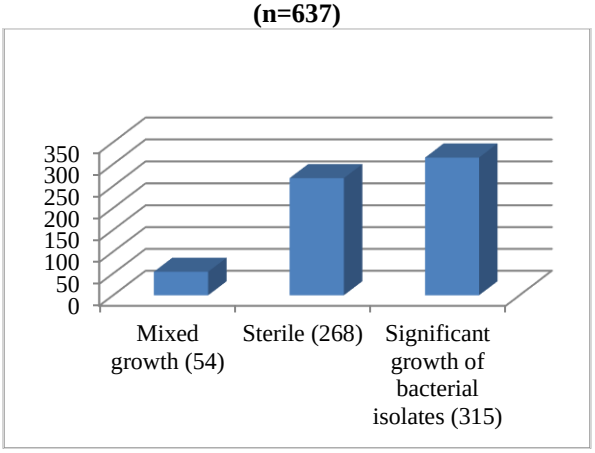


Table 1: Age wise distribution of positive asymptomatic bacteriuria cases

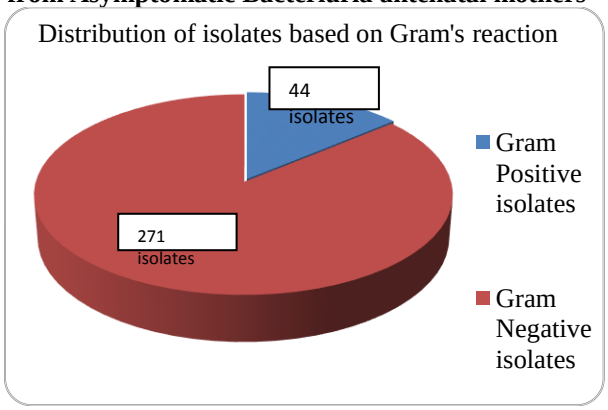
Age of the antenatal women with asymptomatic bacteriuria	Distribution (n=315)
Less than 20 years	24% (76)
21-35 years	66% (208)
More than 36 years	10% (31)

Table 2: Distribution of positive asymptomatic bacteriuria cases based on gestational weeks

Gestational age	Percentage distribution
1st trimester	22% (69)
2nd trimester	29% (91)
3rd trimester	49% (155)

Out of these 315 antenatal mothers urine samples, 44 showed growth of Gram positive cocci, and 271 samples showed growth of Gram negative bacilli (Chart 1). The proportion of asymptomatic bacteriuria in our study group antenatal mother was 49.5%.

Chart 1: Distribution of bacterial isolates- isolated from Asymptomatic Bacteriuria antenatal mothers



Out of 271 significant Gram negative isolates, the majority were, *E.coli* of 56% followed by, *K.pneumoniae* 23%, Non fermenting Gram negative

bacilli (NFGNB) of 11%, *Citrobacter* sp. 7%, and others (Table 3).

Table 3: Distribution of Gram negative isolates

S. No:	Gram Negative Isolates	Total	Percentage
1.	*E.coli*	152	56%
2.	*K. pneumoniae*	61	23%
3.	*NFGNB*	31	11%
4.	*Citrobacter* sp.	19	7%
5.	*Proteus* sp	4	1.55
6.	*Pseudomonas aeruginosa*	3	1.10%
7.	*Enterobacter* sp.	1	0.40%
Total		271	100%

Among 44 Gram positive isolates, 89% were of *Enterococcus* sp., 4.5% were each of *Staphylococcus epidermidis* and *Beta haemolytic Streptococci* and 2% were of *Coagulase negative Staphylococci* (Table 4).

Table 4: Distribution of Gram positive isolates

S. No.	Gram positive isolates	Total	Percentage
1.	*Enterococcus* sp.	39	89 %
2.	*Staphylococcus epidermidis*	2	4.5%
3.	*Beta haemolytic Streptococci*	2	4.5%
4.	*Coagulase negative Staphylococci(CONS)*	1	2%
Total		44	100%

The antibiotic resistance pattern of all Gram negative isolates showed 71% resistance towards Gentamicin, Cotrimoxazole 56%, Cefotaxime 59%, Amikacin 34%, Imipenem 2%, Cefoperazone sulbactam 8%, Norfloxacin 37%, Nadixic acid 63% and 44% on Nitrofurantoin (Table 5).

Table 5: Antibiotic resistance pattern of Gram negative bacilli isolated

Organism	Gen	Cot	Ctx	Ak	Imp	Cfs	Nx	Na	Nf
E.coli (152)	80%	60%	59%	30%	3%	6%	39%	77%	22%
Klebsiella sp. *(61)*	62%	62%	55%	38%	4%	9%	22%	40	64%
Citrobacter sp. (19)	69%	44%	44%	50%	0%	13%	13%	38%	50%
NFGNB (31)	52%	24%	69%	38%	0%	14%	48%	52%	86%
Proteus sp. (4)	100%	100%	75%	25%	0%	0%	100%	100%	75%
Pseudomonas aeruginosa (3)	67%	100%	100%	67%	0%	0%	100%	100%	67%
Enterobacter sp. (1)	0%	0%	100%	100%	0%	0%	100%	100%	100%
Total (271)	71%	56%	59%	34%	2%	8%	37%	63%	44%

Table 6: List of Antibiotic used and its resistance pattern

Organisms	P	Cip	E	Tet	Amp	Cx	Hlg	Va	Lz	Tei
Enterococcus sp. (39)	92.3%	86.50%	82%	66%	45.40%		36.80%	5.10%	0%	23%
Staphylococus epidermidis (2)	100%	50%	0%	0%	-	0%	-	0%	0%	50%
Beta haemolytic Streptococci (2)	50%	50%	0%	100%	0%	-	-	0%	0%	0%
CONS (1)	100%	0%	100%	0%	-	0%	-	0%	0%	0%
Total (44)	89%	80%	73%	64%	39%	-	-	5%	0%	23%

Gram positive isolates showed resistance towards Penicillin 89%, ciprofloxacin 80%, Erythromycin 73%, Tetracycline 64%, Ampicillin 39%, Teicoplanin 23% and towards Vancomycin of 5% (Table 6).

Discussion

Presence of bacteriuria is not always associated with symptoms, the picture ranges from asymptomatic bacteriuria, cystitis, pyelonephritis and it may end with several more complications.[11] Risk of developing pyelonephritis is higher in pregnant women with asymptomatic bacteriuria.[12,13] The complications of UTI various from one to another, mainly depends on their gestational age and load of organisms. The risk of UTIs, in pregnancy increases during the beginning of 6 weeks and peaks during 22 to 24 weeks.[14]

Our study population had high incidence of asymptomatic UTI during their 3rd trimester of pregnancy and the majority fall between the age group 21 and 35 years of age. Concurrence with ours, Khan S et al., (64.04%), Sudha et al., (52%) and Sujatha et al., (72.72%) also reported high prevalence among antenatal mothers with 21-30 years of age.[15,16,17] Prasanna et al. reported 49% of their asymptomatic antenatal mothers were on 3rd trimester, which was very similar to our findings.[18]

The proportion of antenatal asymptomatic bacteriuria in our study was 49.5%. Very similar to our study, Imade et al., also reported 45.3% prevalence.[19] A study by Shahira et al., from Egypt reported 31.3% UTI among their study population which was very close to our findings.[20] In contrast to our study, Obirikorang et al., reported 9.5% prevalence.[21] Sandhya et al.,

reported only 17% prevalence, and in yet another Egyptian study, the prevalence of asymptomatic UTI during pregnancy ranged between 22-35%, which was not correlating with our findings.[22,11,23] We reported a high rate of proportion during our study period. A Nigerian study reported very high prevalence of 87% in their population, which was very much contrast to our findings.[24]

In the present study, Gram negative bacteria were more frequently (86%) isolated than Gram positive bacteria (14%), which were very much similar to Prasanna et al.,[18] Out of those isolates from asymptomatic antenatal mothers, we documented that *E.coli* (56%) was the most common Gram negative bacterial pathogen followed by *Klebsiella* sp. (23%) and others. Among Gram positive isolates *Enterococcus* sp. (89%) was the most common pathogen isolated. Obirikorang et al reported *E. coli* (36.8%) as the most commonest bacterial pathogen, followed by *Klebsiella* sp., (26.3%), yet another study by Khan et al., also reported *E.coli* as their most commonest pathogen.[21,15] Prasanna et al., reported majority (62%) were *E.coli* and only 18% were *Klebsiella* sp., which were very similar to our results.[18] In contrast, Shahira et al., reported *Klebsiella* sp. followed by *E.coli* as the commonest pathogens [20].

Antibiotic treatment need to be directed towards the specific pathogen cultured in pregnant women with asymptomatic bacteriuria. Nitrofurantoin and TMP/SMX should be avoided near term (36-42 weeks) and during labour, due to the risk of fetal abnormalities like jaundice, kernicterus and hemolytic anemia in the newborn.[25] Amoxicillin, cephalexin, and cefixime represent safe options that can be given for the full duration of pregnancy.[26] Out of our Gram negative isolates, the highest resistance of 71%, 63%, 59%, 56% and 44% were documented towards gentamicin, nalidixic acid, cefotaxime, cotrimoxazole and nitrofurantoin respectively. Among Gram positive isolates, 89%, 80%, 73% and 64% resistance were noticed towards penicillin, ciprofloxacin, erythromycin and tetracycline. The isolates from Khan et al., showed 100% sensitivity to imipenem and were mostly susceptible to cephalosporin's, cefepime, ceftriaxone, and cefuroxime,[15] which was less similar to our findings.

According to Andabati et al., most bacterial isolates (62%) were resistant to amoxicillin.[27] Close to our data, isolates from Endale Tadesse et al., showed resistance pattern of norfloxacin (64.7%), gentamicin (47.1%), cefotaxime (43.15%), penicillin (30.8%), trimethoprim-sulphamethoxazole (25.5%), vancomycin (23.5%), and ampicillin (17.3).[28] In contrast to our study, Sandhya et al., reported that, their isolates showed more sensitivity towards ceftazidime, nitrofurantoin, amikacin, cefotaxime drugs, which are relatively safe in pregnancy and effective against UTI.[22] Isolates from Prasanna et al., were found to be sensitive to drugs like nitrofurantoin (76%), norfloxacin (72%), ceftriaxone (71%), amikacin (80%), and meropenem (90%).[18] Similar to this result, our isolates also showed very less resistance percentage towards amikacin 34%, imipenem 2%, and cefoperazone/sulbactam 8%, norfloxacin 37%. Trimethoprim/sulfamethoxazole (TMP/SMX) and ciprofloxacin have historically been recommended as empiric agents for the treatment of uncomplicated UTI.[22] According to 2008 VIHA antibiogram, *E. coli* susceptibilities to TMP/SMX and ciprofloxacin were 81% and 77%. Resistance to TMP/SMX is also a concern although it remains an alternative in cases of contraindications to other effective therapies.[29]

Conclusion

Due to various factors, asymptomatic bacteriuria became more common during antenatal period, and it's known to cause various obstetric complications, if not properly intervened. Thus it is imperative to have screening for bacteriuria periodically in every trimester of pregnancy. Following routine urine culture, all the bacteriuria antenatal women's need to be given adequate and appropriate treatment, thus all the untoward effects can be prevented. Resistance pattern of bacterial pathogen may differ from region to region, which mainly depends upon the local antibiotic prescription pattern among the doctors. *E.coli, Klebsiella* sp., and *Enterococcus* sp. were found to be the most common bacterial pathogen to cause asymptomatic bacteriuria. And we documented high percentage of resistance towards gentamicin, cotrimoxazole, penicillin, ciprofloxacin and erythromycin. Thus we conclude by hoping that, our data will be informative to the antibiotic policy and treatment strategy formulators to prevent and to treat the asymptomatic bacteriuria among antenatal populations.

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