ORIGINAL ARTICLE

Screening for Group B Streptococci in Pregnant women by culture and polymerase chain reaction methods

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ABSTRACT

Background: *Group B Streptococcus (GBS) inhabits pregnant women's genitourinary tracts and spreads to the offspring.* ***Objective:*** *This study aimed to screen for GBS colonization in pregnant women, to compare between GBS culture, VITEK® 2 and PCR, and to assess the antimicrobial susceptibility of GBS isolates.* ***Methodology:*** *Vaginal and rectal swabs were collected from 82 pregnant women. GBS was isolated by culture on Strep B Select. Confirmation by VITEK® 2 and PCR for atr and 16S rRNA genes was done.* ***Results:*** *Sixteen vaginal (19.5%) and 22 rectal colonized cases (26.8%) were positive for GBS by culture on Strep B Select agar. GBS was detected in 10 vaginal (12.2%) and 16 rectal colonized cases (19.5%) by VITEK® 2 and PCR for both atr and 16S rRNA genes.* ***Conclusion:*** *It is suggested that pregnant women should be screened for GBS colonization. The best precise tests to detect GBS are PCR and VITEK® 2.*

INTRODUCTION

Colonization of Group B Streptococcus (GBS) is associated in pregnant women with a higher rate of neonatal illness and death [1]. Group B streptococci are one of the commonest etiology of neonatal meningitis and sepsis worldwide [2]. As a result, detecting and preventing this streptococcal colonization is a critical goal [3].

Recent guidelines are emphasized on GBS screening during pregnancy [4]. Records of decreased susceptibility of GBS to penicillin are worrying [5]. Increased erythromycin and clindamycin resistance rates have been recorded in various areas of the world, including Egypt [6]. There are no local guidelines in Egypt for GBS detection or prevention in pregnant women [7].

The usage of novel chromogenic media could increase GBS yield while decreasing delivery and turnaround time [8]. StrepB Select (Bio-Rad Laboratories, France) was tested for GBS probable detection in vaginal and vaginal-rectal samples [9].

Though the good sensitivity of the StrepB Select medium for the GBS isolation was noticed, there were a few noticed drawbacks. The media must be stored and incubated in the dark, with minimum light exposure required for optimal result due to the presence of the chromogenic substrates. Enterococcus spp., *Streptococcus pseudoporcinus*, *Streptococcus bovis* and group A Streptococcus are examples of non-GBS species that can be seen as turquoise blue colonies. Even after 48 hours of incubation, some GBS strains produce no color or produce a very pale purple color, rather than the usual turquoise blue [8].

The *atr* gene is a perfect target for GBS amplification since it has been studied extensively in this species. The *atr* gene is expressed in all cells of this species, so it is a necessary gene. This gene encodes gs0538, a protein amino acid transporter that is only found in *S. agalactiae* species. Since *atr* gene is a housekeeping gene, the chance of mutations is relatively low [10].

Precise and rapid identification of GBS colonization in pregnant women, mainly those who have not received adequate antenatal care, would be beneficial and would enable us to successfully prevent GBS infection. Conferring to the reports mentioned above, a specific and sensitive test for GBS detection in clinical specimens is important for developing easier and efficient preventive guidelines. So, this study aimed to study *16S rRNA* and *atr* genes as potential targets for GBS detection.

METHODOLOGY

Ethical statement

The research was approved by the Ethical Committee of the Faculty of Medicine at Assiut University, Egypt, in accordance with the World Medical Association's code of ethics (Declaration of

Helsinki) with IRB number: 17300275 dated 5/3/2019.All participants gave their informed consent.

Study design

This cross-sectional study was conducted to compare various diagnostic techniques for detecting vaginal and rectal GBS colonization in a sample of Egyptian pregnant women to minimize the risk of vertical transmission to neonates according to CDC [11]. Between September 2019 and December 2020, 164 samples (one from the rectum and another sample from the vagina) were taken from 82 women between 35 and 37 gestational weeks who came for antenatal treatment in the Outpatient Clinic of the Obstetrics and Gynecology Department, Maternity Hospital at Assiut University, Egypt. The pregnant women in this study were not taking any antibiotics [12].

GBS isolation by culture method

From every woman in this study, vaginal and rectal swabs were taken and sent to Department of Medical Microbiology and Immunology in Assuit University. To prevent other bacterial growth and obtain pure growth of *Streptococcus agalactiae*, the swabs were put in tubes of selective enrichment culture media (Todd Hewitt broth; Conda, Pronasida, Spain) with nalidixic acid (15 mg/L) and gentamicin (8 mg/L), which was important for primary isolation of GBS [13]. The tubes were incubated aerobically 37˚C for 18- 24 hours.All broth cultures were subcultured onto StrepB Select agar and incubated aerobically for 48 hours at 37°C. GBS appeared as turquoise blue colonies. According to morphology of colonies, Gram staining, catalase test and CAMP test, isolates were detected.

The isolated detected strains were stored at 70°C in Todd-Hewitt broth with 15% glycerol till PCR testing.

VITEK ® 2 identification and susceptibility

Using the VITEK® 2 GP ID card and the AST-GP67 card, VITEK® 2 identification and susceptibility testing were done on the suspected bacterial suspension according to the manufacturer's instructions.GBS isolates were tested for their susceptibility to list of antibiotics including penicillin, erythromycin and clindamycin. The results were evaluated and interpreted by AES 8.01 software after a period of 15 hrs of incubation.

Conventional polymerase chain reaction (PCR)

Extraction of DNA:

Thermal lysis was used to extract DNA (boiling method) [10].

Detection of 16SrRNA gene:

16S rRNA gene was used for PCR as GBS primer. The primers had the following forward and reverse sequences: (5'- CGCTGAGGTTTGGTGTTTACA-3') and (5'- CACTCCTACCAACGTTCTTC-3') [14].

Detection of atr gene:

The primers of *atr*gene had the following forward and reverse sequences: atrF (5'-CGATTCTCTCAGCTTTGTTA-3') and atrR (5'-AAGAAATCTCTTGTGCGGAT-3') were used in the PCR reactions.

Invitrogen Company in the United Kingdom synthesized these primers. The BLAST database was used to verify the homology of each primer.

The PCR reaction

The PCR reaction had a volume of 20 µL and was prepared as follows:10 µL of 2x master mix (Promega Co., USA), 3 µL of the DNA sample, 1 µL of each primer, and 5 µL sterile double-distilled water. The amplification was done for *16S rRNA* gene in a DNA thermal cycler (Thermo Fisher scientific, England) as follows: An initial denaturation for 5 minutes at 94° C then 35 cycles of 94° C for 45 seconds, 60° C for 60 seconds, 72° C for one minute, and 72° C for seven minutes. The amplification was done for *atr gene* as follows: An initial denaturation for one minute at 94° C then 30 cycles of 94° C for one minute, 55° C for 45 seconds, 72° C for one minute, and 72° C for 10 minutes. Group B Streptococcus was detected in samples with a 405-bp amplicon for *16S rRNA* gene and a 779-bp amplicon for *atr* gene.

Statistical analysis:

The Statistical Package for Social Sciences, version 16 (SPSS Inc., Chicago, USA) was used to conduct the statistical analysis. To compare categorical and continuous variables, the Chi-square test and Student's t-test were used. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

Identification of GBS by culture, VITEK® 2 and PCR:

The prevalence of GBS vaginal colonization among pregnant women (between 35-37 weeks) by culture on Strep B Select agar (colonies with a turquoise blue color) (Fig.1) was 19.5% . However, using PCR method (*16s rRNA*) (Fig.2), 10 of 82(12.2%) samples were positive for GBS . It was interesting that all positive samples for *16s rRNA* gene were positive also for *atr* gene (Fig.3) and VITEK® 2. So, it can be concluded that all these 10 samples were true positive. The prevalence of GBS rectal colonization by culture was 26.8%. However, using PCR method (*16s rRNA*), 16 of 82(19.5%) samples were positive for GBS.

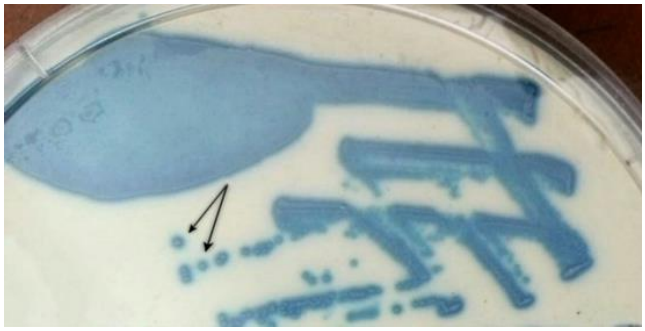

Fig. 1: Colony appearance of GBS on Strep B Select Agar

Fig. 2: Amplification results of 16s rRNA gene in group B Streptococcus (GBS) isolates. Lane 1, positive control strain; Lane 2, negative control (water); Lanes 3,4 and 6, GBS isolates from clinical samples (405 bp); and Lane 5, a clinical sample lacking GBS.

Fig 3: Amplification results of *atr* gene in GBS isolates. Lane 1, positive control; Lane 3, GBS isolate from clinical samples (779); Lane 2, negative control (water); Lanes 4 and 5, clinical samples lacking GBS.

The results for *16s rRNA* gene were compared to the results for *atr* gene and VITEK® 2. All samples that had positive results for 16s *rRNA* gene were positive for *atr* gene and VITEK® 2. So, it can be concluded that all these 16 samples were true positive. There were high statistically significant differences in GBS colonization prevalence between vaginal and rectal samples (Table 1).

Table 1: Comparison between vaginal and rectal GBS colonization based on culture & VITEK® 2 and PCR results:

		Vaginal		Rectal		P value
		No	%	No	%	
Strep B Select Agar	Positive	16	19.5	22	26.8	0.001**
	Negative	66	80.5	60	73.2	
VITEK® 2	Positive	10	12.2	16	19.5	0.003**
	Negative	72	87.8	66	80.5	
16s rRNA	Positive	10	12.2	16	19.5	0.003**
	Negative	72	87.8	66	80.5	
atr gene	Positive	10	12.2	16	19.5	0.003**
	Negative	72	87.8	66	80.5	
Total		82	100	82	100	

Results of antimicrobial susceptibility testing:

GBS isolates were tested for susceptibility to penicillin, erythromycin and clindamycin by VITEK® 2 (Table 2).

Susceptibility to penicillin: Of the 26 GBS isolates, 22 (84.6%) were sensitive and 4 (15.4%) were resistant.

Susceptibility to erythromycin: Of the 26 GBS isolates, all of them were resistant to erythromycin.
Susceptibility to clindamycin: Of the 26 GBS isolates, 2 (7.7%) were sensitive and 24 (92.3%) were resistant.

Table 2: The different patterns of susceptibility of GBS isolates to penicillin, erythromycin and clindamycin by VITEK® 2

The antimicrobial agent	Susceptibility	VITEK®2 (N = 26)	%
Penicillin	**S***	**22**	**84.6**
	R**	**4**	**15.4**
	I***	**-**	**-**
Erythromycin	**S**	**-**	**-**
	R	**26**	**100.0**
	I	**-**	**-**
Clindamycin	**S**	**2**	**7.7**
	R	**24**	**92.3**
	I	**-**	**-**

* Sensitive, ** Resistant, *** Intermediate

Demographic data of the participants:
The mean age of pregnant women with vaginal colonization of GBS (by PCR) was (31.20±7.35), while mean age of non colonized cases was (29.33±5.67) years with no statistically significant difference in the age between colonized and non colonized cases (P-value=0.35). The mean age of pregnant women with rectal colonization of GBS (by PCR) was (32.25±5.88), while mean age of the non colonized cases was (28.91±5.73) years with statistically significant difference in the age between colonized and non colonized cases (P-value=0.041*) as summarized in table (3). The higher prevalence of GBS vaginal and rectal colonization (by PCR) occurred within the age group (>30 years) while lower prevalence occurred in the age group (20-30 years) with no statistically significant difference between the two age groups (P values = 0.41,0.244) respectively as shown in tables (4) and (5).

Table 3: Mean age and Age ranges among vaginal and rectal samples:

Ages (years)	Negative PCR (Colonized)		Positive PCR (Non colonized)		P value
	M±SD	Median (Range)	M±SD	Median (Range)	
Vaginal	29.33±5.67	30 (20-41)	31.20±7.35	34 (23-40)	0.35
Rectal	28.91±5.73	30 (20-41)	32.25±5.88	33 (23-41)	0.041*

Gestational age of the participants:
GBS vaginal colonization (by PCR) was higher at 35weeks of gestation, lower at 37 weeks of gestation while no colonization among the group of 36 weeks of gestation with no statistically significant difference in the prevalence between 3 classes of gestational age (P-value=0.41). GBS rectal colonization (by PCR) was higher at 35 and 37 weeks of gestation, lower at 36 weeks of gestation with no statistically significant difference in the prevalence between 3 groups of gestational age (P-value= 0.117).

Parity of the participants:
The prevalence of GBS vaginal colonization was high among the multigravida but this was not statistically significant (p = 0.41). The prevalence of GBS rectal colonization was high among the multigravida but this was statistically significant (p = 0.05*).

Abortion and intrauterine fatal deaths (IUFD) in previous pregnancies:
There is no statistically significant relation between GBS colonization & the presence of previous abortions and intrauterine fetal deaths.
The results of demographic characteristics are summarized in tables (4) and (5).

Table 4: The demographic and obstetric factors associated with GBS vaginal colonization

Demography	Class	Total		Positive GBS culture		Positive VITEK® 2		PCR	%	PCR	%	P value
		No	%	No	%	No	%	Positive *16s rRNA*		Positive *atr* primer		
Maternal age (years)	20-30	46	56.1	6	13.0	4	8.7	4	8.7	4	8.7	0.41
	>30	36	43.9	10	27.8	6	16.7	6	16.7	6	16.7	
Gestational age(weeks)	35 w	20	24.4	6	30.0	6	30.0	6	30.0	6	30.0	0.41
	36 w	24	29.3	2	8.3	-	-	-	-	-	-	
	37 w	38	46.3	8	21.1	4	10.5	4	10.5	4	10.5	
Parity	Primigravida	10	12.2	4	40.0	4	40.0	4	40.0	4	40.0	0.41
	Multigravida	72	87.8	12	16.7	6	8.3	6	8.3	6	8.3	
Abortion	No	58	70.7	8	13.8	4	6.9	4	6.9	4	6.9	0.41
	Yes	24	29.3	8	33.3	6	25.0	6	25.0	6	25.0	
IUFD*	No	78	95.1	16	20.5	10	12.8	10	12.8	10	12.8	
	Yes	4	4.9	-	-	-	-	-	-	-	-	-
Medical disorders	No	68	82.9	8	11.8	6	8.8	6	8.8	6	8.8	0.41
	Yes	14	17.1	8	57.1	4	28.6	4	28.6	4	28.6	
Total		82	100	16	19.5	10	12.2	10	12.2	10	12.2	

***Intrauterine fetal death

Table 5: The demographic and obstetric factors associated with GBS rectal colonization

	Class	Total		Positive GBS culture		Positive VITEK® 2		PCR	%	PCR	%	P value
		No	%	No	%	No	%	Positive *16s rRNA*		Positive *atr* primer		
Maternal age (years)	20-30	46	56.1	8	17.4	6	13.0	6	13.0	6	13.0	0.244
	>30	36	43.9	14	38.9	10	27.8	10	27.8	10	27.8	
Gestational age(weeks)	35 w	20	24.4	6	30.0	6	30.0	6	30.0	6	30.0	0.117
	36 w	24	29.3	8	33.3	4	16.7	4	16.7	4	16.7	
	37 w	38	46.3	8	21.1	6	15.8	6	15.8	6	15.8	
Parity	Primigravida	10	12.2	4	40.0	4	40.0	4	40.0	4	40.0	**0.05***
	Multigravida	72	87.8	18	25.0	12	16.7	12	16.7	12	16.7	
Abortion	No	58	70.7	14	24.1	10	17.2	10	17.2	10	17.2	0.244
	Yes	24	29.3	8	33.3	6	25.0	6	25.0	6	25.0	
IUFD*	No	78	95.1	22	28.2	16	20.5	16	20.5	16	20.5	
	Yes	4	4.9	-	-	-	-	-	-	-	-	-
Medical disorders	No	68	82.9	12	17.6	10	14.7	10	14.7	10	14.7	0.244
	Yes	14	17.1	10	71.4	6	42.9	6	42.9	6	42.9	
Total		82	100	22	26.8	16	19.5	16	19.5	16	19.5	

***Intrauterine fetal death

DISCUSSION

The results were affected by the exact site of swabs. Among 82 pregnant women, 19.5 % were identified as carriers of GBS on the basis of the results of culture of vaginal specimens as compared with 26.8 % of rectal specimens. This is in agreement with study reported 17.9% of vaginal specimens and 26.8 % of rectal specimens were identified as carriers of GBS by culture[15].

In disagreement with our study, Nomura et al.[16] found no significant difference in detection rates between vaginal and rectal samples. Votava et al. [17] reported that the GBS detection rate by usage of rectal and vaginal swabs separately yielded 24.1% and 22.7%, respectively.

Our results showed that rectal samples culture is more effective than vaginal swabs in detecting GBS. By contrast, Milyani and Rokbah [18] found that GBS was isolated from vaginal samples more than rectal samples by culture method (32.8% vs 21.7%). This difference may be due to the type of culture medium used. Kadanali et al.[19] isolated GBS from 48 patients

including 16 (10.7%) cases only from the vagina, and 7 (4.7%) only from rectal swabs.

Louie et al. [8] reported that 37.5% of samples which gave blue colonies on Strep B Select plates were not confirmed as GBS by PCR.

Our results demonstrated that most GBS positive pregnant women were from 23 to 41 years old which is in accordance with previous studies conducted in Africa[20]. Eren et al.[21] showed that GBS carriage rate was highest among age group (21-30 years). Kovavisarach et al.[22] stated that older maternal age was a risk factor for GBS colonization in pregnant women. This agrees with our study which found statistically significant difference in the age between rectal colonized cases and non colonized cases. In this study, no statistically significant difference was reported in the age between vaginal colonized cases and non colonized cases which agrees with the other studies [23].

In disagreement with this study, Milyani and Rokbah [18] reported that age was found to affect GBS vaginal but not rectal colonization rate.

This study reported no significant differences in GBS colonization among pregnant women between 35-37weeks of gestation. These findings are in agreement with those from other studies [20].

This study reported non significant difference in the detection rate of vaginal GBS colonization between cases of high parity and cases of low parity. This is similar to other studies [23] stated that the relation between gravidity and GBS positivity was statistically insignificant, while significant difference was reported in the detection rate of rectal GBS colonization between cases of high parity and cases of low parity.

All positive cases by PCR were also positive by culture. This indicates that molecular methods are more rapid and more specific.

Of the 26 GBS isolates,22 isolates (84.6%) were sensitive to penicillin and 4 (15.4%) were resistant to penicillin. Makled et al.[24] and Tazi et al. [9] reported that all GBS isolates were sensitive to penicillin. All GBS isolates (100%) were resistant to erythromycin. As regards clindamycin, 2 isolates (7.7%) were sensitive and 24 (92.3%) were resistant. GBS resistance rates to erythromycin and clindamycin differ according to different regions of the world, being low in northern Europe [25], high in southern Europe [26] and higher in the Far East[27]. In New Zealand, 7.5% and 15% of GBS isolates were resistant to erythromycin and clindamycin, respectively [28]. In contrast to our results, a previous study showed a higher rate of resistance to clindamycin than to erythromycin [29]. In a Turkish study[30], all isolates were susceptible to penicillin, while 22.4% were resistant to erythromycin. Our results are different from another study conducted in Egypt reported 13.15% resistance to erythromycin and 23.68% to clindamycin [31]. Resistance has been reported between 4 to 58.3% and 2.3% to 57.9% for erythromycin and clindamycin respectively in other studies [6, 31, 32].

The positive results for *atr* and *16s rRNA* genes targeting PCR were 12.2 % for vaginal samples and 19.5% for rectal samples. These proportions were lower than those obtained by the culture. This in agreement with studies from other countries [33 , 34]. Fatemi et al. [35] in Iran found that the prevalence of GBS colonization was 19.7% and 20.6 % using *16s rRNA* gene targeting PCR and the culture, respectively. de-Paris et al.[10] in Brazil found that the prevalence of GBS colonization was 26.99% and 15.96% percent using *atr* gene targeting PCR and the culture, respectively.

CONCLUSION

It is suggested that pregnant women should be screened for GBS. The results demonstrated that the best antibiotic to prevent intrapartum infection is penicillin or ampicillin. The best precise tests to detect GBS are PCR and VITEK® 2. There were a few noticed drawbacks in Strep B Select medium for the GBS isolation.

- The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article has not been published anywhere and is not currently under consideration by another journal or a publisher.

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