Detection of genital colonization of group B streptococci during late pregnancy

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ABSTRACT

Objective: To detect group B streptococcal carrier state of Saudi females during 3rd trimester of pregnancy and to assess type of specimens and the techniques used for the organism detection.

Methods: A total of 867 consecutive vaginal and rectal swabs were obtained from 217 pregnant women at ≥28 weeks of gestation and their follow up testing from King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia. Swab-specimens were cultured comparatively on Islam and Edwards blood agar plates, and into selective Lim broth. Enrichment Lim broth cultures (≥12 hours) with and without positive modified coagglutination test were then subcultured on Islam and Edwards sheep blood agar plates. Presumptive colonies were then tested for group B streptococcus identity by convential biochemical reactions, serogrouping and serotyping. Collected neonatal swab-specimens (184) were also treated similarly.

Results: In comparison to Lim broth enrichment culture, the direct swab specimen culture on Edwards blood agar or Islam agar plates technique revealed 84% sensitivity and 100% specificity, whereas modified coagglutination test after selective Lim broth enrichment revealed 100% sensitivity and 96% specificity. Group B streptococcus was isolated in at least one of the specimens from the 217 patients in 66 cases. Of these 66 cases, group B streptococcus was isolated from both vaginal and rectal swabs in 33 (50%) cases and only from vaginal swabs in 22 (33%) and rectal swabs in 11 (17%) cases. Of the group B streptococcus positive cases, 10 (15%) cases had spontaneously lost their carriage, upon follow up testing, whereas out of the 151 negative cases, 4 (2.6%) cases became positive for group B streptococcus colonization upon follow up testing with an overall carriage rate of (60/217) 27.6%. Certain demographic factors were found to alter such rate of carriage. Additionally, 50% of group B streptococcal colonized mothers vertically transmitted the homologous serotypes of the organism to their newborns, but clinical infection was not recorded during the study period.

Conclusions: Group B streptococci colonization rate among term Saudi pregnant women is relatively high (27.6%); and thereby constitutes a group of women whose infants are at great risk of early-onset invasive disease. The modified coagglutination test after growth amplification seems rapid and cost-effective to detect lightly or heavily group B streptococcal colonized women. Vaginal and rectal swab specimens at late pregnancy appeared necessary to accurately identify group B streptococcus maternal colonization.

Keywords: Group B streptococci, maternal colonization, demographic factors, neonatal-transmission, prenatal screening, detection-techniques.

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Despite recent advances in prenatal care, Group B Streptococcus (GBS) still remains a leading cause of early and late-onset neonatal sepsis. It also causes significant maternal morbidity.[1] The overall cost of GBS related infections in the United States of America (USA) were estimated to be U$ 727 million annually.[2] Group B Streptococcus is transmitted vertically in up to 70% of infants born to colonized woman, subsequently sepsis develops in 1-2% of these infants. Early–onset GBS disease (septicemia,

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pneumonia, or meningitis occurring within 7 days of life) has a mortality rate of up to 50%, with permanent neurologic sequelae occurring in 15% to 50% of infants surviving the meningeal infection, thus emphasizing the importance of GBS early identification and treatment.[1-6] Sepsis caused by GBS occurs in 2 to 4 of every one thousand newborns, with a 10-fold increase in incidence when there is maternal colonization and up to 25-fold increase, in combination with other risk factors.[5] Prolonged delivery after spontaneous rupture of the membranes (SROM), fever during labor, and premature deliveries are the most important additional risk factors.[6-7] Several investigators have investigated the incidence of GBS-maternal colonization worldwide, it was generally found to be between 5% to 35% of gravidas.[1-3] In the Kingdom of Saudi Arabia (KSA), however, there is scarce information in the literature regarding GBS carriage, apart from a communication reported by Agius et al,[8] who found GBS in only 3% of term pregnant women and in only one percent of newborn infants. In a recent report[9] from our laboratory, we have described antibiotic-susceptibility of GBS clinical isolates, their serotypes, genetic-basis of tetracycline resistance and prevalence of penicillin tolerance among studied strains. We now describe GBS-colonization rate in Saudi pregnant women at ≥28 weeks of gestation in the Riyadh region, and to assess the type of specimens and techniques used for GBS detection and the demographic factors affecting the carriage.

Methods. Pregnant women (≥28 weeks) attending Obstetrics and Gynecology clinics at King Khalid University Hospital (KKUH), Riyadh, KSA were recently recruited for the study. A history sheet was completed where data pertaining to personal and demographic factors-data were recorded. The women who had received antibiotics in the present pregnancy were excluded. Informed written consent was obtained from all qualified patients in accordance with institutional review board guidelines. Vaginal and rectal swabs (Becton Dickinson Microbiology Systems, Cockeysville, MD) were collected initially at around ≥ 28 weeks and also twice thereafter at 32-36 weeks of gestation for follow up. Neonatal-throat, nose, ear, eye, rectal and umbilical swabs were also collected for GBS cultures within 30 minutes of birth, based on the availability of one of the co-investigators for processing of laboratory specimens. From 217 consecutive pregnant women and 56 examined newborns, a total of 867 and 184 swab-specimens were collected (**Table 1**). Upon receipt of specimens in the laboratory, swab specimens (**Figure 1**) were directly streaked on Islam (Oxoid), and Edwards (Oxoid) sheep blood agar plates, then dipped and broken in Todd-Hewitt (TH, Oxoid, UK) broth to which 10 ug/ml colistin, 15 ug/ml nalidixic acid, and one percent yeast extract were added (Lim Broth). The Islam-medium plates were incubated anaerobically and the blood agar plates aerobically at

Table 1 - Group B streptococcal colonization status in 217 pregnant women and their 56 randomly examined neonates.

Subject	N of GBS carriage (%)		
	Positive	Negative	Total
Pregnant women ≥ 28 weeks	66 (30)	151 (70)	217
Newborns of GBS positive mothers	6 (50)	6 (50)	12
Newborns of GBS negative and false positive mothers	0.0 (0.0)	43+1 (100)	44
GBS positive pregnant women later turned negative	56 (85)	10 (15)	66
GBS negative pregnant women later turned positive	4 (2.6)	147 (97.4)	151
Overall GBS women carriage during late pregnancy	60 (27.6)	157 (72.4)	217

GBS - group B streptococcus; N - number

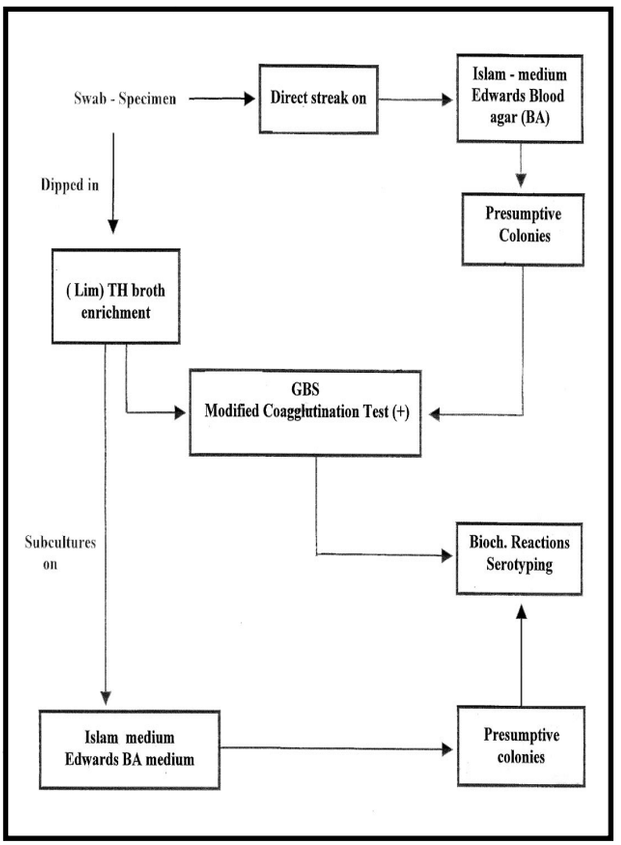

Figure 1 - Protocol for group B streptococcus (GBS) detection in swab specimens. TH - Todd-Hewitt.

Table 2 - Number and percentages of GBS type of specimen in 217 pregnant women and their follow up.

Type of Specimen	N	GBS Isolated (%)
Primary specimens (28-30 wks)		
High vaginal swabs	212	47 (22)
Low vaginal swabs	149	31 (21)
Rectal swabs	203	45 (22)
2nd specimens (30-32 wks)		
High vaginal swabs	94	24 (25)
Low vaginal swabs	67	14 (21)
Rectal swabs	91	19 (21)
3rd specimens (>34 wks)		
High vaginal swabs	20	2 (10)
Low vaginal swabs	11	1 (9)
Rectal swabs	20	3 (15)
Total	**867**	**186 (22)**
Newborn - specimens (rectal, nose, ear, eye, and umbilical swabs	184	12 (7)

GBS - group B Streptococcus, N- number, wks - weeks

37^{0}C overnight. Enrichment Lim broths were also incubated overnight at the same temperature prior to direct latex coagglutination test (Phadebact Streptococcus test, Boule Diagnostic, AB, Huddings, Sweden) rather than testing on pure isolates. For comparison, Edwards sheep blood agar plates and Islam agar plates were inoculated with the same Lim broth cultures. Following overnight incubation, the plates were examined for characteristic large beta hemolytic colonies or pigmented ones on respective used media. All presumptive colonies were confirmed as GBS by convential biochemical reactions, serogrouping, and serotyping as previously described.[9] The obtained results constitute the established GBS isolates of Lim broth culture enrichment procedure.[1]

Results. Of the 867 swab specimens collected from the 217 pregnant women, GBS was isolated from at least one of the specimens from these patients in 66 cases, with an overall colonization rate of 27.6% (**Tables 1 & 2**). Group B Streptococcus was isolated from both vaginal and rectal swabs in 33 (50%) cases and only from vaginal swabs in 22 (33%) and rectal swabs in 11 (17%) cases. Group B streptococcus isolation rate did not seem to vary significantly among high and low vaginal swab specimens (**Table 2**). Of these 66 cases, 10 (15%) cases had spontaneously lost their GBS carriage status, upon their follow up testing. In comparison, only 4 (2.6%) cases out of 151 cases became positive for GBS colonization upon follow up testing (**Table 1**). Of the 184 swab specimens from the examined 56 newborns, GBS was isolated from 6 (11%) neonates whose mothers were positive for GBS; though 6 GBS positive mothers failed to transmit the organism to their newborns. Meanwhile, GBS was not detected in any of the specimens collected from 43 newborns whose mothers were negative for GBS colonization (**Tables 1 & 2**). A specimen of one infant (1/56) revealed false-positive test for GBS as did the mother, but the organism was not isolated by standard enrichment culture procedure from either mother or her baby. When taken by specimen, swabs of external ear (57), throat (30), eye (30), and umbilical (49) yielded 11%, 7%, 7%, and 2% positive GBS.

Table 3 - Results of 867 specimens of 217 pregnant women for GBS screening techniques and their sensitivity and specificity.

Screening test	Established GBS N after overnight Lim broth enrichment			(%)	
	Positive	**Negative**	**Total**	***Sensitivity**	****Specificity**
Direct culture on Islam Medium or Edwards blood Agar					
Positive	157 (TP)	00 (FP)	157		
Negative	28 (FN)	682 (TN)	710		
Total	**185**	**682**	**867**	**(84)**	**(100)**
Modified coagglutination test with overnight Lim broth enrichment					
Positive	185 (TP)	29 (FP)	214		
Negative	00 (FN)	653 (TN)	653		
Total	**185**	**682**	**867**	**(100)**	**(96)**

GBS - group B streptococcus, N - number, *Sensitivity - number of true positive/ true positive + false negative x 100, **Specificity - number of true negative/ false positive + true negative x 100, TP - true positive, TN - true negative, FP - false positive, FN - false negative

Table 4 - Group B streptococcal carriage by demographic factors.

Characteristics	N	(%) Positive	P Value
Nationality			
Saudi	190	(27)	0.19
Non Saudi	27	(16)	
Age			
20-24	64	(25)	
25-30	72	(22)	0.01*
31-35	43	(38)	
35+	38	(25)	
Parity			
0	44	(27)	
1-2	61	(16)	0.004*
3-5	76	(13)	
5+	36	(00)	
Length of Marriage			
0-10	151	(28)	
11-20	60	(17)	0.12
21+	6	(0)	
N of Abortion			
None	104	(22)	
1-2	98	(9)	0.10
3-4	10	(29)	
5+	5	(0)	
History of SROM			
Yes	6	(40)	0.26
No	211	(22)	
Vaginal Discharge			
Yes	101	(26)	0.004*
No	116	(18)	
Socio-Economic Status			
Poor	41	(35)	0.17
Good	176	(18)	
Rhesus factor			
Rh-negative	18	(44)	0.24
Rh-positive	199	(24)	
Milk and cheese ingestion			
Nil to rarely	70	(37)	0.09
moderate to exessive	147	(24)	

N - number, SROM - Spontaneous rupture of the membranes, * significant P value < 0.05, Rh -Rhesus,

Group B Streptococcus was not detected in any of neonatal nose (14) or rectal (4) swabs.

In this study as compared with specimen culture enrichment, 2 techniques were used for the detection of GBS, direct specimen culture on Edward blood agar and Islam agar medium revealed 84% sensitivity, with 100% specificity (**Table 3**). The 2nd technique is a modified coagglutination test which was directly used to detect GBS on mixed enriched culture rather than on pure isolates which revealed high sensitivity of 100% and 96% specificity (**Table 3**). **Table 4** presents the effect of different clinical and demographic factors that may alter GBS colonization. The Saudi women apparently have a higher carrier rate compared to non-Saudi women. Multiparity appears to be protective against GBS carriage (P<0.004). This holds also true for increased years of marriage, good socio-economic status, and moderate to excessive diet intake of milk and cheese. Conversely, advancing age (P<0.01), poor socio-economic status, Rhesus-negative status, and rare to no milk and cheese diet intake were apparently associated with increased risk of colonization. Isolation of GBS was also apparently associated with a history of spontaneous rupture of membranes (SROM), and vaginal discharge (P<0.004), but it was apparently not associated with a history of repeated spontaneous miscarriages (**Table 4**).

Discussion. The present study revealed an overall GBS colonization rate of 27.6% of pregnant women at ≥ 28 weeks of gestation. This result is consistent with those of Yancey et al,[10] Easmon et al[11] and Sunna et al[12] who found carriage rates of 26%,

28% and 30% in USA, England and Jordan. However, in a survey in Israel on pre-term Israeli and Arabic pregnant women, the colonization rates were 5.4% and 1.6%.[13] A similar study in France revealed a rate of 11%; whereas in 3 hospitals in Italy[14] 7.5% of mothers and 4.9% of babies were found to be colonized. Of interest, in Asir province, KSA, Agius et al,[8] found 3% rate of term pregnant women and only one percent of newborns. This discrepancy is most likely explained by our use of Lim broth culture enrichment as the standard method instead of only direct plating on blood agar. Nevertheless, the authors attributed their observed lower rate, to differences in age, parity, contraception methods, sexual mores, diet and climate. In Jordan, Sunna et al[12] reported that these demographic factors did not seem to influence the rate of GBS-colonization. Our study evaluated these factors, and showed no significant difference in GBS-prevalence among Saudi and non-Saudi women. Women of Caribbean origin and black women, however, were previously reported to be at greater risk of colonization than those of Mexican origin and white women.[15] Our results also seem to support previous findings[3] that multi-parity, increased years of marriage, and good socio-economic status appear to be protective against GBS-carriage. The observed protective effect of rich milk and cheese diet might be attributed to the enrichment of lactobacilli-flora in the vagina and thereby would suppress GBS-colonization at least in terms of economy and surface area competition. That advancing age and poor socio-economic status are associated with increased risk of GBS-colonization,[3] has also been confirmed in this study. Our results also confirm previous findings that GBS-colonization is apparently associated with a history of spontaneous rupture of membranes, Rhesus-negative status, and vaginal discharge. No correlation however, was observed with a history of repeated spontaneous miscarriages. Moller et al[16] and Yancey et al[10] found an increase in the incidence of premature rupture of the membranes and preterm labor with GBS carriage, while Maniats et al[17] demonstrated that GBS is highly associated with vaginal discharge and should be considered as a vaginal pathogen. The considerations of these risk factors would have significant impact on both medical management and antibiotic therapy of pregnant women during labor and their newborns, since intrapartum antibiotic prophylaxis (IAP) of GBS-positive women after the onset of labor or membrane rupture but before delivery, has been shown[1,5,10] to decrease neonatal colonization and early onset GBS disease (EOGBSD). The importance of rectal cultures in defining maternal GBS carriage is well recognized. Our finding of a rectal to vaginal carriage ratio of 0.80 to 1.0 confirms previous reports[6,18] that not only does rectal carriage serve as a reservoir of organisms for maternal genital colonization, but it may also serve as a source for neonatal colonization. These findings support the concept that both rectal and vaginal swab-specimens should be examined to accurately identify GBS-women carriage.[5] The fact that 85% and 2.6% of the studied patients retain or acquired GBS carriage late in pregnancy, emphasized the belief that antepartum surveillance programs remote from delivery are inadequately predictive of maternal colonization at delivery.[1-5] Hence the recent approach of the American Academy of Pediatrics (AAP)[19] to prevent EOGBSD with IAP now requires prenatal screening at 35 to 37 weeks of gestation, but not at 26 to 28 weeks as previously suggested[20] in 1992. Clearly in women who deliver before term, screening would be missed and preterm infants are at greater risk of EOGBSD. This explains why the recent AAP approach[19] is not only based on GBS cultures but also combined with other risk factors.[5,19] Our study did not permit the follow-up of all parturient newborn infants, since our emphasis was on prenatal GBS carriage. Nevertheless, of the examined 56 newborns, GBS was not detected in any of multiple site specimens from 43 newborns whose mothers were negative for GBS colonization as expected. Whereas specimens of one newborn revealed false-positive GBS carriage as did those of the mother. Although limited in their number, our results revealed that 50% of GBS colonized mothers vertically transmitted the homologous serotypes of the organism to their newborns. In a similar study, Boyer et al[18] found 17% to 65% transmissions depending on the density of maternal colonization. There was no attempt to evaluate the clinical impact of this study neither was routine screening policy for GBS in pregnant women in effect during the study, but retrospective data revealed its major cause of neonatal sepsis primarily among preterm deliveries (27-36 weeks), twins, and SROM-cases (unpublished data).

The recommendations of the AAP[19] to address the problem of GBS infection emphasized culture techniques, which maximize the likelihood of GBS recovery. According to Kircher et al[1] this includes a single swab or 2 separate vaginal and rectal swabs which are inoculated into selective broth medium and incubated for 18-24 hours; followed by growth subculture onto sheep blood agar plate. The selective broth culture presumably improves the sensitivity of the technique and thereby detects more readily lightly colonized mothers. Obviously, heavily colonized women are at higher risk of transmitting GBS to their newborns but several reports have also demonstrated that sepsis can develop in infants[1,10,18] born to lightly colonized mothers. Although the selective broth enrichment culture is quite sensitive, it has an average turnaround time of 36 to 72 hours.[1] Hence several rapid tests for GBS-colonization based on antigen detection have been attempted.[1,5,21,22] Although these tests were highly sensitive for the detection of heavily colonized women, they require at least 5-8 hour growth amplification before testing

to accurately identify lightly colonized women.[5,6,21] In general, as the time of broth enrichment increases the sensitivity of the antigen-detection test parallel increases. Thus a recently introduced deoxyribonucleic acid (DNA) probe based system[1,21] detected only 71%-73% of GBS positive women after broth enrichment for 4 hours, but sensitivity reaches 100% (as did our modified coagglutination test) after 24 hours enrichment. Also Tuppurainen and Hallman,[22] found that the sensitivities of streptolatex test were 70% and 93% with specimen broth enrichment for 3 and 24 hours. To eliminate false positive results, the authors re-tested all positive samples on previously boiled broth, and found that only 26% of culture positive vaginal specimens were streptolatex positive. In comparison, our modified coagglutination test revealed only 4% of specimens (29/682) that gave false positive tests; mostly from rectal specimens, specifically due to *Enterobacter cloacae*. Following the manufacturer's instructions we did not boil the broth before testing, doing so, may further have improved the specificity of test (96%). The results presented here illustrate the usefulness of modifying a commercially available test kit, for detecting GBS from mixed culture rather than purified isolates which take longer to produce and are less economic. With further modifications to shorten the time of broth enrichment, the test may well prove useful, comparatively rapid, and cost-effective for the implementation of a routine GBS screening programme,[5] among Saudi pregnant women in view of observed high carriage rate and the annual cost of EOGBSD. The proper identification of a woman's GBS colonization status would be useful in selecting pregnant women who should receive chemoprophylaxis during labor. This strategy, combined with other risk factors[19] has been estimated to prevent 86% of EOGBSD,[1,5,23] therefore it is highly recommended for wide implementation across the Kingdom.

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