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Research Article

Comparing Urine Samples with Vaginal Swabs in Detecting *Streptococcus Agalactiae* Carriage in Pregnant Women Using Chromogenic Media

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Abstract

Background: Although Group B *Streptococcus* (GBS) is part of the normal microflora in the vagina of many women; it is well recognized as a human pathogen, especially for causing septicemia, meningitis and other serious invasive disease in neonates due to vertical transmission from mother during labor. The purpose of this study was to evaluate urine specimens in comparison to vaginal swabs as effective samples for screening GBS carriage among pregnant females using chromogenic media. Also to compare the sensitivity of chromogenic media; Granada agar (GRAN) and ChromID Strepto B (STRB) with that of a conventional medium; Tryptic soy agar with 5% sheep blood (TSA) in detecting GBS colonization in pregnant females.

Methods: Fifty females at 35 - 37 weeks of pregnancy were included in the study. One vaginal swab and one urine sample were taken from each female and they were inoculated onto selective Lim broth and incubated for 18 - 24 hours followed by subculture on TSA, GRAN and STRB. The plates were incubated for 24 hours and if culture was negative, incubation was extended to 48 hours. GBS growth confirmation was done using latex agglutination test.

Results: GBS was detected in 16 (32%) cases on both TSA and GRAN, whereas on STRB, GBS was detected in 19 (38%) cases in at least one sample type. There was no difference regarding the sample types in detecting GBS as 17 (34%) of the vaginal swabs and 17 (34%) of the urine samples showed positive cultures on at least one of the three media, and on each medium there was no statistically significant difference between vaginal swabs and urine samples in detecting GBS. The sensitivity of STRB, GRAN and TSA in detecting GBS in the vaginal swabs was 94%, 88% and 82%, while in the urine samples it was 100%, 76.5% and 76.5%, respectively.

Conclusions: Urine samples are as effective as the vaginal swabs in detecting GBS colonization in pregnant women. STRB was more sensitive than the other media in detecting GBS especially in the urine samples, while the sensitivity of GRAN was comparable to TSA.

Keywords: *Group B Streptococcus; Urine Samples; Vaginal Swabs; Chromogenic Media*

Abbreviations

GBS: Group B *Streptococcus*; GRAN: Granada agar; STRB: ChromID Strepto B; TSA :Tryptic soy agar with 5% sheep; (CDC): Centers for Disease Control and Prevention

Introduction

Streptococcus agalactiae, usually termed group B Streptococcus (GBS) is one of the most important causes of early-onset neonatal infection [1,2]. Approximately 10 – 30% of pregnant women are colonized with GBS in the vagina or rectum [3]. Centers for Disease Control and Prevention (CDC) guidelines, 2010, recommend screening vaginal or rectovaginal GBS colonization in pregnant women at 35 to 37 weeks of gestation. For pregnant women colonized by GBS, intrapartum administration of antibiotics is recommended to prevent GBS transmission to the newborn during delivery. As a result of prevention efforts, incidence of GBS has declined dramatically over the past 15 years, from 1.7 cases in the early 1990s to 0.37 cases in recent years per 1,000 live births [4].

Maternal GBS bacteriuria, including pure and predominant growth of GBS in the urine, has been associated with GBS colonization and an increased risk for early-onset disease in the newborn [5]. CDC guidelines recommend that women with GBS isolated from the urine at any time during the current pregnancy should receive intrapartum antibiotic prophylaxis and do not need third trimester screening for GBS colonization [4]. Urine samples are easier to collect than vaginal swabs, so if they are as effective as vaginal swabs in detecting GBS carriage in pregnant females, it would save effort.

According to CDC guidelines 2010 which has not been changed till now, inoculation into selective enrichment broth and further subculture onto solid media as TSA is the gold standard for GBS isolation [4]. One of the most important requirements for a screening protocol for GBS during pregnancy is the minimization of false negatives which, in this case, can produce a high risk of neonatal GBS infection. Blood agar is widely used around the world for the culture of hemolytic GBS, however, growth of two or more species of bacteria in rectovaginal samples can make detection of GBS difficult especially if those organisms produce large β-hemolytic zones. Also a greater level of experience is needed to identify GBS on blood agar from characteristics alone, increasing the possibility of variability in interpretation [6]. The performance of microbiological methods for GBS screening has been greatly improved by the use of selective chromogenic media which undergo color change in the presence of GBS [7]. STRB and GRAN media (bioMérieux, France) are two commercially available chromogenic media selective for this bacterium. GRAN is a selective differential chromogenic medium used to screen for GBS in pregnant women, on which GBS colonies appear orange, while other bacteria are either inhibited or form white colonies. GRAN is a good medium for detecting GBS in urine specimens [8]. STRB is another selective chromogenic agar that was developed to screen for GBS in pregnant women, but it has not been tested for urine samples yet. GBS colonies on it appear pink or red, while other bacteria are either inhibited or form blue, violet or colorless colonies [6].

This study aimed to compare urine samples with vaginal swabs in detecting GBS colonization in pregnant females and to compare the sensitivity of chromogenic media (GRAN and STRB) with that of a conventional medium (TSA) in detecting GBS colonization in pregnant females.

Subjects and Methods

This study was conducted on fifty pregnant women in the late third trimester, 35 - 37 weeks of gestation, coming for antenatal care at outpatient clinic of Obstetrics and Gynecology Department, Kasr El-Aini University Hospitals during the period from August to October 2015. An informed consent was taken from all participants. A full history was taken including name, age and obstetric history; as number of pregnancies and normal deliveries, history of previous preterm labour, previous premature rupture of membranes and previous neonatal sepsis. Females who had received any systemic or local vaginal antimicrobial agents during the preceding 2 weeks or those with any abnormal vaginal discharge or vaginal bleeding or having known or suspected urinary tract infection were excluded.

One lower vaginal swab and one mid-stream urine sample were collected from each female and they were inoculated onto Lim selective enrichment broth (bioMérieux, France) which were used as both transport and enrichment media [9] and incubated for 18 - 24 hours followed by subculture on TSA, GRAN and STRB. TSA was incubated at 37°C with 5-10% CO_2, while GRAN was incubated anaerobically using GENbox anaer (bioMérieux, France) at 37°C, whereas STRB was incubated aerobically at 37°C. The plates were incubated for 24 hours

and if culture was negative, incubation was extended to 48 hours.

Colonies on plates were identified by colony morphology (white colonies with moist or glistening features and a narrow zone of β-hemolysis or no hemolysis on TSA / orange-coloured colonies on GRAN / pale pink to red colonies on STRB) figures (1-4), gram stain (gram positive cocci arranged in short chains or pairs), catalase test (catalase negative) and finally confirmed to be GBS by latex agglutination test using Slidex Strepto Plus B Kit (bioMérieux, France).

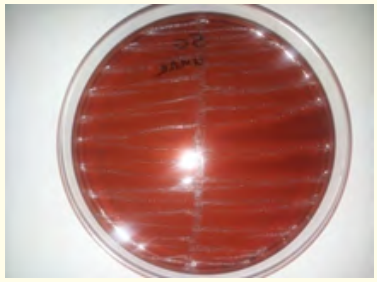

Figure 1: *GBS growth on TSA in a urine sample showing β-hemolysis.*

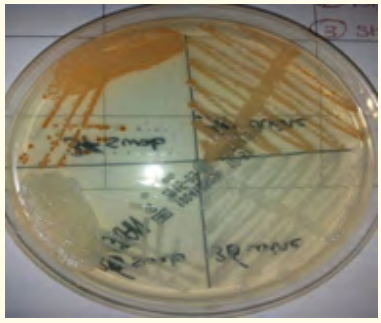

Figure 2: *Positive (orange colonies) and negative (whitish colonies) result on GRAN.*

Figure 3: *A positive result (red colonies) on STRB.*

Figure 4: *A Negative result (blue colonies) on STRB.*

Statistical Analysis

Data were statistically analyzed by using the Statistical Package of Social Science (SPSS) software program, version 21. Data were summarized using mean and standard deviation for quantitative variables and frequency and percentage for qualitative ones. The data recorded were statistically analyzed by using z-score test for two sample proportions to know whether two populations or groups differ significantly. P-values less than 0.05 were considered statistically significant and less than 0.01 were considered highly significant. The Evaluation of screening tests validity was conducted through measurements of sensitivity, specificity, positive and negative predictive values.

Results and Discussion

Prevalence of GBS colonization among the studied group

Over the duration of the study, 19 (38%) out of the 50 examined pregnant women were found to be colonized by GBS in either the genital tract, the urinary tract or both. Fifteen cases showed positive culture of GBS in both the vaginal swabs and urine samples, while 2 cases showed positive culture only in the vaginal swabs and 2 cases showed positive culture only in the urine samples figure 5.

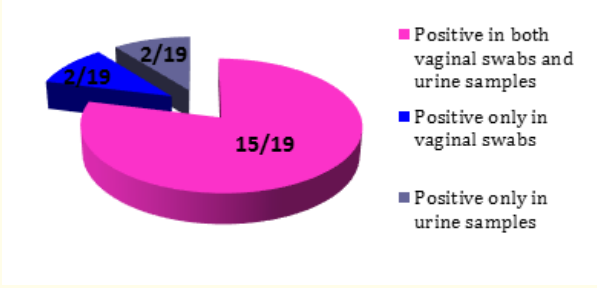

Figure 5: *Distribution of GBS-positive cases among the sample types.*

There was no difference regarding the sample types in detecting GBS as 17 (34%) of the vaginal swabs and 17 (34%) of the urine samples showed positive cultures on at least one of the three media, and on each medium there was no statistically significant difference between vaginal swabs and urine samples in detecting GBS.

GBS colonization in relation to participants characteristics

There was no statistically significant difference between positive and negative cases for GBS colonization as regards the age, the parity, the occurrence of neonatal sepsis, stillbirth and PROM in previous pregnancies, while there was statistically significant difference between positive and negative cases as regards preterm labor and abortion as shown in tables 1 and 2.

Age group (years)	GBS-positive cases		GBS-negative cases		*P*-value*
	N =19	(100%)	N = 31	(100%)	
15 - < 25 (21)	10	(52.6%)	11	(35.4%)	0.23
25 - < 35 (24)	6	(31.5%)	18	(58%)	0.06
35 - < 45 (5)	3	(15.7%)	2	(6.4%)	0.28
No. of deliveries					
Nullipara (11)	6	(31.6%)	5	(16%)	0.2
Para1& 2 (28)	9	(47.4%)	19	(61%)	0.33
Para ≥ 3 (11)	4	(21%)	7	(22.5%)	0.89

Table 1: *GBS colonization in relation to different age groups and parity.*
**P-value ≤ 0.05 is considered significant.*

	GBS-positive cases		GBS-negative cases		P-value
	N = 19	(100%)	N = 31	(100%)	
Neonatal sepsis (1)	0	(0%)	1	(3.2%)	0.4
Stillbirth (3)	2	(10.5%)	1	(3.2%)	0.29
PROM (4)	2	(10.5%)	2	(6.5%)	0.6
Preterm labor (3)	3	(15.8%)	0	(0%)	0.02
Abortion (5)	4	(21%)	1	(3.2%)	0.04

Table 2: *GBS colonization in relation to neonatal sepsis, PROM, preterm labor, abortion and stillbirth.*

Results of culture for GBS

GBS was detected in 16 (32%) cases on both TSA and GRAN, whereas on STRB, GBS was detected in 19 (38%) cases in at least one sample type (Table 3).

Medium	Sample	True +ve	False +ve	False -ve	True -ve	Sensitivity*	Specificity	PPV**	NPV***
STRB	Vaginal swabs	16	5	1	33	94	86.8	76	97
	Urine samples	17	3	0	33	100	91.6	85	100
GRAN	Vaginal swabs	15	0	2	33	88	100	100	94
	Urine samples	13	0	4	33	76.5	100	100	89
TSA	Vaginal swabs	14	0	3	33	82	100	100	91
	Urine samples	13	0	4	33	76.5	100	100	89

Table 3: *Characterization of the vaginal swabs' and urine samples' testing results by the 3 different media.*

**Sensitivities were calculated by comparing the proportion of positive samples for each medium in relation to composite positives for all test media (n = 17) confirmed by latex agglutination test.*

*PPV**= Positive predictive value*

*NPV*** = Negative predictive value*

Early-onset infections by GBS as pneumonia, sepsis and meningitis are acquired vertically through exposure to this organism from the vagina of a colonized woman [10]. In the absence of a licensed GBS vaccine, universal screening and intrapartum antibiotic prophylaxis continue to be the cornerstones of Early-onset GBS disease prevention [4].

In the present study, 19 (38%) out of the 50 examined pregnant women were found to be colonized by GBS in either the genital tract, the urinary tract or both. For the detection of GBS colonization among normal Egyptian women during the childbearing period, other studies were done using vaginal swabbing. El-Shimy [11] detected GBS in 13% of cases using sheep blood agar, while Rashwan [12] detected GBS in 25% of cases using TSA. The difference in GBS carriage rate between the previous studies done in Egypt and the present one may be because of using different media for GBS isolation after broth enrichment or it may be due to an actual increase in the GBS carriage rate among the normal healthy Egyptian women. Also, these studies detected colonization only in the vaginal swabs, while the present one detected colonization in the vaginal swabs and urine samples.

In this study, 15 out of the 19 cases showed positive culture of GBS in both the vaginal swabs and urine samples, while 2/19 cases showed positive culture only in the vaginal swabs and 2/19 cases showed positive culture only in the urine samples. In the study conducted by Wali., *et al*. [13], GBS was detected in the vaginal swabs and urine specimens of 21 (15.67%) and 11 (7.4%) females, respectively, so there were 10 cases detected in the vaginal swabs and missed in the urine samples, however, all the cases that were detected in the urine samples were detected in the vaginal swabs. In the present study, the reason for the 2 cases that were positive in the vaginal swabs and missed in the urine samples may be the light colonization in the vagina. On the other hand, the 2 cases that were positive in the urine samples and missed in the vaginal swabs did not have any symptoms of urinary tract infection, so they are thought to be colonized by GBS. These 2 cases may have been colonized before by GBS in the vagina as GBS colonization can be transient and can change over the course of a pregnancy [4]. In addition, the presence of GBS as a colonizer of the gastrointestinal and genitourinary tracts does not necessarily mean its presence in these sites at the same time, so it may be found in the gastrointestinal, genital or urinary tracts.

In the present study, 17 (34%) of the vaginal swabs and 17 (34%) of the urine samples showed positive cultures on at least one of the three media with no difference regarding the sample types in detecting GBS. On each medium, there was no statistically significant difference between vaginal swabs and urine samples in detecting GBS as STRB, GRAN and TSA detected GBS in 16 (32%), 15 (30%) and 14 (28%) cases in the vaginal swabs versus 17 (34%), 13 (26%) and 13 (26%) cases in the urine samples, respectively. A study conducted by Wali., *et al*. [13] aimed at evaluating urine specimens in comparison to vaginal swabs as possibly reliable samples for screening GBS carriage among Egyptian pregnant females using direct plating on sheep blood agar, direct antigen detection from Lim broth and subculture from Lim broth onto sheep blood agar. The study showed that GBS was detected in the vaginal swabs and urine specimens of 21/134 (15.67%) and 11/134 (7.4%) females, respectively. So, GBS could not be detected in the urine of about half the cases tested positive using vaginal specimens. The difference in GBS carriage rate and yield of GBS from the urine samples between the previous study and the present one may be because of using more selective media for GBS isolation after broth enrichment or it may be due to an actual increase in the GBS carriage rate among the normal healthy Egyptian women.

In the present study, no significant difference was found between positive and negative cases for GBS colonization in different age groups. This was similar to the results of Rashwan [12] who found that there was statistically insignificant difference between age of positive and negative cases.

In the current study, no significant difference was found in the carriage rate of GBS between cases of high parity and cases of low parity. This finding is similar to that of Atkins., *et al*. [14], Costa., *et al*. [15], Dechen., *et al*. [16] and Rashwan [12] who found that the relation between parity and GBS culture positivity was statistically insignificant.

In the present study, there was statistically significant difference between positive and negative cases as regards preterm labor and abortion, while there was no statistically significant difference as regards the occurrence of neonatal sepsis, PROM and stillbirth in previous pregnancies. In partial agreement with our results Jahromi., *et al*. [17] found that preterm birth and PROM were more common among GBS colonized mothers. Dechen., *et al*. [16] also reported that GBS infection among pregnant women was significantly related with PROM and preterm labor.

In the present study, we aimed at comparing the performance of two chromogenic media; STRB and GRAN, with TSA in detecting GBS colonization in pregnant females. GBS was detected in 16 (32%) cases on both TSA and GRAN, whereas on STRB GBS was detected in 19 (38%) cases in at least one sample type. The sensitivity of STRB in detecting GBS was the highest among the three media used. Both GRAN and TSA showed the same sensitivity in detecting GBS.

Regarding vaginal swabs, on STRB GBS was detected in 16 (32%) cases with a sensitivity of 94%, while on GRAN GBS was detected in 15 (30%) cases with a sensitivity of 88%, whereas on TSA GBS was detected in 14 (28%) cases with a sensitivity of 82%.

Gil., *et al*. [18] conducted a study to evaluate GRAN for detection of vaginal and rectal GBS in pregnant women in comparison with selective Columbia blood agar. GRAN was as sensitive as Columbia agar plate, but GRAN provided a clear advantage; the characteristic red-orange colonies produced overnight by GBS can be identified by the naked eye and is so specific that further identification is unnecessary.

El Aila., *et al*. [7] conducted a study to evaluate different sampling techniques and different culture methods for detection of GBS carriage in pregnant women. The rectovaginal swabs of 100 pregnant women cultured on Columbia CNA agar, GRAN and STRB after Lim broth enrichment yielded 77, 95 and 100% sensitivity, respectively.

Morita., *et al*. [6] conducted a study to evaluate STRB as a screening media for GBS in comparison with 5% sheep blood agar in 1425 anovaginal swabs. GBS was recovered from 319 (22.4%) samples with one or both media; 318 on STRB compared to 299 using blood agar. One false negative was observed on STRB, while 20 false negatives were observed on blood agar.

In the present study, on STRB five (10%) false positive cases were obtained in the vaginal swabs. Thus, the specificity of the medium in detecting GBS in the vaginal swabs was 86.8%. All the false positive results showed streptococci other than group B. El Aila., *et al*. [7] found that *S. porcinus* and *S. anginosus* gave false positive results on STRB. Morita., *et al*. [6] found some *Streptococcus* spp. other than GBS and *Enterococcus* spp. strains that showed pink colonies and gave a false positive results. Because false positives are a possibility, an additional confirmatory test using a biochemical or immunological assay should be performed on each isolate [6]. On the other hand, in the present study, the specificity of GRAN in detecting GBS in the vaginal swabs was 100%. Gil., *et a*l. [18] reported that GRAN was so specific in detecting GBS that further identification is unnecessary.

Regarding the urine samples, in the current study GBS was detected in 17 (34%) cases on STRB with a sensitivity of 100%, while on both GRAN and TSA GBS was detected in 13 (26%) cases with a sensitivity of 76.5%.

Although in the present study both GRAN and TSA showed the same sensitivity in detecting GBS in the urine samples, the identification of GBS was much more easier on GRAN than TSA and no confirmatory tests are needed to confirm GBS growth with corresponding savings of time and money. In contrast to our results, a study done by Tamayo., *et al*. [8] compared blood agar to GRAN on 834 urine specimens without using the enrichment medium and GBS was detected in 50 (5.9%) cases using blood agar and in 103 (12%) using GRAN. This difference may be due to the use of enrichment medium in our study.

As regards STRB, to our knowledge no reported studies were done to evaluate it as a screening media for GBS detection in urine samples.

In the present study, STRB showed 3 (6%) false positive cases from urine samples. Thus, the specificity of the medium in detecting GBS in the urine samples was 91.6%. All the false positive results showed streptococci other than group B. On the other hand, the specificity of GRAN in detecting GBS in the urine samples was 100%. Similar result was reported by Tamayo., *et al*. [8] who found that the specificity of GRAN was 100%.

One of the main advantages of GRAN over blood agar is the easy visual identification of GBS (orange-colored colonies) even when the specimen contains a small number of GBS or when GBS is mixed with other microorganisms (whitish colonies which usually correspond to enterobacteria or staphylococci), with no specialized personnel being required for its identification. The pigmentation on GRAN is very specific for *S. agalactiae*, and does not occur with streptococci other than group B or other organisms. The specificity of GRAN (100%) eliminates the need of further latex agglutination confirmation, with corresponding savings of time and money [8]. A disadvantage of GRAN is its relatively short shelf life, which is due to the easy degradation of some of its components and its extreme sensitivity to changes in its storage conditions [19]. Therefore, it should be used within a short period of time and be adequately refrigerated until use to ensure full efficiency of the test. Another disadvantage of GRAN is the need of anaerobiosis for incubation. Also, pigment production requires the colony to be β-hemolytic. Occasional strains of *S. agalactiae* that are non-hemolytic, will grow on the medium but will not appear as orange colonies.

While for STRB, the first advantage in the detection mechanism is the easy visual identification of GBS; pale-pink to red colonies, compared with blood agar, even when the specimen contains a low level of GBS or when GBS is mixed with other microorganisms. Bacteria other than GBS display different colors or are inhibited by antibiotics. Therefore, GBS stands out against background flora. The second advantage is the detection of non-hemolytic GBS. STRB can identify non-hemolytic GBS with the same simple color scheme that is used for hemolytic strains. The advantages of STRB over GRAN include that it is incubated in an aerobic environment, whereas GRAN agar is incubated anaerobically and blood agar is generally incubated in 5% CO_2. Also, the shelf life of STRB is relatively longer than that of GRAN. Although the positive rate of the STRB screen is excellent, there are some disadvantages. False positives are a possibility, so an additional confirmatory test using a biochemical or immunological assay should be performed on each isolate. Also, STRB contains light-sensitive components and should not be exposed to light except when necessary during the inoculation and reading steps [6].

Conclusion

From this study, it can be concluded that: 1) GBS colonization in the genitourinary tract is common among pregnant women in Egypt. 2) Urine samples are as effective as the vaginal swabs in detecting GBS colonization in pregnant women with the advantage of being easier in collection. 3) Both STRB and GRAN are more economic and allow for easier visualization of the pathogen than does the TSA, especially from mixed cultures. 4) The sensitivity of GRAN is comparable to TSA, while STRB is more sensitive in detecting GBS especially in the urine samples.

We also recommend that this study would be done on a larger scale to validate the results.

Conflict of Interest

No conflict of interest exists.

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