The Gram's stain was another method used to estimate bacteriuria.A drop of uncentrifuged well mixed urine was taken on a clean grease free slide and stained by gram's method of staining and examined under the oil immersion microscope. Presence of >1 bacteria per oil immersion field correlates with the significant bacteriuria of $>10^5$ CFU/ml of urine.[7]

Urine from a healthy person does not contain nitrite. The detection of nitrite in urine is a useful test in the investigation of urinary tract infection caused by nitrate reducing bacteria. The presence of leukocytes in urine indicated inflammation of urinary tract.[10]A nitrite reagent strip which also detects leukocyte esterasesUROCOLOR 10 was used. The test detects nitrite in a concentration as low as 11micromol/l[10].

3. Results

100 pregnant women attending the antenatal clinics of ChigateriGeneralHospital and Bapuji Hospital, Davangere, during the two months period of June – July, 2014 were taken for study.Out of 100 samples, 87 (87%) were sterile. Organisms in pure culture in significant number were obtained in 13 cases (13%). In 13 samples pure growth of organism $>10^5$ CFU/ml were obtained. None of the samples showed growth contamination.

Table 1: Effect of age on asymptomatic bacteriuria

Age in years	Total no.of patients	asymptomatic bacteriuria	Percentage %
15 – 20	21	4	31
21 – 25	61	6	46
26 – 30	11	2	15
31 – 35	7	1	8

According to Table – 1, occurrence of ASB varies with age. It occurs more among women with age group of 21-25 years 6 (46%), in 15-20 years 4 (31%), 26-30 years 2 (15%), 31-35 years 1 (8%). No cases were found in age group 35 and above in our study.

Table 2: Effect of socio-economic status on ASB

Socioeconomic status	Total no.of patients	asymptomatic bacteriuria	Percentage %
Low	71	10	77
Middle	24	2	15
Upper	5	1	8
Total	100	13	100

According to table – 2, antenatal women from low socioeconomic status had highest (77%) of incidence followed by middle (15%) and upper (8%).

Table 3: Result of wet film preparation with respect to culture

Wet film	Culture		
	+	-	Total
+	5	7	12
-	8	80	88
Total	13	87	100

According to table – 3, the number of samples showing wet film positive – culture positive, wet film positive- culture negative, wet film negative – culture positive, wet film negative- culture negative are 5, 7, 8 and 80 respectively.

Table 4: Result of Gram stain with respect to culture

Gram stain	Culture		
	+	-	Total
+	10	12	22
-	3	75	78
Total	13	87	100

According to table – 4, the number of samples showing gram stain positive – culture positive, gram stain positive – culture negative, gram stain negative – culture positive, gram stain negative – culture negative are 10, 12, 3 and 75 respectively.

Table 5: Result of Leukocyte esterase test with respect to culture

Leukocyte esterase	Culture		
	+	-	Total
+	7	8	15
-	6	79	85
Total	13	87	100

According to table – 5, the number of samples showing Leukocyte esterase positive – culture positive, Leukocyte esterase positive – culture negative, Leukocyte esterase negative – culture positive, Leukocyte esterase negative – culture negative are 7, 6, 8 and 79 respectively.

Table 6: Result of Nitrite test with respect to culture

Nitrite	Culture		
	+	-	Total
+	6	2	8
-	7	85	92
Total	13	87	100

According to table – 6,the number of samples showing Nitrite positive – culture positive, Nitrite positive – culture negative, Nitrite negative – culture positive, Nitrite negative – culture negative are 6, 7, 2 and 85 respectively.

Table 7: Organisms present in ASB

Total no. of patients ASB	Organism present	No. of patients	Percentage %
13	*E.coli*	5	38
	Staphylococcus aureus	3	23
	Klebsiella pneumonia	2	15
	Acinetobacter	1	8
	Proteus mirabilis	1	8
	Pseudomonas	1	8

According to table 7, the most common bacteria found in ASB is *E.coli* (38%) followed by *Staphylococcusaureus(23%), Klebsiella pneumonia* (15%),*Acinetobacter*(8%), *Proteus – mirabilis(* 8%), *Pseudomonas(8%).*

Table 8: Antibiotic susceptibility of the isolates

Organisms	No.of isolates	Amikacin (30µg)	Amoxycillin (25µg)	Ampicillin (10µg)	Cefotoxime (30µg)	Cephalexin (30µg)	Gentamycin (10µg)	Cefuroxime (30µg)	Nitrofurantoinin (300µg)
E.coli	5	4	-	-	2	1	3	3	2
Staphylococcus aureus	3	2	-	-	1	-	2	1	1
Klebsiella pneumonia	2	1	-	-	-	-	2	1	1
Acinetobacter	1	1	-	-	1	-	1	1	1
Proteus mirabilis	1	1	-	-	-	1	1	1	1
Pseudomonas	1	1	-	-	-	-	1	1	1

According to table 8, all the strains were resistant to Ampicilin,Amoxycilin. There was varying susceptibility to other antibiotics.

Table 10: Comparison of screening tests at significant bacteriuria

Tests	*Sensitivity*	*Specificity*	*PPV*	*NPV*
Wet film	38%	92%	47%	90%
Gram stain	76%	86%	45%	96%
LE	46%	91%	47%	92%
Nitrite tests	54%	98%	75%	92%

According to table 10, PPV – positive predictive value, NPV – negative predictive value comparing the various screening tests, it was seen that gram stain had the maximum sensitivity 76% and highest negative predictive value 96% and low positive predictive value of 45%. Wet film has high specificity of 92% and least sensitivity of 38%. Leukocyte esterase has high specificity of 91% and low sensitivity of 46%. Nitrite test has highest specificity of 98% and highest positive predictive value of 75%.

4. Discussion

The urinary tract is second only to the respiratory tract in acquiring microbial infections, especially in females. The gold standard for the detection of bacteriuria is urine culture. However, the full bacteriological analysis is both time consuming and expensive and a vast majority of antenatal urine specimens will be negative. Thus a number of other screening methods have been proposed like Wet mount,Gram's staining and combination of Leukocyte esterase and Nitrite reduction test.[8]

In our study of 100 pregnant women, 13 (13%) were found to be suffering from asymptomatic bacteriuria. BalamuruganS et al(2014) reported that the prevalence of asymptomatic bacteriuria as 13% which is correlating with our study.

In our study incidence of urinary infection is more in the age group of 21-25 years i.e. 46%. Chandel et al (2012) also observed incidence of bacteriuria was more in the age group of 20-25 years.

In our study incidence of bacteriuria was more in low socioeconomic status group that is 77% compared to 15% in middle class group and 8% in upper class group. Muktikesh et al (2013) reported that the prevalence of bacteriuria is more in low socioeconomic group 62.4%.

In our study of 100 pregnant women, 13 (13%) were found to be suffering from asymptomatic bacteriuria and the organisms isolated were *E.coli 5* (38%), *Staphylococcus aureus* 3 (23%), *Klebsiella pneumonia* 2 (15%), *Acinetobacter*1(8%), *Proteus mirabilis* 1 (8%), *Pseudomonas* 1 (8%). In our study we found *E.coli* 5 (38%) whereas CA Turpin et al (2007) 37%, Muktikesh, et al (2013) (39%) which were similar to our study. In our study *Staphylococcus aureus* 3 (23%) whereas CA Turpin, et al (2007) had 31% which is closer to our studies.

In our study we got *Klebsiella pneumonia* 2 (15%) whereas ShanweelAhamad et al (2011) had 17% which were almost similar to our study.

Acinetobacter, Proteus mirabilis,Pseudomonas i.e. 8% each found from our study when compared to the other studies they are almost similar. In our present study 4 rapid tests were used to screen asymptomaticbacteriuriain pregnant women.

a) Wet film : of uncentrifuged urine

In our study, this test had poor sensitivity 38% and PPV 41% but good specificity 92%.

Previous study like Taneja N et al have shown this test to have sensitivity 40%, specificity of 90.9% and PPV 28.5% which were similar to our study.

b) Gram's stain :

Gram staining of uncentrifuged urine samples in our study had a sensitivity of 76%, specificity of 86% NPV 96%, but poor PPV 45%. Study of Tanejaet,al have shown this test to have sensitivity 70%, specificity 77.5%, NPV 96.6%, PPV 22% which showscentrifuged urine samples have high false positive result. Hence uncentrifuged urine is better.

c) Leukocyte esterase test :

Leukocyte esterase test in our study had moderate sensitivity of 46% but high specificity 91%. Other study like Balamurugan S et al has shown this test to have sensitivity of 85% and specificity of 71% which were almost similar to our study.

d) Nitrite test

Nitrite test in our study had the highest specificity 54% and sensitivity 94%. Other study like Balamurugan S et al, have shown this test to have sensitivity of 62% and specificity of 71% which were similar to our study.

5. Conclusion

From the present study it is observed that ASB is present in 13% of antenatal women in our hospital. The earlier

diagnosis and proper antimicrobial treatment in these women would prevent the obstetric complications. Culture is the gold standard but it is time consuming and expensive, though many screening tests are available, no one is 100% sensitive. Leukocyte esterase and Nitrite test is a rapid and inexpensive method to ruleout UTI in antenatal women. Alternatively Wet film and Gram's stain can be used as screening method.

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References

[1] ShamweelAhemd, ShehlaShakooh. Prevalence of asymptomatic bacteriuria among pregnant women in Kashmir. Srilanka Journal of Obstetrics and Gynecology 2011; 33:158-162.
[2] Muktikesh Dash, SusmitaSahu, IndraniMohanty, MoningiVenkat, Jyotimayee, Rani Sahu. Prevalence, risk factors and antimicrobial resistance of asymptomatic bacteriuria among antenatal women. Journal of Basic and Clinic Reproductive Sciences 2013; 2(Issue 2):92-96.
[3] ChandelLata R,Karga Anil, Thakur Kamlesh, MoktaKiran K, SoodAnuradha, ChauhanSmriti. Prevalence of pregnancy associated asymptomatic bacteriuria: A study done in a Tertiary care hospital. The Journal of Obstetrics and Gynaecology of India 2012; 62(5):511-514.
[4] Emilie Katherine Johnson. Urinary tract infections in pregnancy. Medscape reference, Drug Diseases and Procedures 2014.
[5] Akinola B. Ajayi, Charles Nwabuisi, AbiodunP,Nanji S,Nanji S Ajayi,Adeola F, Olurotimi O. Asymptomatic bacteriuria in antenatal patients in Ilorin, Nigeria. Oman Medical Journal 2012; 27(1).
[6] Gayathree L, Shetty S, Deshpande S R, Venkatesha D T. Screening for asymptomatic bacteriuria. In Pregnancy: An evaluation of various screening tests in Hassan District Hospital, India. Journal of Clinical and Diagnostic Research 2010; 4:2702-06.
[7] Jayalakshmi J. Evaluation of various screening tests to detect asymptomatic bacteriuria in pregnant women. Indian Journal of Pathol – Microbiol 2007; 51(3): 379-81.
[8] Balamurugan S, Chaitannya Shah, Jayapriya S, Priyadarshini S, Jeya M, Ramesh Rao K. Reagent strip testing (RST) for asymptomatic bacteriuria (ASB) in pregnant women: A cost-effective screening tool in under – resourced settings. Journal of Clinical and Diagnostic Research 2012; 6:671-673.
[9] KonemanColor Atlas and Text book of Diagnostic Microbiology. Part-1, pp.20-25.
[10] District laboratory practice in tropical countries. Part-1, Monica Cheesbourg, pp.380-382.
[11] District laboratory practice in tropical countries. Part – 2, Monica Cheesbourg pp.106-115.
[12] Topley and Wilson's microbiology and microbial infections. pp.677-680.
[13] Gradwohl's clinical laboratory methods and diagnosis. Volume 2, Part VII, page 1136-1139.
[14] Colle J.G, Marimon BP, Fraser AG, Simmons A, Mackie and McCartney practical Medical Microbiology, 14th ED, London, Elesvier 1996.
[15] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty third Informational supplements. Clinical and Laboratory Standards Institute Wayne PA 2014:M100-523
[16] Vaishali Jain, Vinita Das, AnjooAgarwal, AmithPandey. Asymptomatic bacteriuria and obstetric outcome following treatment in early versus late pregnancy in North Indian women. Indian Journal of Medical Research 2013; 137:753-758.
[17] CA Turpin, BridgeetMirkah, KA Danso, EH Frimpong. Asymptomatic bacteriuria in pregnant women attending clinic at KomfoAnokyeTeaching Hospital, Kumsai, Ghana. Ghana Medical Journal 2007; 41(1):26-29.
[18] Taneja N, Kavya M, Rangmei M, Deo N, Sharma M. Evaluation of three screening methods for detection of urinary tract infection in antenatal women. J ObstetGynocol India,2004; 54(3):267-270.