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Original Research Article

Microbiological profile of asymptomatic bacteriuria in pregnancy

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ABSTRACT

Background: Asymptomatic bacteriuria is the presence of actively multiplying bacteria within the urinary tract in the absence of any symptoms. Anatomical and physiological changes make women more susceptible to UTI in pregnancy. The objective of present study was to determine the percentage of pregnant women with asymptomatic bacteriuria and its microbiological profile.
Methods: A total of 555 antenatal women who had no clinical features of urinary tract infection were recruited for this study over a period of one year. Clean catch mid-stream urine sample was collected and semi quantitatively cultured immediately. Significant bacteriuria was identified and antibiotic sensitivity found out by conventional methods.
Results: Significant growth was observed in 26 samples. There was no association between age, parity, gravidity, period of gestation and asymptomatic bacteriuria. Gram stain was found to be the best screening test. Escherichia coli were the commonest organism isolated. Escherichia coli and Staphylococcus saprophyticus were the most resistant organisms.
Conclusions: Escherichia coli, the most common organism isolated, was resistant to most of the commonly used antibiotics. Wet film examination, the most commonly used screening test in our set up, was less sensitive and specific. So culture has to be done in all antenatal cases for screening asymptomatic bacteriuria of pregnancy.

Keywords: Asymptomatic bacteriuria in pregnancy, Pregnancy, Screening tests, Semi-quantitative culture, UTI

INTRODUCTION

Urinary tract infections are amongst the most common infections encountered in clinical practise. About 50% of women experience at least one episode of urinary tract infection during their life time.[1] UTI in pregnancy can be symptomatic or asymptomatic.[1-4] Asymptomatic bacteriuria (ASB) is defined as persistently and actively multiplying bacteria in significant numbers i.e., 10^5 bacteria per ml within the urinary tract without any obvious symptoms.[1-5] It is also known as Covert bacteriuria. Females are more susceptible for these infections because of the short length of urethra along with proximity to warm, moist anal canal.[4] Sexual intercourse facilitate the ascent of bacteria into bladder. The pregnant females are two times more commonly affected than age matched non-pregnant females. The reason behind this is urinary stasis due to progesterone effect in pregnancy in addition to different anatomical changes occurring during pregnancy.[2,4]Various studies from the west have documented the prevalence of asymptomatic bacteriuria in pregnancy to be between 2 and 7% while in India it was found to be on higher side i.e., between 5 and 17%.[6-10] Studies in African region showed higher prevalence than both these regions.[11] Commonest organisms responsible are *Escherichia coli* (80–85%), followed by coagulase negative

Staphylococcus species, *Klebsiella* species, *Pseudomonas* species, and *Proteus* species.[6-11]

The Gold standard investigation for detection of asymptomatic bacteriuria is urine culture.[1-3] Therefore, urine culture at first prenatal visit or between 12 and 16 weeks of gestation should be considered as a screening test of choice.[1,2] Detection of asymptomatic bacteriuria during pregnancy is important as subsequently it may lead to symptomatic infection during that pregnancy in 25% of infected women, chronic infection resistant to chemotherapy, pyelonephritis, low birth weight (LBW) babies and preterm delivery.[9] This study is designed to determine the bacterial profile and antibiotic susceptibility pattern of uropathogen among pregnant women, along with the outcome of urinary tract infection in pregnancy.

The objective of present study was to find out the percentage of women with asymptomatic bacteriuria in pregnancy, coming to Obstetrics OPD in Government Medical College, Kottayam, Kerala, India and to find out the common isolates and their antibiotic sensitivity pattern.

METHODS

It is a descriptive study carried out at Department of Microbiology, Government Medical College, Kottayam and Department of Obstetrics and Gynaecology, Government Medical College, Kottayam, Kerala, India. The study was carried out for one year from February 2014. Study population includes antenatal women who attended Obstetrics OPD during 12-16 weeks of pregnancy or for first time during whole pregnancy.

Inclusion criteria

Asymptomatic pregnant females who were willing to participate in study.

Exclusion criteria

Those with signs and symptoms of UTI, those with diabetes, those with any structural anomalies of urinary tract, those patients who took antibiotics in past one week and those who were not willing to participate were excluded from this study.

Sample size was 550 and it is the minimum sample size as calculated with the formulae $4pq/d^2$ using prevalence (p) =15, d=20 % of p. Sampling methodology was non-probability convenient sampling.

Sample collection and processing

The study was conducted after obtaining necessary permission from institutional ethics committee, over a period of one year from February 2014, on a total of 555 asymptomatic pregnant females at their first visit in whole pregnancy or during 12-16 weeks of pregnancy.[1,2] Informed consent was taken. The pregnant females were instructed how to collect clean catch mid-stream urine sample. The samples were taken in sterile leak proof universal containers and immediately transferred and processed within 2 hours of collection.

Samples were subjected to macroscopic examination, wet film examination for pyuria, Gram stain, catalase test, nitrate reductase (Griess) test and semi quantitative culture method. The semi quantitative culture was done by surface streaking method on 5% sheep Blood agar, Cysteine Lactose Electrolyte Deficient agar with bromo-thymol blue and MacConkey agar. HiCrome UTI agar and HiCrome Strep B Selective Agar (both by Hi Media Laboratories, Mumbai) was also used to aid in early identification of isolates with significant bacteriuria. For the inoculation, sterile standard nichrome wire loop, with volume holding capacity of 0.001 ml was used. The plates were incubated at 37°C for 24 hours.

HiCrome UTI agar media was prepared as per manufacturer's instructions. Sample was inoculated and incubated at 37^0C overnight. Differentiation is aided by different colour of different bacteria like *E. coli* → dark pink to reddish, *Proteus* → brown halo, *Klebsiella*, Enterobacter and *Serratia* → metallic blue, *S. aureus* → golden, opaque, small, *Citrobacter* → metallic blue with red halo, *S. saprophyticus* → pink, opaque, small, *Candida albicans* → colourless, Streptococcus agalactiae → light blue, Pseudomonas aeruginosa → Translucent, cream to blue.

HiCrome Strep B selective agar media was prepared according to manufacturer's instructions. Blue coloured colonies with luxuriant growth of Streptococcus agalactiae was observed after incubation at 37°C overnight.

Screening tests done

Wet film examination of uncentrifuged urine for pyuria

A 0.05 ml (50µl) of uncentrifuged well mixed urine sample was transferred carefully on to the middle of a microscope slide. A coverslip, 22 X 22 mm was applied immediately avoiding trapped bubbles so that the film should show a small excess of fluid along the edges of the coverslip. The prepared film was examined under microscope in high power field. The finding of more than 1 leukocyte per 7 high power fields corresponds with more than 10^4 leukocytes per ml-which was considered as significant pyuria.[12,13]

Gram stain of well mixed uncentrifuged urine for microorganisms

A drop of well mixed urine is allowed to air dry, the smear is fixed, stained and examined under oil immersion (1000 X).[3] The presence of at least one bacterium per oil

immersion field in a midstream, clean-catch, Gram-stained, uncentrifuged urine correlates with 10^5 bacteria per ml of urine or more. Smear is discarded as negative after examining at least 20 oil immersion fields.[4,9]

Urinary nitrite detection

The Griess reagent was prepared by dissolving 1.5 gm of sulphanillic acid in 450 ml of 10% acetic acid. This was then added to a solution of 0.6 gm of ∝- naphthylamine dissolved in 60 ml of boiling distilled water. The reagent remains stable for several months when stored in a stopper amber bottle and refrigerated. Deterioration of the test reagent is detected by the development of a pink color. If pink color develops; reagent can be regenerated by shaking it vigorously with small amounts of metallic zinc powder and filtering it.

Urine is tested by adding 1.0 ml of urine to 1.0 ml of the reagent in a clean test tube. The immediate development of a pink color to dark red color indicates the presence of nitrites in urine and is called a positive reaction. The ∝-naphthylamine was handled carefully because of its possible hazards.[14]

Urinary catalase detection in uncentrifuged well mixed urine sample

1.5 to 2 ml of urine was placed in a test tube. Four drops of 10% hydrogen peroxide was added to the test tube, and the mixture was shaken gently for 5 seconds. A positive finding was defined as the formation of effervescence sufficient to form a complete ring or layer on the surface of the liquid within 1 to 2 minutes of the addition of the hydrogen peroxide. The test result was considered negative in the absence of effervescence or when the ring of effervescence was incomplete after two minutes.[9,15]

Accuracy of screening tests

All the screening tests were evaluated for its accuracy as screening tests by calculating sensitivity, specificity, positive predictive value and negative predictive value.[16]

Definitions and formulae

- True positive (TP): Screening test and culture, both positive
- False positive (FP): Positive screening test finding and negative culture
- True negative (TN): Screening test and culture, both negative
- False negative (FN): Screening test negative and culture positive
- Sensitivity = TP/(TP+FN): Probability that screening test will be positive in patients with UTIs (positive culture)
- Specificity = TN/(TN+FP): Probability that screening test will be negative in patients without UTIs (negative culture)
- Positive predictive value (PPV): TP/(TP+ FP): Probability that a UTI is present when screening test is positive.
- Negative predictive value (NPV): TN/(TN+FN): Probability that a UTI is not present when screening test is negative

Semi quantitative culture of urine

After mixing thoroughly, the top of the container was removed. The calibrated loop was introduced vertically into the urine and taken out. Loop was touched to the centre of the plate, from which the inoculum was spread in a line across the plate. Without flaming or re-entering urine, loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies. Plates were incubated at 37^0C for 24 hours. Number of colonies were counted and multiplied by 1000 to get the colony count.[3]

Interpretation

- No bacteriuria - If no growth was present.
- No significant bacteriuria - If the colony count was $<10^5$ colony-forming units/ml.
- Significant bacteriuria - If colony count was $>10^5$ colony-forming units/ml of a particular organism except in case of growth of gram-positive cocci where even 10^2 cfus per ml were taken as significant.

Antibiotic sensitivity testing of bacterial isolates

Antibiotic sensitivity testing was done using Kirby-Bauer method (disc diffusion), with 0.5 McFarland standard turbidity of the inoculum on blood agar for Streptococcus species. and Mueller-Hinton agar for other isolates. Standardization of antibiotic susceptibility testing was done by using standard strans of *Escherichia coli* - ATCC 25922, *Enterococcus faecalis* - ATCC 29212 and *Staphylococcus aureus* - ATCC 25923 as control strains. E-test, Vitek 2 systems and agar dilution was done to detect the MIC of Vancomycin for all Staphylococcus species. Antibiotics were tested and zone of inhibition of growth was measured and interpreted according to CLSI guidelines.[17]

All antibiotics were not reported. Only antibiotics which can be safely given in pregnancy and those which attain good urinary concentration were reported. Antibiotics which can be given orally were preferred for reporting. Antibiotics used were Ampicillin, Amoxicillin-clavulanic acid, Cephalosporin 1st generation(Cephalexin), Cephalosporin 3rd generation (Cefotaxime), Cotrimoxazole (Trimethoprim / Sulphamethoxazole), Nitrofurantoin, Norfloxacin and Ciprofloxacin depending upon the isolates.[18]

Test for ESBL production was also done for relevant isolates. The data was numerically coded and was entered in Microsoft excel spread sheet. Further analysis was

done using the software SPSS 16 and tables and charts were created using excel. The level of statistical significance was noted if p value was <0.05 and high significance was p <0.01.

RESULTS

As per the inclusion and exclusion criteria, mid-stream clean catch urine sample was collected from 555 asymptomatic pregnant females. Out of the total 555 subjects, 26 (4.68 %) were having asymptomatic bacteriuria of pregnancy (Figure 1).

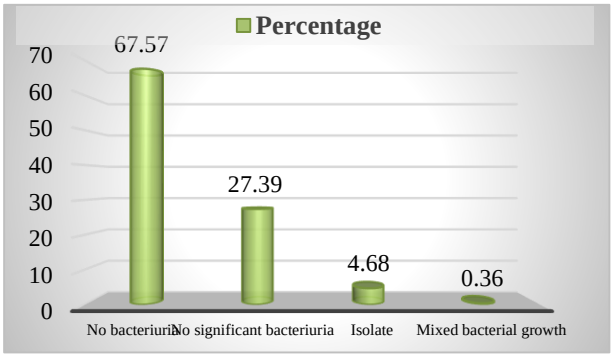

Figure 1: Report of all the samples studied.

Most common age group affected was 26-30 years followed by 21-25 years and 31-35 years (Figure 2).

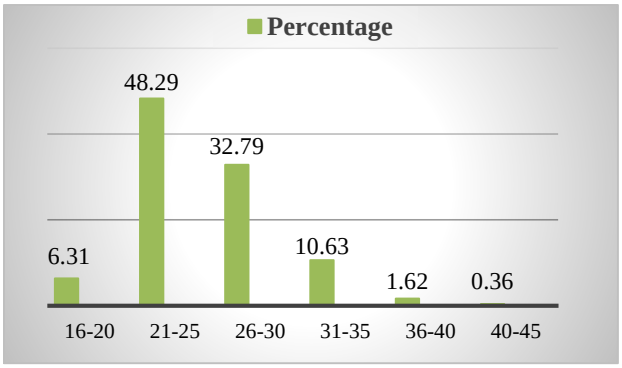

Figure 2: Age distribution.

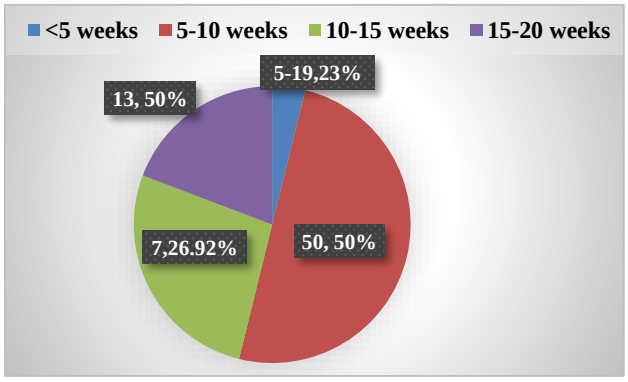

Figure 3: Period of gestation wise distribution of those with ASB.

ASB was more common among primi gravida (53.85%) followed by gravida 2 (30.77%). Parity wise distribution of ASB was as follows: nulli para (57.69%) >para1 (30.77%) >para 2 (7.69%) >para3 (3.85%). Maximum case of ASB was reported during 5-10 weeks (50%) followed by 7 weeks (26.92%) (Figure 3).

Out of the 26 cases with asymptomatic bacteriuria of pregnancy, 23 (88.46 %) were not having any risk factors. Only 2 (7.69%) cases had associated UTI (before pregnancy or during previous pregnancy) and one (3.85%) had UTI in early period of present pregnancy. So overall a 10 % of subjects were having history of UTI.

Sensitivity, specificity, positive predictive value and negative predictive value of the screening tests are shown in Table 1.

Table 1: Sensitivity, Specificity, Positive predictive and negative predictive value of screening tests.

	Wet film for pyuria	Gram stain	Urinary catalase	Urinary nitrite
True positives	173	168	144	13
False negative	07	10	36	167
True negative	268	374	375	375
False positive	107	01	0	0
Sensitivity	86.5 %	94.38 %	80.0%	7.2 %
Specificity	71.4 %	99.73 %	100%	100 %
Positive predictive value	60.2%	99.4 %	100%	100%
Negative predictive value	97.4%	99.7 %	93.5 %	84.8 %

Table 2: Microbiological profile of asymptomatic bacteriuria.

Isolate	No	%
E. coli	07	26.92
K. pneumoniae	06	23.08
Citrobacter koseri	02	7.69
Staphylococcus aureus	02	7.69
Methicillin resistant coagulase negative *Staphylococci* (MRCoNS)	03	11.54
Enterococcus faecalis	02	7.69
Candida tropicalis	01	3.85
Streptococcus agalactiae	03	11.54
Total	**26**	**100.00**

Out of the 26 samples which were having ASB, 15 (57.7%) were gram negative bacteria, 10 (38.5%) were gram positive bacteria and one (3.8%) was yeast. Most

common isolate was *Escherichia coli* followed by *K. pneumoniae, Staphylococcus saprophyticus, Streptococcus agalactiae, Citrobacter koseri, Staphylococcus aureus, Enterococcus faecalis* and *Candida tropicalis* respectively (Table 2).

Table 3: Sensitivity of gram positive isolates.

Drugs tested	*Staphylococcus aureus*	Methicillin resistant coagulase negative *Staphylococci*	*Enterococcus faecalis*	*Streptococcus agalactiae*
Ampicillin	0 (0%)	0 (0%)	2 (20%)	3 (30%)
Gentamicin	1 (10%)	1 (10%)	0 (0%)	0 (0%)
Erythromycin	1 -NR (10%)	0 -NR (0%)	2 -NR (20%)	3 -NR (30%)
Cephalexin	2 (20%)	0 (0%)	0 – NR (0 %)	3 (30%)
Amikacin	2 (20%)	3 (30%)	0 (0%)	0 (0%)
Vancomycin	2 (20%)	3 (30%)	2 (20%)	3 (30%)
Cloxacillin	2 (20%)	0 (0%)	NT	NT
Mox-Clav	1 (10%)	0 (0%)	2 (20%)	2 (20%)
Linezolid	2 -NR (20%)	3 -NR (30%)	2 -NR (20%)	2 -NR (20%)

NR- Not reported, NT- Not tested

Table 4: Sensitivity of gram negative isolates.

Drugs	*Escherichia coli*	*Klebsiella pneumoniae*	*Citrobacter koseri*
Ampicillin	1 (6.25%)	0	0
Gentamicin	6 (37.5%)	6 (37.5%)	2 (12.5%)
Cephalexin	3 (18.75%)	5 (31.25%)	0
Amikacin	6 (37.5%)	6 (37.5%)	2 (12.5%)
Cefotaxime	5 (31.25%)	6 (37.5%)	2 (12.5%)
Cotrimoxazole	3 (18.75%)	5 (31.25%)	2 (12.5%)
Ciprofloxacin	4 (25%)	6 (37.5%)	2 (12.5%)
Cefoperazone-sulbactum	7 (43.75%)	6 (37.5%)	2 (12.5 %)
Piperacillin- tazobactum	7 (43.75%)	6 (37.5%)	2 (12.5%)
Meropenem	7 (43.75%)	6 (37.5%)	2 (12.5%)
Norfloxacin	4 (25%)	5 (31.25%)	2 (12.5%)
Nitrofurantoin	5 (31.25%)	3 (18.75%)	0
Imipenem	7 (43.75%)	6 (37.5%)	2 (12.5%)
Mox-Clav	3 (18.75%)	5 (31.25%)	0

Antibiotic sensitivity of the isolates is shown in Table 3 and Table 4. Complications detected due to asymptomatic bacteriuria are shown in Table 5.

Table 5: Complications of asymptomatic bacteriuria detected on follow up.

Complications	No.	%
No complications	14	53.80 %
Recurrent ASB	623	0.08 %
Symptomatic bacteriuria (except pyelonephritis)	5	19.2 %
Pre- term labour	1	3.84%
Total	**26**	**100**

DISCUSSION

A one year study was conducted on microbiological profile of asymptomatic bacteriuria in pregnancy, Government Medical College, Kottayam. A total of 555 samples were studied. Significant bacteriuria was noted in 26 (4.68%) of isolates.152 (27.39%) showed no significant bacteriuria and in 375 (67.57%), there was no growth. The low percentage of asymptomatic bacteriuria may be attributed to the good ante natal care hygiene and awareness among people of Kerala.

The prevalence of asymptomatic bacteriuria in the following studies were similar to our study- Lata R Chandel et alfrom Shimla (7.34%) and J Jayalakshmi et al from Bangalore (7.4%).[8,19] Maximum pregnant females with asymptomatic bacteriuria was noted in age group 26-30 years (30.77%) followed by 21-25 years and 31-35 years (both 26.92%). The p value of association between age group and asymptomatic bacteriuria of pregnancy was 0.15 >0.05. So, the association was not statistically significant. Similar results were obtained in study conducted by Ananthi Kasinathan et al from Pondicherry in 2014, with maximum subjects with ASB in the age

group of 26-30 years.[20] Many studies show advancing age as a risk factor for acquiring ASB in pregnancy because there is decrease in glycogen deposition and reduction in the lactobacillus as a part of ageing process which enhances bacterial adherence and invasion by pathogens and make them more susceptible.[21]

Of the total 555 samples, 293 (52.8%) was primi gravda. Asymptomatic bacteriuria in pregnancy was more common among primigravida (53.85 %) compared to multigravida. This association was also not found to be statistically significant (p =0.115>0.05). The findings were correlating with the findings of Sudha Kerure et al from Karnataka in 2012.[22] Out of the total 555 samples, 335 (60.36%) were nullipara, 167 (30.09%) were para-1 and rest 10 % were para-2 and para-3. In samples with asymptomatic bacteriuria in pregnancy (26 samples), 15 (57.69 %) were from nulliparous women and 8 (30.77%) were para-1. A study by Lavanya and Jogalakshmi from India also gave similar results.[9] As p value of association between parity and significant bacteriuria was more than 0.05 (0.11), this association was also not found to be statistically significant.

Out of the 555 samples, 263 (47.39%) were in 5-10 weeks of gestation and 230 (41.44%) were in 15-20 weeks of gestation. Maximum number of asymptomatic bacteriuria was detected in 5-10 weeks of gestation (50%) followed by 10-15 weeks (26.92%) and 15-20 weeks (19.23%). This association was also not found to be statistically significant (p value= 0.465 >0.05) this study was correlating with a study conducted by Lata R. Chandel et al from Shimla in.[19]

Age, parity and gestational age were not associated with UTI, like in this study, according to a study conducted by Hamdan et al in.[23] According to Paul Erhunmwunse Imade et al from Nigeria there was no significant difference in the prevalence of asymptomatic bacteriuria with respect to trimester.[6]

Out of the 26 cases with asymptomatic bacteriuria of pregnancy, 23 (88.46%) were not having any risk factors. Only 2 (7.69%) cases had associated UTI before pregnancy or during previous pregnancy and 1 (3.85%) had UTI in early period of present pregnancy. So overall 10% of subjects were having history of UTI. According to a study by Agersew Alemu et al from Ethiopia, thirteen (3.4%) and 47 (12.2%) of study subjects had history of previous catheterization and urinary tract infection, respectively.[24] Of all considered variables only history of catheterization and previous history of UTI were significantly associated with UTI. There was no association between maternal age, parity, gravidity, trimester, occupation, marital status and education with bacteriuria.

Accuracy of screening tests

According to this study Gram stain was the most sensitive test, i.e. there is high probability that gram stain will be positive in those with asymptomatic bacteriuria of pregnancy. Urinary catalase, urinary nitrite and Gram stain were highly specific according to this study. That means there is high probability that urinary catalase, nitrite and Gram stain will be negative in subjects without asymptomatic bacteriuria of pregnancy. Urinary catalase, urinary nitrite and Gram stain were also having a high positive predictive value which means in a subject with asymptomatic bacteriuria of pregnancy there is high chance that these tests will be positive. Gram stain was having the highest negative predictive value followed by wet film for pyuria which means in a subject without asymptomatic bacteriuria of pregnancy there is high chance that these tests will be negative. Gram stain examination was having similar sensitivity (96.0%), specificity (99.2%), positive predictive value (97.6%) and negative predictive value (98.7%) according to a study conducted by Celso Lui´Z Cardoso et al from Brazil.[25]

As there was no consensus in the methods used for wet film examination and the criteria for significant pyuria, different studies showed different values on analysis as a screening test. A study conducted by Taneja et al showed sensitivity, specificity, positive predictive value and negative predictive value of wet film examination for pyuria to be 68.4%, 60.8%, 32.7% and 87.3% respectively.[26] Another study conducted by Willy Fred Nabbugodi et al sensitivity (67.5%), specificity (88.2%), positive predictive value (67.5%) and negative predictive value (88.2%).[27] These two studies were similar to our study on wet film examination. According to Morike Ngobe Mokube et al from Cameroon, sensitivity, specificity, positive predictive value and negative predictive value of urinary (Griess) nitrite test were respectively 8%, 98.7%, 67% and 77.8%.[28] The lower sensitivity of nitrite tests compared to previous reports may be due to the fact that this test is more accurate when the first voided specimen is collected or when urine has been stored in bladder for over four hours. It was difficult to ensure such conditions in our study. The presence of abnormal amounts of urobilinogen and ascorbic acid in urine as well as a urinary pH of < 6 and bacteriuria due to gram-positive cocci or nonfermentative bacilli, such as Pseudomonas species. account for some of the false-negative results.[29,30] False negatives can also occur when the organism present does not produce nitrate reductase and when dietary nitrate is absent.[28] Catalase test was having similar sensitivity (83%) in a study conducted by Stephen A. Berger et al from Israel and Shilpa et al from Bangalore, India.[30] Many organisms causing urinary tract infection contain the enzyme catalase.[31] The presence of catalase may be due to bacteria, but also to erythrocytes, WBC, or kidney cells. Therefore, this test is not specific for bacteria.[32] But in this study no false positivity was recorded for catalase reaction. This may be because of decreased number of WBC's and reduced immune

response in asymptomatic bacteriuria of pregnancy. Catalase can also be false negative for catalase negative organisms and yeasts which were present in our study.

Microbiological profile of asymptomatic bacteriuria in pregnancy

Out of the 26 samples with significant growth, 15 were Gram negative bacteria (57.7%), 10 were Gram positive bacteria (38.5%) and 1 was fungi (3.80%). *Escherichia coli* was the most common organism isolated (57.7 %), followed by *Klebsiella pneumoniae sub species. pneumoniae* (23.08%), *Staphylococcus saprophyticus* and *Streptococcus agalactiae* (both 11.54%), *Citrobacter koseri, Staphylococcus aureus* and *Enterococcus faecalis* (all three 7.69%) and *Candida tropicalis* (3.85%). Most studies report *Escherichia coli* as the most common organism isolated followed by *Klebsiella species.* According to a study conducted by Gayathree et al from Hassan, Karnataka, *Escherichia coli* emerged as the most frequent cause of ASB with 32 cases (51.61%), followed by *Proteus mirabilis* with 9 cases (14.51%), *Staphylococcus aureus* and *Klebsiella pneumonia* with 6 cases (9.67%) each, *Acinetobacter species*., with 5 cases (8.05%), *Pseudomonas aeruginosa* with 3 cases (4.83%) and *Enterococcus faecalis* with 1 case (1.61%).[33]

According to another study conducted by Kheya Mukherjee et al from West Bengal, Gram negative organisms were predominant (80.95%) causative agents than Gram positive organisms (19.05%) and *E. coli* (57.14%) was the most common organism isolated.[34] According to a study by Madhu Udawat et al in India, there was an increasing trend in the prevalence of *S. aureus* infection (15.28%).[35] This dramatic increase in prevalence rate can be attributed to the emergence and global spread of S*taphylococcus aureus*. Hence, recognizing this change in the spectrum of uropathogens remains important to guide changes in empirical antimicrobial therapy. According to Nithyasree et al from Tamilnadu, majority of isolates were Gram negative bacteria (81.39%).[36] Gram-positive organisms were responsible only for 18.6%. *E. coli* (65.11%) was the most prevalent uropathogen isolated followed by *Klebsiella pneumonia* and S*taphylococcus aureus*, accounted 11.62% for each. The least prevalent bacteria isolated were *Proteus mirabilis* (4.65%).

Antibiotic sensitivity pattern

Gram positive isolates

Staphylococcus aureus isolates was 100% sensitive to Cephalosporin 1st generation drugs (Cephalexin). None of the *Staphylococcus species* was sensitive to Ampicillin and Nitrofurantoin. 50% of *Staphylococcus aureus* strains were sensitive to Amoxicillin- Clavulanic acid. *Staphylococcus saprophyticus* was 100% sensitive to Amoxicillin- Clavulanic acid but none were sensitive to first generation Cephalosporins. *Enterococcus faecalis* and *Streptococcus agalactiae* was 100% sensitive to Ampicillin**.** All the *Enterococci* were sensitive to Amoxicillin- Clavulanic acid. *Streptococcus agalactiae* strains were 100% sensitive to Cephalexin and Clindamycin and 66.67% sensitive to Amoxicillin - Clavulanic acid. As *Streptococcus agalactiae* strain was Erythromycin sensitive no inducible Clindamycin resistance was tested and Clindamycin was reported as sensitive for intra partum prophylaxis. As the strain was Penicillin, Ampicillin and Cephalexin sensitive, Cefazolin was reported as sensitive for using as an alternative drug for prophylaxis.

Gram negative isolates

Escherichia coli isolates had more resistance rates than other gram negative isolates. *E. coli* isolates were 100% sensitive only to third generation injectable drugs like Cefoperazone- Sulbactum, Piperacillin- Tazobactum, Meropenem and Imipenem. Ampicillin was the least sensitive drug (14.2%) followed by Cephalexin (42.86%), Cotrimoxazole (42.86%), Norfloxacin (42.86%) and Amoxicillin- Clavulanic acid (42.86%). Ciprofloxacin was 57.14% sensitive, Cefotaxime and Nitrofurantoin (both 71.43%) and aminoglycosides (85.71%). For the two Cefotaxime resistant strains, ESBL production was tested and was found to be negative. *Klebsiella species* was 100% sensitive to Aminoglycosides, Cefotaxime, Ciprofloxacin and all the third-generation injectable drugs like Cefoperazone- Sulbactum, Piperacillin-Tazobactum, Meropenem and Imipenem. Cephalexin, Cotrimoxazole, Norfloxacin and Amoxicillin- Clavulanic acid was 83.33 % sensitive. Nitrofurantoin was the least sensitive drug (50%). None of *Citrobacter koseri* strains were sensitive to Cephalexin, Nitrofurantoin and Amoxicillin- Clavulanic acid. It was 100 % sensitive to Aminoglycosides, Third generation Cephalosporins, Cotrimoxazole, Fluoroquinolones and higher generaion injectables.

Only low level of resistance was found against Nitrofurantoin, and this might be due to its narrow range of clinical indications, which results in less usage. Resistance to Ciprofloxacin, Norfloxacin, Ampicillin and Amoxicillin- Clavulanic acid could have been contributed by an increase in prescribing practices of these drugs for uncomplicated UTI and other indications in recent years. These drugs are very common due to low cost and often purchased without prescription in different areas.

According to Chidre Yogiraj Vaijanathrao et al from Hyderabad, highest sensitivity was noted to Ciprofloxacin, Gentamicin, Nitrofurantoin, Ofloxacin and Amikacin.[37] For Gram positive cocci, highest sensitivity was seen with Amoxyclav, Nitrofurantoin, and increasing resistance pattern were seen with Cephalosporins and Ampicillin. Another study by Roopa et al from Karnataka, all the isolates of asymptomatic bacteriuria were sensitive to Gentamicin and 62.5% isolates were sensitive to Nitrofurantoin.[38] Study done by Ayoade et

alfrom Nigeria, showed that all the isolates were resistant to Amoxycillin, Augumentin and Cotrimoxazole but most of the isolates were assessed to be highly susceptible to Ofloxacin.[39] Three antibiotics namely Ofloxacin, Gentamicin and Nitrofurantoin were found to be most effective for Gram negative bacteria while Gentamicin, Chloramphenicol and Cotrimoxazole were effective for Gram positive bacteria. A study by Amete Mihret Teshale et al from Ethiopia, Nitrofurantoin, Clindamycin and Ciprofloxacin were the most effective antibiotics for Gram positive isolates with less efficacy of Sulfamethoxazole/Trimethoprim for this bacteria.[40] Gentamicin, Amoxicillin/Clavulanic acid, Norfloxacin and Ciprofloxacin were the antibiotics of choice for Gram negative isolates.

Complications of asymptomatic bacteriuria of pregnancy detected on follow up

All of the patients with significant growth were informed about the growth and was advised to take treatment immaediately. All 26 patients took treatment. No complications were detected in 14 (53.80%) of subjects. Recurrent asymptomatic bacteriuria and symptomatic bacteriuria was present in 23.08% and 19.2% of subjects respectively. These were detected as the most common complications. Pretem labour was noted in only one subject (3.84%). But the patient was having other associated complications towards end of delivery. The decreased rate of complications may be due to prompt treatment under taken by the patient. In a study conducted by Lavanya and Jogalakshmi in Vishakapatnam out of 42 subjects with significant growth, 38 cases (90.46 %) complied with treatment and 4 (9.54%) did not.[9] Three out of 4 (75%) who did not take treatment delivered prematurely and two (50%) delivered low birth weight babies.

CONCLUSION

The percentage of asymptomatic bacteriuria of pregnancy is less towards this part of the country. The probable reason could be the prompt antenatal care and good hygienic living of people. Age, parity, gravidity and period of gestation were not found to be associated with asymptomatic bacteriuria of pregnancy. Gram stain was found to be the best screening test with high sensitivity and specificity. In our set-up urine microscopy is the one that is given importance in antenatal screening. If the wet film is positive, then only a sample is sending for culture. But as wet film examination is not having a good sensitivity and specificity according to this study and many other studies, urine culture should always be done as recommended by standard guidelines ideally between 12-16 weeks of pregnancy to decrease asymptomatic bacteriuria and its morbidity. Gram-negative bacteria were isolated from more than half of the samples. *Escherichia coli* was the most common organism isolated closely followed by *Klebsiella spp.* Gram-positive bacteria including Group B *Streptococci* were also isolated in large numbers. Even though pregnancy is considered as an immunocompromised condition, only one *Candida* isolate was obtained until this period of gestation. *Staphylcoccus aureus* strains were 100% sensitive to Cephalexin and all the *Staphylococcus saprophyticus* strains were sensitive to Amoxycillin-Clavulanic acid. All the *Streptococci* were sensitive to Ampicillin. *Escherichia coli*, the most common organism isolated, was comparatively resistant to the most common antibiotics used like Ampicillin and Cephalexin. Cefotaxime was found to be the best drug which can be used against gram-negative bacteria. Nitrofurantoin was another oral formulation found to be useful for *Escherichia coli*. But Nitrofurantoin was not that effective for other gram-negative bacilli. Resistance to Ciprofloxacin, Norfloxacin, Ampicillin, Cephalexin and Amoxicillin- Clavulanic acid could have been contributed by an increase in prescribing practices of these drugs for uncomplicated UTI and other indications in recent years. Complications of asymptomatic bacteriuria like preterm labour and low birth weight were almost not detected in this study. This may be due to the prompt treatment and good antenatal care.

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