SUBCULTURE ON ISLAM AGAR: RAPID AND RELIABLE METHOD TO DETECT GROUP B STREPTOCOCCI COLONIZATION STATUS

Mujahid Munir Bhatti,[1] Munir Ahmad,[2] Afshan Khalid,[3] Khalid Munir Bhatti[4]

ABSTRACT

Background: Various methods have been used to detect the colonization status of near term pregnant females but there is lack of consensus on the most appropriate method. In this study we have evaluated three different techniques in terms of their reliability and easiness to perform. **Objective:** The objective of our study was to determine the validity of 3 laboratory methods namely direct culture on selective blood agar, direct culture on Islam agar and subculture after enrichment with Lim broth on Islam agar in diagnosis of group B streptococcus (Streptococcus agalactiae) (GBS) in near term pregnant females. **Materials & Methods:** This prospective study was conducted at microbiology section of department of the Pathology, King Edward Medical University and outpatient department of Lady Atchison and Lady Willington Hospital, Lahore. 200 near term, pregnant women of all ages between 35 to 37 weeks of gestation were included in the study. Swabs from the vaginal introitus were subjected to analysis by culture on 4 medias and sensitivity and specificity were calculated for each media against the gold standard. **Results:** On gold standard media 32 cases were found to be positive for GBS. The prevalence was 16 %. The calculated sensitivity and specificity for different techniques were; Blood agar (93.3%, 98%), Islam Agar (93.3%, 98%) and LIM enriched Islam Agar (100%, 100%). **Conclusion:** Subculture on Islam Agar after LIM enrichment is superior to other techniques in terms of its performance and cost related issues.

Key words: Group B streptococci, Islam agar, Blood Agar, Chromogenic Agar, Pregnant Women.

INTRODUCTION

Group B streptococci (GBS) are gram positive cocci. They may colonize the genital tracts especially those of pregnant females.[1] During normal vaginal delivery Group B streptococcus may be transmitted from mother to the neonate in about 80-85 % of the cases. This may result in neonatal sepsis, meningitis and pneumonias. Hence detection of pre delivery GBS colonization status may help to reduce complications related to this organism.[2]

Recently PCR[3] and LCR[4] have also been investigated as a screening tool to determine GBS colonization status. Though their performance is good but in our setup where cost of the screening is a major concern it seem inapplicable. So in low resource settings, culture on selective blood agar after LIM enrichment is considered as gold standard as culture result on this media is taken as final.[5] The other conventional methods include culture on selective blood agar, direct culture on chromogenic agar and subculture after enrichment with Lim broth on Islam agar.[6] Although all of these three methods have comparable performance, superiority of one method over the other is not well established. In this study we have evaluated the validity of these methods in terms of sensitivity, specificity, positive predictive value and negative predictive value.

1.Department of Microbiology & Serology, King Edward Medical University, Lahore.
2.Department of Pathology King Edward Medical University, Lahore.
3.Department of Obs/Gyane, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, University of Health Sciences, Lahore.
4.Department of Pediatric Surgery, Sultan Qaboos university Hospital, Muscat, Oman.

Correspondence: Dr. Khalid Munir Bhatti, Specialist Registrar
Department of Pediatric Surgery, Sultan Qaboos University Hospital
Muscat, Oman.

Email: drkhalidmunirbhatti@yahoo.com
Phone: 00968-94200767

MATERIALS AND METHODS

Study site and population: After approval by ethical committee, this cross sectional prospective study was conducted at microbiology section of department of the Pathology, King Edward Medical University and outpatient department Lady Atchison and Lady Willington Hospital, Lahore, over the period of 6 months starting from 1st March, 2011. Specimen collection: By random purposive sampling, 200 near term, pregnant women of all ages between 35 to 37 weeks of gestation were included in the study after taking informed consent. Females with known vaginal infection and or taking antimicrobial for any reasons were excluded from the study. Vaginal specimen was collected by using sterilized disposable cotton swab, another vaginal specimen was collected by using cotton swab with Amies agar gel medium. Swabs from the vaginal introitus were subjected to analysis by culture on selective blood agar and Islam agar. Sub-culturing on these two medias was repeated after enrichment by Lim broth**.** Diagnostic Tests: Recognition of colony was done by visualizing characteristic features (Fig 1) and detection of organism was done by applying confirmatory test like gram staining, catalse test and CAMP test. Performance and cost analysis: 2x2 table

was used to calculate sensitivity, specificity, positive predictive value and negative predictive value for each test. Cost was not calculated directly because of lack of information related to labor cost. However factors which could affect the cost were taken into account. Duration to complete each procedure was also calculated. Data was entered and analyzed in SPSS version 10.

RESULTS

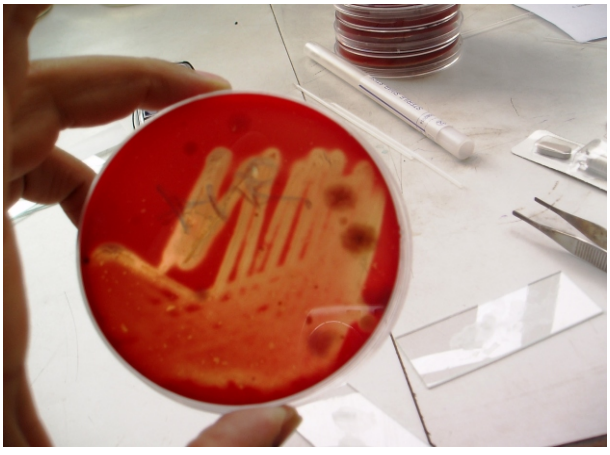
On gold standard media 32 cases were found to be positive for GBS. 168 cases were declared negative when no colony was formed on LIM/Selective Blood agar after 72 hours and confirmatory tests were also found to be negative. Direct culture on Islam Agar detected 30 cases as positive. 2 cases detected negative on Islam Agar were found to be positive on LIM/SBA.

Similarly, 30 cases were positive on Selective blood Agar and 2 of the negative cases were found positive on LIM/SBA.

Table I: Performance of 3 culture methods compared to LIM/SBA Culture

Method	Number of				Statistics			
	True positive	False Negative	True Negative	False Positive	Sensitivity	Specificity	Positive Predictive value	Negative Predictive value
Selective Blood Agar	30	2	168	0	93.3%	100%	100%	98%
Islam Agar	30	2	168	0	93.3%	100%	100%	98%
Lim/Islam Agar	32	0	168	0	100%	100%	100%	100%

Fig I: Orange colony formation on Islam Agar



However, 32 cases which were detected on LIM/SBA were found positive on LIM/Islam Agar. None of the cases detected positive on 3 medias was found to be negative on LIM/SBA or confirmatory tests. So, the calculated sensitivity and specificity for different techniques were Blood agar (93.3%, 98%), Islam Agar (93.3%, 98%) and LIM enriched Islam Agar (100%, 100%) (Table I) .

DISCUSSION

For the last one decade newer techniques have been introduced to determine GBS colonization status of near term pregnant females. Few of the examples include PCR[3] and LCR[4]. Undoubtedly cost related to peri-natal morbidity and mortality is a major concern. Nevertheless cost on screening is also considerable. So a test which has reliable performance, easy to perform and has less reporting time and cost should be an ideal.

The factors which are related to the cost of a test include cost of kit, reagents and materials, confirmatory tests and labor. Labor associated cost is directly related to time required to complete a test. Similarly if a method requires confirmatory test on culture it increases the cost in two ways i.e. cost of confirmatory tests and cost of labor related to extra time utilized. The results of this study showed that the sensitivity of direct culture on Selective blood agar (SBA) and Islam agar are equal but less than those of subculture on Islam Agar after Lim enrichment. This 2 percent extra detection has many implications. One of the basic principal of the screening test is that none of the positive case should be missed. Secondly if we develop such an algorithm that at 1st step direct culturing is done followed by sub culturing in case of being negative, it is expected to increase cost by many factors. Firstly, as prevalence in low, so most of the samples will be cultured twice. Secondly, total time required to complete will be increased hence increasing the cost. According to the results of our study we have found that subculture after LIM enrichment has many advantages. In all most all of the case average time to complete was 36 hours including 12 hours of LIM enrichment. Secondly it avoids use of second culture as it would be required in case on negative samples on direct culturing. Thirdly as it produces orange colored recognizable colonies by naked eye, this will help to reduce the cost related to confirmatory tests. Our results are in harmony with the CDC recommendations, which recommend Lim enrichment.[6] The other authors have also shown similar results.[7,8,9]

CONCLUSION

In our setup as the colonization status of the females is not very high, subculture on Islam Agar after LIM enrichment is expected to provide most cost effective method as it has sensitivity, specificity and positive/ negative predictive values equal to Conventional blood agar. Reports are at least 12 hours earlier available. Moreover, cost related to second culture, extra duration and confirmatory tests can be saved.

REFERENCES

1. Krohn MA, Hiller SL, Baker CJ. Maternal paripatum complication associate with vaginal group B streptococcal colonization. J Infect Dis 1999; 179:419-23.
2. Schuchat A. Group B streptococcus. Lancet 1999; 53:51-6.
3. Edward MS, Baker CJ. Infectious Diseases of the Fetus and Newborn Infant 2001.Philadelphia: WB Saundres: 1091-1156.
4. Park, C. Detection of group B streptococcal colonization in pregnant women using direct latex agglutination testing of selective broth. Med Sci 2001; 22:97-112.
5. Shibuya R, Yamashita T, Shibuya S, Hasegawa M, Kanayama A, Ikeda F et al. Evaluation of three selective enrichment media for detection of Group B Streptococcus in vaginal swabs. Kansenshogaku Zasshi 2009; 83:52-5.
6. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC, 2010. *MMWR*. 2010;59 (RR-10):1-32.
7. Heelan JS, Struminsky J, Lauro P, Sung CJ. Evaluation of a new selective enrichment broth for detection of group B streptococci in pregnant women. J Clin Microbiol 2005; 43**:**896-7.
8. El-Kersh TA, Al-Nuaim LA, Kharfy TA, Al-Shammary FJ, Al-Saleh SS, Al-Zamel FA. Detection of genital colonization of group B streptococci during late pregnancy. Saudi Med J 2002 Jan;23:56-61
9. Fatemi F, Chamani-Tabriz L, Pakzad P, Zeraati H, Rabbani H, Asgari S. Colonization rate of Group B Streptococcus(GBS) in pregnant women using GBS Agar Medium. Acta Medica Iranica 2009; 47:25-30