GROUP-B STREPTOCOCCUS IN PREGNANT WOMEN: Prevalence of Colonization and Sensitivity Pattern in Denpasar during June 2007-May 2008

by Ni Nyoman Sri Budayanti

FILE	GBS_DR._SRI.PDF (226.49K)		
TIME SUBMITTED	21-APR-2014 06:59PM	WORD COUNT	2275
SUBMISSION ID	418882128	CHARACTER COUNT	12177

Bali Medical Journal (BMJ) 2013, Volume 2, Number 1: 17-20
P-ISSN.2089-1180, E-ISSN.2302-2914

GROUP-B STREPTOCOCCUS IN PREGNANT WOMEN: Prevalence of Colonization and Sensitivity Pattern in Denpasar during June 2007–May 2008

[1]Sri-Budayanti, N. and [2]Hariyasa-Sanjaya, N.

[1]Department of Microbiology, Faculty of Medicine, Udayana University/ Sanglah General Hospital, Bali-Indonesia

[2]Department of Obstetrics and Gynecology, Faculty of Medicine Udayana University/ Sanglah General Hospital, Bali-Indonesia

Objective: Group-B Streptococci (GBS) are Gram-positive cocci that are the most common cause of early onset neonatal sepsis. The mortality rate of early onset neonatal sepsis has been reported up to 50%. One of the major risks of early onset neonatal sepsis is GBS colonization in birth canal of pregnant women that can infect the baby during process of vaginal delivery. Antibiotic chemoprophylaxis for pregnant women that is colonized by GBS can reduce the risk of early onset neonatal sepsis. The detection of GBS colonization needs Todd Hewitt (TH) enrichment medium to reduce false negative result. Until now, there is no report about either prevalence of colonization or sensitivity pattern of Group B Streptococcus among pregnant women in Denpasar. The aims of this research were to determine the prevalence of GBS colonization and sensitivity pattern of GBS among pregnant women with Todd Hewitt enrichment medium. **Method**: This research was a descriptive cross-sectional study. Vaginal swab specimens from 35–37 weeks gestation pregnant women were collected and 32 samples that met the inclusion criteria were cultured on Blood agar (BA) plates, Chromagar (CA) plates, and Todd Hewitt (TH) broth. The GBS colonization that grew in culture medium was followed by antibiotic sensitivity test. **Results**: In the present study, we found that the prevalence of GBS colonization in pregnant women detected with culture method using BA and CA without TH broth was 9.4%, whereas the prevalence with culture method using BA and CA enriched by TH broth was 31.3%. Moreover, GBS showed resistance to penicillin, erythromycin, and cefazolin. It is indicated that TH enrichment medium seems to be promising as a screening method for GBS colonization in pregnant women in Bali. **Conclusion:** There was an enrichment detection of GBS prevalence colonization in pregnant women detected the swab with culture method using BA and CA enriched by TH compare to BA and CA without TH broth. Moreover, GBS showed resistance to penicillin, erythromycin, and cefazolin. It is indicated that TH enrichment medium seems to be promising as a screening method for GBS colonization in pregnant women in Bali.

Keywords: GBS, early onset neonatal sepsis, culture method, Todd Hewitt broth, sensitivity test

INTRODUCTION

Infection is the leading cause of death in the first month of life, accounting for 13-15% of all neonatal deaths. One type of infection that often occurs in early life of the infant is early-onset sepsis. The mortality rate of early-onset neonatal sepsis can reach 50% in infants who are not properly treated.[1,2] Group-B Streptococcus (GBS) and *Escherichia coli* are the most common bacteria causing neonatal infection. GBS colonization is the major risk for the occurrence of neonatal sepsis.[1,3]

Correspondence: *Sri-Budayanti, N*
Addres: Department of Microbiology, Faculty of Medicine, Udayana University/ Sanglah General Hospital, Bali-Indonesia

Recommended chemoprophylaxis in pregnant women to prevent perinatal GBS diseases have been published since 1996 by The American College of Obstetricians and Gynaecologists (ACOG) and The Center for Disease Control and Prevention (CDC). In 1997, the same recommendations were published by The American Academic of Pediatrics. The CDC study used a sample population of more than 600.000 babies born alive. Results of that study showed that screening approached 50% was more effective than at risk approach in pregnant women. In addition, some theoretical predictions stated than screening approach reduced the incidence of early-onset sepsis more than at risk approach. Although this recommendation has been successful in reducing the incidence of early-onset sepsis in developed

countries, chemoprophylaxis was not routinely performed in health services in Indonesia.[3,5]

Several studies suggested that prenatal culture screening was accurate for identifying intrapartum colonization status.[3] The accuracy of colonization status can be improved by culture timing, swab location and the proper method for culture and detection of organism. In a culture that uses only direct platting or blood agar media without selective media found about 50% false negative culture in colonized GBS women.[3,4,5]

Until now, there is neither prevalence data nor antibiotic sensitivity pattern of GBS in pregnant women in Bali. Lacks of this data affect the implementation of chemoprophylaxis program in pregnant women. Therefore, it is necessary to determine the prevalence of GBS colonization in pregnant women and antibiotic sensitivity pattern of GBS in Bali, especially in Denpasar, in order to reduce the risk of early-onset neonatal sepsis.

PATIENTS AND METHOD

Study design

This is a descriptive cross-sectional study conducted at Microbiology Laboratory Faculty of Medicine Udayana University and Nikki Medika Laboratory in Denpasar since June 2007 until May 2008. Institutional review board/ethics committee approval was obtained from Medical Ethic Commission Sanglah General Hospital and this study adhered to the tenets of the Declaration of Helsinki.

Sample inclusion criterion of the study was vaginal swab from pregnant women with gestational age 35–37 weeks. Samples were collected with consecutive sampling.

Specimen collection, bacterial cultures, and antimicrobial resistance test

All specimens were collected by an obstetrician with swab method, and then were inoculated in Amies transport medium before sending to the Microbiology Laboratory or Nikki Medika Laboratory to avoid the overgrowth of contaminants bacteria and to ensure the GBS survival during samples transportation. Samples processing were conducted within 2 hours after samples collection. The sample swabs were streaked onto Blood agar (BA) plates, Chromagar (CA) plates (Biomerieux, inc), and Todd-Hewitt (TH) broth, and then incubated at 37°C for 24 h. About 10 µl of cloudy TH broth was inoculated onto BA and CA, and was continued to be incubated at the same conditions. White colonies that were surrounded by a clear zone on BA after 24 hour incubation were determined by Gram staining. Catalase test and Strepkit® tests (Biomerieux, inc) for grouping were performed for confirming Gram positive cocci colonies were Streptococci. The growth of pink to red colonies on Chromagar plates was evaluated, and was determined by Gram staining. The colonies on Chromagar plate were judged as GBS if the Gram staining result showed Gram positive cocci.

All isolates that grew on agar plates employed in this study were tested for sensitivity pattern using Kirby-Bauer method based on Clinical and Laboratory Standards Institute Guidelines (CLSI). Briefly, one single colony of GBS was inoculated in nutrient broth to 0,5 McFarland and was streaked on Mueller-Hinton with 5% sheep blood agar plates. Penicillin (10 units), Augmentin® (20/10µg), Cefazolin (30 µg), Imipenem (10µg), Amoxycillin (30 µg), Cotrimoxazole (1.25/23.75 µg), Chlorampenicol (30 µg), Erythromycin (15 µg), Ceftriaxone (30 µg), Ampicillin (10 µg) antibiotic disks were used for antibiotic sensitivity test. The cut off values of each antibiotic disks were determined as described in CLSI.

RESULTS

Swab of the sample onto Blood Agar (BA) plates produced white colonies surrounded by a clear zone after 24 hour incubation and applying gram staining as can be seen in Figure 1.

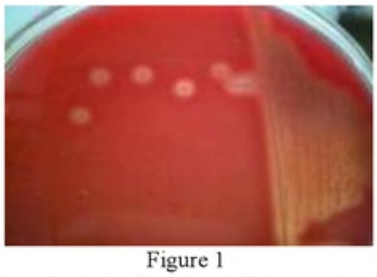

Figure 1
GBS Colonies on BA after 24 hour incubation: small and white colonies surrounded clear zone

Swab sample on Chromagar plates produced growth of pink to red colonies and determined by Gram staining as indicates by Figure 2. The colonies on Chromagar plate were judged as GBS if the Gram staining result showed Gram positive cocci.

Figure 2
GBS colonies on a chromagar plate after 24 hour incubation: small, pink to red color colonies

During study period, there were 32 samples met inclusion criteria. The prevalence of GBS colonization in pregnant women with 35–37 weeks gestation were higher in combination of TH+BA method and TH+CA (31.3%) than the method that used only BA or CA (9.4%) (Table 1).

Table 1
Growth of bacterial colonies on culture media (n=32)

Media	Colonies		Total
	GBS	Non-GBS	
Blood Agar (BA)	3 (9.4%)	29 (90.6%)	32 (100%)
Chromagar (CA)	3 (9.4%)	29 (90.6%)	32 (100%)
Blood Agar + Todd Hewitt (BA+TH)	10 (31.3%)	22 (68.7%)	32 (100%)
ChromAgar + Todd Hewitt (CA+TH)	10 (31.3%)	22 (68.7%)	32 (100%)

All GBS isolates were still sensitive to chlorampenicol and ceftriaxone. Antibiotic that was commonly used for GBS chemoprophylaxis has been decreased in the sensitivity to GBS isolate, namely penicillin (90%), erytromycin (80%) and cefazolin (80%) (Table 2).

Table 2
Sensitivity test result of GBS isolates (n=10)

Antibiotic	% Sensitivity (n)		
	Sensitive	Intermediate	Resistant
Penicillin	90% (9)	0% (0)	10% (1)
Augmentin®	90% (9)	0% (0)	10% (1)
Cefazolin	80% (8)	0% (0)	20% (2)
Imipenem	90% (9)	0% (0)	10% (1)
Amoxycillin	90% (9)	0% (0)	10% (1)
Cotrimoxazole	60% (6)	0% (0)	20% (2)
Chlorampenicol	100% (10)	0% (0)	0% (0)
Erythromycin	70% (7)	10% (1)	20% (2)
Ceftriaxone	100% (10)	0% (0)	0% (0)
Ampicillin	80% (8)	0% (0)	20% (2)

DISCUSSION

In the present study, we succesfully isolated 32 GBS isolates using a combination of BA+CA enriched by TH broth. In addition, the sensitivity pattern of these isolates were mapped as described in Table 2.

The prevalence of GBS found in the present study was in accordance with the prevalence of GBS colonization in pregnant women in the USA (30%) and the research conducted by the CDC (10–30%). Without chemoprophylaxis, 50% of babies born would get GBS colonization and 1–2% of them will undergo invasive diseases. Pregnant women were severe colonized with GBS which is characterized by positive culture by using BA and CA without TH had baby with higher risk to develop early-onset neonatal sepsis.[3,4] In addition, these result were also consisted with previous studies conducted by Nomura *et al*. in Brazil. They compared between BA agar plates that was not previously incubated in TH medium mixed with gentamycin and nalidixic acid, and BA media with previously incubated in TH medium with antibiotics. The prevalence of GBS with TH medium mixed with antibiotic was 24.1% (49/203 specimens), whereas positive GBS culture result in media which was not incubated on TH with antibiotic was 16.7% (34/203 specimens). Improvement in GBS detection capabilities of TH selective medium can occur because this medium can optimalize GBS isolation and suppress the growth of other contaminant organism. The addition of gentamycin and nalidixic acid on research conducted by Nomura *et al*. aims to suppress the growth of Gram negative bacteria that can increase sensitivity of culture.[6,7]

Penicillin resistance rate in this study did not match with the CDC study, which did not find any resistance to penicillin. The difference of this situation might be caused by widely used of antibiotics, especially beta-lactams, in Indonesia. Resistance rate of GBS to erytromycin in this study was comparable to the prevalence of erytromycin resistance in the USA and Canada which was about 7–25%.[3]

Since the number of samples were not large enough, the sensitivity test results obtained in this study could not reflect the real situation yet, therefore it is needed to interpret it carefully. However, these findings might be used as preliminary data for the next study using large numbers of samples. Moreover, it provides that TH enrichment medium could be a promising method in screening of GBS among pregnant women.

CONCLUSION

In this present study, we observed that there was an enrichment detection of GBS prevalence colonization in pregnant women detected the swab with culture method using BA and CA enriched by TH compare to BA and CA without TH broth. Moreover, GBS showed resistance to penicillin, erythromycin, and cefazolin. It is indicated that TH enrichment medium seems to be promising as a screening method for GBS colonization in pregnant women in Bali.

REFERENCES

1. Eschenbach, D. A. 2002. Prevention of Neonatal Group-B Streptococcal Infection. N. Engl J Med vol 347 (4).
2. Schrag, S., Gorwitz, R., Fultz-Butts, K., and Schuchat, A. 2002. Prevention of Perinatal Group-B Streptococcal Disease. MMWR 2002/51 (RR11); 1-22.
3. Gilson, G. J., Christensen, F., Romero, H., Bekes, R., Silva, L., and Qualls, C. R. 2002.

Prevention of Group B Streptococcus Early-onset Neonatal Sepsis : Comparison of the Center of Disease Control and Prevention Screening-Based protocol to a Risk-Based Protocol in Infants at Greater that 37 weeks' Gestation. Journal of Perinatology; 20;491-495.

4. Baltimore, R. S., Huie, S. M., Meek, J. I., Schuchat, A., and O'Brien, K. 2001. Early-onset Neonatal Sepsis in the Era of Group B Streptococcal Prevention. Pediatrics; 108; 1094-1098.
5. Gupta, C., and Briski, L. E. 2004. Comparison of Two culture Media and Three Sampling techniques for Sensitive and Rapid Screening of Vaginal Colonization by Group B Streptococcus in Pregnant Women. Journal of Clinical Microbiology. Vol 42(9); 3975-3977.
6. Nomura, M. L., Junior, R. P., and Oliveira, U. M. 2006. Selective versus Non-selective culture medium for Group B Streptococcus Detection in Pregnancies Complicated by Preterm labor or preterm-Premature Rupture of Membrane. The Brazilian Journal of Infectious Diseases. 10(4); 247-250.

GROUP-B STREPTOCOCCUS IN PREGNANT WOMEN: Prevalence of Colonization and Sensitivity Pattern in Denpasar during June 2007-May 2008

ORIGINALITY REPORT

14%	6%	13%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.library.nhs.uk Internet Source	2%
2	"Posters", Clinical Microbiology and Infection, 5/2008 Publication	1%
3	Nasri, Khadijeh; Chehrei, Ali and Manavi, Mahdokht Sadat. "Evaluation of vaginal group B streptococcal culture results after digital vaginal examination and its pattern of antibiotic resistance in pregnant women", Iranian Journal of Reproductive Medicine, 2013. Publication	1%
4	www.ncbi.nlm.nih.gov Internet Source	1%
5	A. Adler. "Culture-based methods for detection and identification of Streptococcus agalactiae in pregnant women—what are we missing?", European Journal of Clinical Microbiology & Infectious Diseases, 03/2008 Publication	1%

6	Zakaria El Astal. "Increasing Ciprofloxacin Resistance Among Prevalent Urinary Tract Bacterial Isolates in Gaza Strip, Palestine", Journal of Biomedicine and Biotechnology, 2005 Publication	1%
7	Submitted to Massey University Student Paper	1%
8	Barcaite, E.. "Group B streptococcus and Escherichia coli colonization in pregnant women and neonates in Lithuania", International Journal of Gynecology and Obstetrics, 201204 Publication	1%
9	Jonas, J.B. Budde, W.M.. "Is the nasal optic disc sector important for morphometric glaucoma diagnosis? (Scientific Correspond", British Journal of Ophthalmology, Nov 2002 Issue Publication	<1%
10	Surya, I.G.P.. "Prediction of intrauterine HBV infection transmission", International Hepatology Communications, 199503 Publication	<1%
11	Tantoro Harmono M. "GASTRODUODENITIS, ULCER AND HELICOBACTER PYLORI IN SURAKARTA", Journal of Gastroenterology and Hepatology, 12/2000 Publication	<1%

12	R. Gilbert. "Prenatal screening for group B streptococcal infection: gaps in the evidence", International Journal of Epidemiology, 02/01/2004 Publication	<1%
13	www.balimedicaljournal.com Internet Source	<1%
14	Gary Sutkin. "Antibiotic Prophylaxis and Non–Group B Streptococcal Neonatal Sepsis :", Obstetrics and Gynecology, 03/2005 Publication	<1%
15	"The 21st Conference of the Asian Pacific Association for the Study of the Liver : Oral Presentations 17 February, 2011 (Thursday)", Hepatology International, 03/2011 Publication	<1%
16	Benitz, W.E.. "Perinatal treatment to prevent early onset group B streptococcal sepsis", Seminars in Neonatology, 200208 Publication	<1%
17	www.cdc.gov Internet Source	<1%
18	F. Rallu. "Sensitivities of Antigen Detection and PCR Assays Greatly Increased Compared to That of the Standard Culture Method for Screening for Group B Streptococcus Carriage in Pregnant Women", Journal of Clinical Microbiology, 03/01/2006	<1%

Publication

19	T. H Williamson. "A (quote)throttle(quote) mechanism in the central retinal vein in the region of the lamina cribrosa", British Journal of Ophthalmology, 9/1/2007 Publication	<1%
20	Pierre Kuhn. "Incidence and distribution of pathogens in early-onset neonatal sepsis in the era of antenatal antibiotics : Distribution of early-onset neonatal sepsis", Paediatric and Perinatal Epidemiology, 06/2010 Publication	<1%
21	"The 20th Conference of the Asian Pacific Association for the Study of the Liver Poster Presentation", Hepatology International, 03/2010 Publication	<1%
22	Khosravi, Nastaran; Noorbakhsh, Samileh; Tabatabaei, Azardokht and Ghavami, Yaser. "Prevalence of streptococcus group B in tracheal tube secretions of neonates with respiratory distress: a brief report", Tehran University Medical Journal, 2013. Publication	<1%
23	R POLIN. "Bacterial Sepsis and Meningitis", Avery s Diseases of the Newborn, 1994 Publication	<1%
24	Shirazi, Mahboobeh; Abbariki, Ezat; Hafizi, Ali; Shahbazi, Fatemeh; Bandari, Mozhgan and	<1%

Dastgerdy, Ebrahim. "The Prevalence of Group B Streptococcus Colonization in Iranian Pregnant Women and Its Subsequent Outcome", International Journal of Fertility & Sterility, 2014.
Publication

---

25 F. Jaureguy. "Effects of Intrapartum Penicillin Prophylaxis on Intestinal Bacterial Colonization in Infants", Journal of Clinical Microbiology, 11/01/2004 <1%
Publication

---

26 Ronald S. Gibbs. "Perinatal Infections Due to Group B Streptococci :", Obstetrics and Gynecology, 11/2004 <1%
Publication

EXCLUDE QUOTES	OFF	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	ON		