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Research Article

Efficacy of Dip slide test in assessing the asymptomatic bacteriuria in pregnancy

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ABSTRACT

Background: Asymptomatic bacteriuria is identified only when significant number of bacteria is demonstrated in urine. As bacterial culture takes at least 3 to 4 days, early diagnosis is a problem. Hence a reliable and cost effective screening test for bacteriuria is of great value. The aim of the study was conducted to know the prevalence of asymptomatic bacteriuria and to evaluate the diagnostic efficacy of Dip slide test.
Methods: This study was conducted at Government Maternity Hospital - Upgraded institute for Obstetrics and Gynaecology, Nayapool, Hyderabad between May 2004 and October 2004. All women who attended the antenatal clinics of this hospital were taken as study group. A total of 300 urine specimens were taken and urine culture was done by both standard and also Dip slide method. The results were compared.
Results: The main objective of this study was to compare the results of, traditional culture method and Dip slide method for screening bacteriuria. The incidence of asymptomatic bacteriuria was found to be 6.3%. E coli were the commonest organism, which was isolated in 63.16% of cases. All the positive culture in standard method also showed pure growth in in Dip slide method, with, a colony count of >10 with more than 200 colonies. The results were found to be identical in both methods.
Conclusions: Dip slide method of culture is simple to use, accurate, inexpensive & reliable method of screening bacteriuria during pregnancy and it can be strongly recommended for screening pregnant women in antenatal clinics, by including it as a routine part of antenatal care.

Key words: Urinary tract infection, Dip slide method, Culture, Bacteriuria

INTRODUCTION

Asymptomatic bacteriuria is identified only when significant numbers of bacteria are demonstrated in urine .In order to avoid the hazards of bacteriuria in pregnancy, detection and treatment of bacteriuria should be included as a part of routine ante natal care. As bacterial culture takes 2-3 days early diagnosis is a problem. Hence a reliable and cost effective screening test for bacteriuria would be of great value. Dip slide is a simple inexpensive indigenous method for screening bacteriuria in pregnancy.[1,2] The present study was conducted to know the prevalence of asymptomatic bacteriuria and to evaluate the diagnostic efficacy of dip slide test in our antenatal population at government maternity hospital - Upgraded institute for Obstetrics and Gyneacology, Nayapool Hyderabad, India.

METHODS

The study population consisted of pregnant women attending ante natal clinic at government maternity hospital - upgraded institute for Obstetrics and Gyneacology, Nayapool Hyderabad, India between May 2004 and October 2004.

Clean-catch mid-stream urine specimen was collected from 300 patients in sterile containers. All the specimens were processed by standard procedure and by dip slide method of culture. Urine specimen was collected only from asymptomatic patients. A detailed history including history of previous urinary tract infection was taken in detail from each patient and was recorded on the proforma. Preliminary haemoglobin estimation was done for all patients before proceeding to culture microscopic and macroscopic examination of urine was done to look for albumin, sugar, pus cells and bacteria.

DIP slide preparation

It includes both dip Slide Preparation and Preparation of Medium - Cysteine lactose electrolyte deficient medium (CLED) was used in our study. The ingredients were suspended in water. It was brought to boil to dissolve the contents .This was then sterilized for 15 minutes at 121 degree C. Later it was mixed well before pouring on the glass slides.

CLED medium

This medium (Machey and Sandys 1966) is considered preferable to Mac conkey-bile salt lactulose medium for the culture of coliform and other bacteria from infected urine.

Like Mac Conkey medium it distinguishes between lactose fermenting (yellow) and non lactulose fermenting (blue, grey and green) colonies, inhibits swarming of proteus and shows the greenish colour, matt surface and rough periphery of pseudomonas colonies. It has the advantage in supporting the growth of certain streptococci, staphylococci and candida strains that fail to grow on Mac-Conkey.

Escherichia coli form yellow opaque colonies usually1-1.5mm in diameter. Klebsiella, mucoid yellow to whitish blue colonies. Staphylococcus deep yellow opaque colonies, other streptococcus feacalis, yellow to white translucent colonies about 0.5mm in diameter. Ingradients of CLED medium are Peptone 4gm, Agar 15gm, Trptone 4gm, Water 1 litre, Lab-Lamco Meat extract 3gm, Lactose10gm, L Cystine 120gm, Bromothymol blue 0.2 gm.

Preparation of DIP slide

7.5 x 2.5 glass slides sterilised by hot air oven, 2ml of cysteine lactose electrolyte deficient medium (CLED) was then pipetted out on to the sterile glass slide in a laminar hood and the agar allowed to set at room temperature. These slides were prepared 24 hours before collection of urine specimens and kept in sterile slide container. Culture was then performed by the following two methods. One is the standard procedure and the other is the DIP slide inoculation method.

DIP slide procedure: In this procedure, some of the slides were inoculated in the laboratory and some were given to the patient for inoculation.

Inoculation of slides in the laboratory: In the beginning of the study about 100 patients were provided with only sterile containers and after thorough vulval toilet they were instructed to collect mid - stream urine in the containers. The specimen was transported to the laboratory within ½ hour and there urine was poured over the dip slide. After removing excess urine from the slide, it was included at 37 degree C for 24 hours.

Inoculation of the slide by the patient: In the latter half of the study, the dip- slides which were kept in sterile container were taken to the ante-natal clinics. After instructing the patients regarding vulval toilet, they were provided with the DIP slide and were asked to expose the DIP slide in the mid-stream urine and immediately, they were transported to the laboratory in sterile containers and incubated at 37 degree centigrade for 24 hours. After 24 hours of inoculation the slides were examined for growth.

The count of viable bacteria in the urine is estimated approximately from the number of colonies. DIP slides with a colony count of more than 200 colonies were taken as significant bacteriuria. This correlated very well with positive cultures done by standard method which showed significant bacteriuria. Slides with less than 200 colonies were taken as insignificant Slides with colony count between 200 and 500 could be read easily. If the count was more than 500 it appeared very confluent and it was very difficult to count the colonies. An experienced microbiologist can read and say whether it is significant or insignificant bacterial growth.

RESULTS

In our present study the incidence of asymptomatic bacteriuria was found to be 6.3% of all the positive cultures, Escherichia coli was the commonest. A total of 300 urine specimens were obtained from pregnant patients who were between 10-32 weeks of gestation .Of the 300 urine specimens, the routine traditional culture method showed 19 samples to be culture positive with pure growth of microorganisms with a colony count of >10 /ml of urine.

Colony count with <10 /ml was taken as insignificant bacteriuria and was discarded. When the traditional method was compared to the Dip slide culture results, it

was found that all the 19 specimens which showed positive culture in standard method also showed pure growth on DIP slide with a colony count of >10/with more than 200 colonies. In our study we did not find any discrepancy with the results of the above two methods. The results were found to be identical in both methods. There were no false negative cases obtained in our study by using Dip-slide. The other parameters which were studied are tabulated in the following tables.

Table 1: Age distribution of cases.

Age of the mother in years	Total number of cases	No of culture positive cases	Percentage
15-19	55	3	15.79
20-24	104	8	42.11
25-29	93	5	26.32
30-34	30	2	10.-53
35-39	15	1	5.26
40 >	3	0	
Total	300	19	100.00%

Table 1 show that age did not have any appreciable influence on the incidence of asymptomatic bacteriuria.

Table 2: Positive culture in relation to gestational age.

Gestational age in weeks	Total number of cases	Number of positive cases	Percentage
10-19	38	1	5.26
20-24	102	8	42.11
25-28	69	7	36.84
29-32	64	3	15.79
Total	300	19	100.00

Table 2 shows the incidence of asymptomatic bacteruria in relation to the period of gestation. Most of the antenatal cases do not come for check-up before 20 weeks and hence only a small no was available for our study. The maximum incidence in the study was found to be between 20 to 28 weeks of gestation.

The Table 3 clearly shows that all positive culture cases, which showed significant growth of bacteria by standard culture method, also showed significant number of colonies on the DIP slide.

One more thing that is noticed from the Table is that, in all significant bacterial growth obtained by standard culture, the corresponding DIP slide showed >200 colonies. Because of this reason >200 colony count on the DIP slide was taken as significant bacteruria. All the other slides which showed a colony count of <200 colonies, did not show significant growth by standard method.

Table 3: Results of standard method Versus DIP slide method.

Organism	Standard method	DIP slide method
Escherichia coli	Significant growth	200 colonies
Escherichia coli	Significant growth	350 colonies
Escherichia coli	Significant growth	Confluent growth
Escherichia coli	Significant growth	500 colonies
Proteus mirabilis	Significant growth	>200 colonies
Klebsiela pneumonia	Significant growth	150 colonies
Escherichia coli	Significant growth	300 colonies
Escherichia coli	Significant growth	200-300 colonies
Staphylococcus	Significant growth	150-200 colonies
Escherichia coli	Significant growth	500 colonies
Klebsiela pneumonia	Significant growth	300-350 colonies
Escherichia coli	Significant growth	>400 colonies
Escherichia coli	Significant growth	Confluent growth
Proteus mirabilis	Significant growth	150-200 colonies
Escherichia coli	Significant growth	150 colonies
Escherichia coli	Significant growth	Confluent growth
Escherichia coli	Significant growth	400-500 colonies
Staphylococcus	Significant growth	250-300 colonies
Klebsiela pneumoniai	Significant growth	200 colonies

DISCUSSION

In spite of all the controversies as acute symptomatic pyelonephritis occurs in the latter stages of pregnancy in a significant percentage of women with symptomatic bacteriuria and is associated with serious implications to the well- being of the mother and fetus, it seems justified to recommend a detection scheme to screen aggressively for bacteriuria in pregnancy and to treat bacteriuria in pregnant women in order to decrease the risk of symptomatic infection. As urine cultures are the most satisfactory method for establishing the diagnosis, the most cost effective and reliable means of both collections and culturing the urine should be employed.

Previous workers have tried many procedures to detect bacteriuria. Unfortunately non culture methods for establishing significant bacteriuria have been unsuccessful. Microscopic examination of a un-centrifuged drop of urine is a rapid inexpensive and simple procedure. Although the presence of bacteria detected in this manner correlates with at-least 1lakh bacteria/ml. This is not sensitive enough to allow its use as a screening procedure for asymptomatic bacteriuria. Later a number of chemical tests have been proposed as a means to establish significant bacteriuria .The most commonly used ones are (1) Griess test (2) Triphenyle Tetrazolium Chloride (TTC) test.

Table 4: Prevalence according to various authors.

Name of the Author	Year	Rate (%)
Kass EH[3]	1960	6.7
Turner GC[5]	1961	7.8
Harris RE[7]	1984	2-10
Hankin & Whalley[8]	1985	2-7
Von Dorston JP et al[6]	1986	6-10
Stenquest et al[9]	1989	2-11
Finh SD[10]	1988	4-7
Buckshee K, Ratan A[11]	1993	6.8
Present study	1995	6.3

Screening methods also depend on the population studied. In low risk high socio-economic status populations, either selective screening on the basis of historical risk factors or universal screening using an expensive test is appropriate The Griess test for instance is a reasonable compromise between accuracy and and low risk Likewise, micro culture technique, in so far as specific identification and sensitivity testing to antibiotics seems unnecessary for empirical treatment, is another effective method of screening with sufficient accuracy and without inordinate cost. In high risk population, screening with bacteriologic culture is recommended as a cost effective, appropriate and sensitive means of diagnosis. Early Kass EH advocated routine screening for bacteriuria in ante natal clinics.[3] Despite this screening has not become a routine part of ante natal care in our country, because the bacteriological facilities in our hospitals are inadequate and the bacterial culture takes at least 3-4 days. This makes early diagnosis a problem. By using DIP slide method for screening bacteriuria all the above problems are overcome.[4,5]

DIP slide is made from easily available material and is very economical. A glass slide is coated with culture media on one or both sides. This slide is inoculated by dipping or passing in the mid-stream urine and then immediately incubated at 37^0C. After 24 hours the slide is observed for growth and colony count. Up to 500 colonies can be easily separated and counted on one side of the slide, the growth becoming confluent only when colony counts approaching 1000 colonies. With a little experience the majority of the DIP SLIDES may be assigned to significantly or insignificantly infected groups by inspection alone.

The quantitative accuracy of the method is greater than that obtained by standard loop inoculation method. As the DIP slide method is simple and inexpensive method for screening bacteriuria in pregnancy, we have selected this technique for our study to once again evaluate the diagnostic efficacy of DIP slide method in our ante natal population at Government Maternity Hospital - Upgraded institute for Obstetrics and Gyneacology, Nayapool Hyderabad and also compare the results obtained by DIP slide method and the routine standard method of culture.

In our study we did not find any discrepancy between the two methods. Culture positive cases by standard loop method also showed significant growth on the DIP slide. There were no false negative cases observed in DIP slide method in our study, thus clearly indicating that DIP slide culture method is equally good and effective. According to Von Dorston JP et al the sensitivity and specificity of DIP slide method was found to be 98.2% and 97.4% respectively.[6]

In the standard culture method as we have used both Mac conkey and blood agar culture plates, there was no chance of missing any urinary pathogens which could have been possible if only one media was used. Therefore the CLED media which we have used on the DIP slide did not offer any additional value in detecting gram negative bacilli. However the advantages of using CLED media on the slide is that no urinary pathogens are missed and the medium being transparent the presumptive colony count is is much easier. The same finding were also observed in a previous study done by Von Dorston JP et al with DIP slide having Mac Conkey media on one side and CLED media on the other side.[6]

Advantages of DIP slide method are results of the DIP slide method correlated very well with the standard culture. There were no false negative cases obtained by DIP slide method. The DIP slide which is used in our study is very easy to prepare and it is very economical and inexpensive. By using CLED medium there is no chance of missing any urinary pathogens. As medium is transparent the colonies could be read and counted easily. Interpretations of results were also not very difficult in DIP slide method. When the colonies are below 500 it is easy to count and >200 colonies when present it is taken as significant and this correlated with the significant bacteriuria of the standard culture method very well. These DIP slides can be inoculated in the laboratory or by the patient themselves. If the patient is properly instructed regarding the procedure, all she has to do is to simply pass the slide in the stream of urine. Only DIP slide that show significant growth need to be sent to laboratory for identification and sensitivity tests. Thus the laboratory is spared from taking the large amount of clerical work required for reception and reporting of the negative specimens.

This method of semi quantitative culture on DIP slide is the least laborious for the laboratory and as the medium is seeded with urine immediately, as soon as it is passed, obviates the difficulty of having bacterial multiplication during transport to the laboratory. Further it can also be recommended whenever there is likely to be delay in delivery of urine specimen to the laboratory. It may be particularly helpful in general practice where the specimen have to be sent by post. Owing to its ease of inoculation and reading it is very well suited for the routine screening of large number of specimens at our ante-natal clinics.

CONCLUSION

DIP slide method of culture is simple to use, accurate, inexpensive and reliable method of screening bacteriuria during pregnancy and it can be strongly recommended for screening pregnant women in antenatal clinics, by including it as a routine part of our antenatal care.

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