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Original Article

Obstetrics and Gynaecology Section

Diagnosis of Asymptomatic Bacteriuria and Associated Risk Factors Among Pregnant Women in Mangalore, Karnataka, India

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ABSTRACT

Background: Asymptomatic bacteriuria (AB) is common in-women and increases in prevalence with age or sexual activity. Prompt detection and treatment of this condition and associated factors decreases complications like acute pyleonephritis, intrauterine growth retardation and preterm labour. Chromogenic media is a versatile tool in rapid primary screening of the causative organisms considerably reducing daily routine workload.

Aim: To determine the prevalence of AB among pregnant women in a tertiary care set-up and analyse the contributory risk factors, its effects on pregnancy and the role of chromogenic media in the laboratory diagnosis of these cases.

Materials & Methods: Urine samples of all pregnant women attending pre-natal check-ups with no genitourinary complaints, history of fever or antibiotic intake were collected for Gram stain, culture and antibiotic sensitivity tests. A second urine specimen for culture and sensitivity testing was obtained from those with significant bacteriuria. The results were compared with patients showing negative urine cultures.

Results: The overall prevalence of this clinical condition in our study was 13.2%. The significant isolates were *Klebsiella pneumonia* and *E.coli* and the most common risk factor was a previous history of urinary tract infection. The isolates were easily identified by using chromogenic agar (HiCrome) but colonies of uncommon pathogens like *Acinetobacter* and *Streptococcus* species appeared white and needed further identification.

Conclusion: Screening of pregnant women for AB at first prenatal checkup helps analyse the associated factors and prevents its effects on pregnancy. The use of a chromogenic media can enhance reporting accuracy and will be an effective tool to monitor these cases routinely.

Keywords: Asymptomatic bacteriuria, Chromogenic agar, *Klebsiella pneumonia* and *E.coli,* Urinary tract infection

INTRODUCTION

Urinary tract infections are relatively common problems during pregnancy. The physiologic changes related to pregnancy make healthy women susceptible to complications such as asymptomatic and symptomatic urinary tract infections. The combination of mechanical, hormonal and physiologic changes during pregnancy contributes to significant changes in the urinary tract, which has a profound impact on the acquisition and natural history of bacteriuria during pregnancy [1]. The purpose of this study was to determine the prevalence of AB among pregnant women in a tertiary care set up and analyse the contributory risk factors in these cases like maternal anaemia, preterm labour, history of previous urinary tract infection, low socioeconomic status, grand multiparity, its effects on pregnancy and evaluate the use of a chromogenic agar medium in rapid presumptive identification of commonly associated uropathogens in this clinical condition.

MATERIALS AND METHODS

A cross-sectional study was done at the obstetrics and gynaecology out-patient department in a tertiary care institute in Mangalore, Karnataka, India in collaboration with the microbiology department from June to September 2013. After ethical clearance and voluntary informed consent, mid stream clean catch urine samples of 107 pregnant women attending pre-natal check-ups were collected into sterile uricol bottles. The samples were transported to the laboratory without delay.

Patients with a history of fever > 38^{0}C, dysuria, urinary hesitancy, urgency, slow stream, incontinence, frequency, incomplete voiding, flank / suprapubic / hypogastric pain and those who have had a history of intake of antibiotics for any indication during the current pregnancy were excluded from the study.

Baseline obstetric demographic data including maternal age, gravidity, parity, period of gestation, history with an emphasis on previous urinary tract infections, previous antibiotic intake, previous pre-natal check-up and a history of diabetes were noted. At the laboratory urine samples were subjected to Gram stain and culture by semi quantitative analysis using chromogenic agar (HiCrome UTI Agar M1353R – HiMedia Labouratories) for isolation and identification.

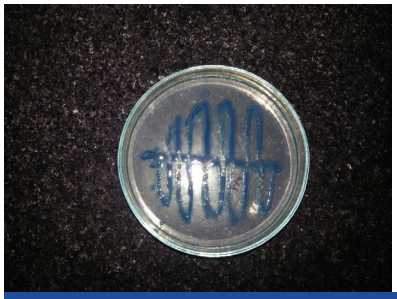
RESULTS

Data collected was analysed using statistical software. Overall prevalence of AB cases in our study was 13.2%. A total of 107 pregnant women were screened for bacteriuria at the first OPD visit. Relevant obstetric demographic data shows the mean age was 25 years, period of gestation 15-16 weeks and 15 (14.1%) cases were from a low socioeconomic status. The significant organisms and their colony characteristics on UTI Crome agar are depicted in [Table/Fig-1] and the blue mucoid colonies of *Klebsiella pneumonia* and pink to purple colonies of *E.coli* are shown in [Table/Fig-2,3] respectively. All colonies that appeared white on HiCrome UTI Agar M1353R came under the group of uncommon urinary tract pathogens and needed to be identified by conventional biochemical tests.

The associated factors significant in our cases are depicted in [Table/Fig-4]. *Klebsiella pneumonia* & *E.coli* showed high resistance to ampicillin among all the isolates. Nitrofurantoin was the most useful alternative to ampicillin. Among gram-positive isolates,

S. No:	Organisms Isolated	Colonies On Hicrome Agar	Number (%) n=14
1	*Klebsiella pneumoniae*	Blue to purple, mucoid	7 (50%)
2	*Escherichia coli*	Pink to purple	2 (14.2 %)
3	Group B *streptococcus*	White	1 (7.1%)
4	*Streptococcus species*	White	1 (7.1%)
5	*Staphylococcus aureus*	Golden yellow	1 (7.1%)
6	*Proteus mirabilis*	Light brown	1 (7.1%)
7	*Acinetobacter*	White	1 (7.1%)

[Table/Fig-1]: Significant isolates ≥ 10^5 CFU/ml of asymptomatic bacteriuria cases & their colony characteristics



[Table/Fig-2]: 10^5 CFU/ml of blue color mucoid colonies of *Klebsiella pneumonia* on Hi Crome UTI agar

[Table/Fig-3]: 10^5 CFU/ml of pink to purple colonies of *E.coli* on HiCrome UTI agar

Staphylococcus aureus and other *Streptococcus species* and *Group B streptococcus* were sensitive to ampicillin.

DISCUSSION

AB is the presence of >100,000 colony forming units (CFU) per ml of urine of a single pathogen in two consecutive mid-stream clean catch urine or one catheterized specimen from an individual without symptoms of urinary tract infection [2-4]. Increased prevalence of AB is seen in low socio-economic status, sickle trait, diabetes mellitus and grand multiparity each is associated with two-fold increase in the rate of bacteriuria [1]. Maternal anaemia is associated with both AB and pyelonephritis, but association with covert bacteriuria has not been confirmed [5]. The significance of AB lies in its potential to cause acute pyelonephritis in one third of the pregnant women with untreated bacteriuria [6]. In addition to this prevalence of AB also increases with higher parity and advancing age [7]. Chromogenic agar media are being used in early differentiation and identification of Gram positive and negative isolates [8]. Several chromogenic agar medium have been developed allowing more specific and direct differentiation of microorganisms on the primary plate itself on the basis of distinct colour and colony morphology [9-12]. Identification on chromogenic media makes it easier requiring less training and improves the quality of urine culture by contributing to a more uniform interpretation by the different personnel at the labouratory [8].

The gold standard for screening for AB is growing bacterial cultures of urine samples from women in early pregnancy (12-16 wk gestation) [1]. Our results showed that early gestational age had a greater likelihood of bacteriuria as was described by Patterson et al., [1]. Smooth muscle relaxation and urethral dilatation facilitates the ascent of bacteria to the kidney [13-15].

AB is detrimental to pregnancy, yet data available to support this contention is limited. Randomized controlled trials and cohort studies have shown that treatment of AB decreases acute pyelonephritis and intrauterine growth retardation.

The prevalence of AB in pregnancy varies from 4-7% (range 2-11%) and is similar to that observed in non-pregnant women [1,16]. The

S.NO:	Associated Factors	Number (%) n=20
1	History of previous UTI	9 (64.2%)
2	Preterm labour	4 (28.5%)
3	Pre-eclampsia	3 (21.4%)
4	Maternal anaemia	2 (14.2%)
5	History of diabetes	2 (14.2%)

[Table/Fig-4]: Associated factors in the cases of asymptomatic bacteriuria

prevalence of AB cases in our study was 13.2% close to studies done by Mignini et al., at 15% [17]. Prevalence rates can vary greatly as in the study by Paul showing 45.3% prevalence in Ghana and Ethiopia [18]. A significant risk factor that played a role in our cases were previous history of UTI's that accounted for 9(64.2%) consistent with the report by Lindsay E. Nicolle [19]. Klebsiella pneumonia was the most predominant isolate in our set up as opposed to various other studies where it was *E.coli* [20].

Previous history of urinary tract infection was an independent risk factor similar to earlier studies and was the most important predictor with 18.9% prevalence [20]. Profound physiologic changes in urinary tract during pregnancy are more likely to occur in women who had their first pregnancies or in women who have pregnancies in rapid succession [20]. In our study, age had no detectable influence on the frequency of bacteriuria.

The association between AB and anaemia during pregnancy was varying. Only two (14.2%) cases were anaemic in our study. Since anaemia tends to be a feature when the bacteria are resistant to treatment it may be related to prolonged chemotherapy or inapparent renal parenchymal disease [20]. Diabetes mellitus is an independent risk factor and pyelonephritis occurs more frequently among diabetic than non-diabetics [21]. In our study two (14.2%) cases were diabetics.

More than 30 other studies have been published since the first report of an association between asymptomatic bacteriuria and low birth weight (less than 2,500 g) in 1962. Some confirmed this association while others disputed it. In our study there were no cases of low birth weight pregnancies [22]. Pre-eclampsia has been reported to increase susceptibility to infection. A significant difference (p-value<0.005) in the rate of AB was found in patients with pre-eclampsia (19%) in a study done by JA Hill et al., [23]. In our study we had one (7.1%) case of pre-eclampsia in a primi from whom Group B *Streptococci was isolated*.

Chromogenic medium had reduced the burden of biochemical characterization and workload for identification of bacteria. Out of 107 urine samples tested, 10 (9.3%) yielded probably significant growth 10^4-10^5 CFU/ml of single organism or two organisms. However, there are studies showing that there is an 80% probability of true infection with single positive urine and 95% with repeat culture [20].

The chromogenic agar media allowed the growth and primary identification in all the samples. The different coloured colonies produced by the breakdown of the chromogenic substrate by the specific enzymes of the bacteria were useful in the presumptive identification of these organisms even from polymicrobial cultures. The medium also supports the growth and differentiation of Gram positive organisms like *Staphylococcus* and *Enterococci*. Although blood agar, MacConkey and cystiene lactose electrolyte deficient (CLED) media are good for the isolation of single pathogens, they do not have the differential capacity to distinguish between mixture of species. These findings reaffirm another study by Sohely S et al., which show that chromogenic media offers far superior means of differentiation of polymicrobial cultures [8].

However, the drawbacks of our study were the difficulty to identify *Streptococcus species* other than *Enterococci* and non fermenters like *Acinetobacter* and *Pseudomonas species*. The inability at our centre to test urine samples for other non cultivable pathogens on a routine basis to demonstrate a possible greater prevalence of this condition was also a conclusion in the study by Awasthi A et al., [24]. Our study pattern was comparable with an evaluation by Gayathree et al., [25] where screening for AB in all the three trimesters was stressed. On the contrary Vaishali et al., states if AB is detected late in pregnancy it leads to maternal and neonatal complications despite treatment. Hence, it's of paramount importance to detect and treat this condition in early pregnancy [26].

CONCLUSION

Screening for AB in pregnant women should be made mandatory in the early trimesters of pregnancy with an emphasis in those with a history of previous urinary tract infection. A recommendation for two consecutive urine cultures growing the same isolate at 1,00,000 CFU /ml or more, the concomitant use of chromogenic media as an effective tool in any health care set up and the use of newer rapid and accurate tests for identifying uncommon and difficult to isolate pathogens can enhance the overall detection of these cases.

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REFERENCES

[1] Patterson T, Andriole V. Detection, significance and therapy of bacteriuria in pregnancy. *Infect Dis Clin North Am*. 1997;11(3):593-607.
[2] Kiningham R. Asymptomatic bacteriuria in pregnancy. *Am Fam Physician.* 1993; 47(5):1232-38.
[3] Task Force on UTI. Philippine Practice Guidelines Group in Infectious Disease. *Urinary Tract Infection in Pregnancy.* 1998;1:16-19.
[4] Zhanel GG, Harding GK, Guay DR. Asymptomatic bacteriuria- which patient should be treated? *Arch Inter Med.* 1990;150(7):1389-97.
[5] Andriole V, Patterson TF. Epidemiology, natural history and management of urinary tract infection in pregnancy. *Med Clin North Am.* 1991;75(2):359-71.
[6] Lucas M, Cunningham FG. Urinary tract infections in pregnancy. *Clin Obstet Gynecol*. 1993;36(4):855-68.
[7] Maranchie JK, Capelouto CC, Loughlin KR. Urinary tract infections during pregnancy. *Infect Urol.* 1997; 10:152-57.
[8] Sohely S, Farhana A, Fahmida B, Jaigirdar QH. Use of Chromogenic Agar Media for Identification of Uropathogen. *Bangladesh J Med Microbiol.* 2010;04(01):18-23.
[9] Hengstler KA, Hammann R, Fahr AM. Evaluation of BBL. Chromeagar orientation for detection and presumptive identification of urinary tract pathogens. *J Clin Microbiol*. 1997;35:2773-77.
[10] Mazoyer MA, Orenga S, Doleans F, Freney J. Evaluation of CPS ID 2 medium for detection of urinary tract bacterial isolates in specimens from a rehabilitation centre. *J. Clin. Microbiol*. 1995;33:1025-27.
[11] Merlino J, Siarakas S, Robertson GJ, Funnell GR, Gottieb T, Braudbury R. Evaluation of Chromagar orientation for differentiation and presumptive identification of gram negative bacilli and Enterococcus spp. *J Clin microbial.* 1996;34:1793.
[12] Samra Z, Heifetz M, Jalmor J, Bain E, Bahar J. Evaluation of chromagar in the detection of uropathogens. *J Clin Microbiol*. 1998;36:990-94.
[13] Sweet RL. Bacteriuria and pyelonephritis during pregnancy. *Semin Perinatol.* 1977;(1):25-40.
[14] Kass EH. Bacteriuria and pyelonephritis of pregnancy. *Arch Intern Med.* 1960;105:194-8.
[15] Smaill F, Vazquez JC. Antibiotics for asymptomatic bacteriuria in pregnancy. *Cochrane Database Syst Rev.* 2007;18(2):CD000490.
[16] Norden CW, Kass EH: Bacteriuria of pregnancy – a critical appraisal. *Ann Rev Med*. 1968;19:431-70.
[17] Mignini L, Carroli G, Abalos E, Widmer M, Amigot S, Nardin JM. World Health Organization. Asymptomatic Bacteriuria Trial Group. Accuracy of diagnostic tests to detect asymptomatic bacteriuria during pregnancy. *Obstet Gynecol.* 2009;113(2 Pt 1):346-52.
[18] Imade PE, Izekor PE, Eghafona NO, Enabulele OI, Ophori E. Asymptomatic bacteriuria among pregnant women. *North Am J Med Sci.* 2010;2:263-66.
[19] Lindsay E. Nicolle, Suzanne Bradley, Richard Colgan, James C. Rice, Anthony Schaeffer, Thomas M. Hooton. *I*nfectious Diseases Society of America Guidelines for the Diagnosis and Treatment of Asymptomatic Bacteriuria in Adults. *Clinical Infectious Diseases.* 2005; 40 :643–54.
[20] Nerissa Isabel C Sescon, Felice D Garingalao-Molina, Carla Elena JY casiano, Mediadora C Saniel, Ricardo M Manalastas. Prevalence of Asymptomatic Bacteriuria and Associated Risk Factors in Pregnant Women.*Phil J Microbiol Infect Dis*. 2003;32(2):63-69.
[21] Pometta D, Rees S B, Younger D, Kass EH. Asymptomatic bacteriuria in diabetes mellitus. *N Engl J Med*. 1967;276:1118-21.
[22] Mittendorf R, Williams MA, Kass EH. Prevention of preterm delivery and low birth weight associated with asymptomatic bacteriuria. *Clin Infect Dis.*1992;14(4):927-32.
[23] Hill JA , Devoe LD , Bryans CL Jr. Frequency of asymptomatic bacteriuria in preeclampsia. *Obstet Gynecol.* 1986;67(4):529-32.
[24] Awasthi A, Prashanth Adiga P, Rao S. Prevalence of asymptomatic bacteriuria and sterile pyuria in pregnant women attending antenatal clinic in a tertiary care center in Karnataka: A pilot study. *Clin Epidemiol and Global Health.* 2013;1(1):44–49.
[25] Gayathree L, Shetty S, Deshpande SR, Venkatesha DT. Screening For Asymptomatic Bacteriuria In Pregnancy: An Evaluation Of Various Screening Tests In Hassan District Hospital, India. *J Clin Diag Res.* 2010;4(4):2702-06.
[26] Vaishali J, Vinita D, Anjoo A, Amita P. Asymptomatic bacteriuria & obstetric outcome following treatment in early versus late pregnancy in north Indian women. *Indian J Med Res.* 2013;137:753-58.

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