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Original article

Prevalence of group B *Streptococcus* colonization in pregnant women and the correspondence of its virulence genes with adverse pregnancy outcome

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ABSTRACT

Background and rationale: Group B *Streptococcus* (GBS) is a Gram-positive, non-motile, encapsulated bacterium classified under Lancefield group B antigen. Predominantly found in the gastrointestinal and genitourinary tracts. GBS is a leading cause of invasive bacterial diseases in neonates, including neonatal sepsis, meningitis, septicemia, and pneumonia. **Aim:** To study the vaginal colonization rate of *Streptococcus agalactiae* and virulence genes in pregnant women with adverse pregnancy outcome. **Methods**: A cross-sectional study was designed included 200 pregnant women at 34–37 weeks of gestation. A total of two hundred vaginal swabs were taken from all pregnant women enrolled in this project by the gynecologist. GBS isolated bacteria was evaluated by means of using classical microbiological approaches. After DNA extraction, the isolated GBS strains were screened for the presence of *cfb* and *scpB* gene by polymerase chain reaction. **Results:** Thirty-six (18%) of 200 pregnant women enrolled in this project were positive for group B *Streptococcus* by culture methods. The majority were 18–36 years old, 20 (20.6%) had a history of abortion, and 10 (9.9%) had rupture membranes of >18 hours. The particular PCR primer detected *scpB* and *cfb* genes. The *scpB* gene was discovered in 17 (47%), whereas the *cfb* gene was detected in 31 (86%). **Conclusion:** There is no statistical significance between repeated abortions with the presence of *scpB* and *cfb* genes as a virulence factor in GBS while there is statistical significance between the presence of these virulence genes and rupture membranes.

Introduction

Streptococcus agalactiae or group B *streptococcus* (GBS) are members of normal flora in human genitourinary and gastrointestinal systems. GBS is the main agent for serious infections such as meningitis in newborns, asymptomatic bacteriuria, urinary system infections, cystitis, pyelonephritis, chorioamnionitis, postpartum endometritis, pre and postpartum bacteremia, and post-cesarean wound infections in pregnant women [1].

Early-onset GBS infections refer to bacterial infections in newborns that occur within the first few days of life, typically during the first week. When mothers are colonized with Group B *Streptococcus* (GBS) in the urogenital or gastrointestinal systems, their infants are at risk [2]. Without preventive measures, approximately 1–2%

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of newborns born to GBS-colonized mothers may develop early-onset GBS infections. These infections often manifest in the first days of life and can progress rapidly to fulminant disease, posing a significant threat to the newborn's health. Intrapartum antibiotic prophylaxis is an effective preventive measure against the majority of these early-onset GBS infections in newborns [3,4].

This colonization is a dynamic condition and represents the main risk factor for early neonatal infection. Notably, the international literature reports maternal GBS colonization rates of 6.5-36.0% in Europe [5,6], 10.0-30.0% in North America [7,8], 16.5-31.6% in African countries [9], and 1.4- 36.7% in South America, including Brazil [10,11], Chile [12], Peru [13], and Argentina [14]. The first guidelines for the prevention of GBS infection by maternal intrapartum antibiotic prophylaxis were created in 1966 [13]. After the initiation of such strategies, an 80% reduction in the incidence of neonatal GBS disease was observed in the United States [15].

Revised CDC guidelines for the prevention of early-onset GBS disease (2010) recommend universal culture-based screening of all pregnant women at 35th and 37th weeks of pregnancy to identify those who should receive prophylactic intrapartum antibiotic treatment [7].

Actually, the identified virulence genes, *scpB* and *cfb*, in Group B *Streptococcus* (GBS) hold pivotal roles in the pathogenesis of adverse pregnancy outcomes, particularly in the contexts of repeated abortions and premature rupture of membranes (PROM). The scpB gene's presence is significant as it encodes *C5a* peptidase, an enzyme pivotal for immune evasion by cleaving C5a, a complement system component [16]. This evasion mechanism may impact the maternal immune response, heightening the susceptibility to GBS-related complications during pregnancy. Conversely, the *cfb* gene, associated with producing a CAMP factor enhancing beta-hemolysis, underscores GBS's ability to evade host defenses and establish infection, potentially contributing to adverse pregnancy outcomes [17]. While statistical significance eluded the link between repeated abortions and *scpB/cfb* genes, their detection implies a potential influence on pregnancy outcomes, collectively shaping an environment conducive to recurrent pregnancy loss. Notably, the statistically significant association between the presence of *scpB* and *cfb* genes and rupture of membranes suggests their potential contribution to the weakening of fetal membranes, leading to PROM. C5a peptidase (*scpB*) and enhanced hemolysis (*cfb*) may collectively play roles in tissue damage and inflammation, contributing to the untimely rupture of membranes during pregnancy [3,7,16]. Although the CDC guidelines indicate culture as the gold standard method for GBS detection, these same guidelines include expanded laboratory methods for detecting this organism. In particular, polymerase chain reaction (PCR)-based assays comprise an additional option for the rapid detection of GBS colonization [7,8]. The goals of this investigation were to study the vaginal colonization rate of *Streptococcus agalactiae* in pregnant women with adverse pregnancy outcome.

Subjects and methods

A cross-sectional study was designed to include 200 pregnant women at 34–37 weeks of gestation. All females were examined clinically by the gynecologist. Data information were collected from each participant in this study including: age, previous abortions, a history of premature rupture of membrane (PROM), premature labor and neonatal anomalies.

Vaginal swabs have been collected from pregnant ladies attending the antenatal clinic of AL-Imammian AL-Kadhmain Teaching Hospital, Baghdad, Iraq during the period from February to October 2018. The age of patients ranged from 15 to 45 years. Exclusion criteria included: pregnant women who were on antibiotic treatment two weeks prior to recruitment, women with vaginal bleeding and pregnant women with UTI. The current research was authorized by the ethical committee of College of Medicine, Al-Nahrain University and it was conducted in the Department of the Medical Microbiology, College of Medicine, Al-Nahrain University.

Sample size

The sample size was calculated for this study by utilizing G*Power with a power of 92%, alpha error probability of 0.05, and a large effect size (0.4), is 80 subjects. With due consideration for a 10% error rate, the adjusted sample size for 200 pregnant women was also determined to be 200 subjects.

Specimens collection and processing

A total of two hundred samples, including 200 Vaginal swabs were taken from all pregnant women enrolled in this project by the gynecologist. Vaginal samples were taken from the lower one-third of the vagina using a sterile swab at lithotomy position. Swabs from each patient were directly inoculated in Todd-Hewitt broth media containing 10 µg/ml colistin and 15 µg/ml nalidixic acid, and aerobically incubated overnight at 37°C.

The appropriate colony was selected by subculturing on blood agar, and then the plates were inspected and identified for GBS organisms using the following criteria: colonies with a narrow zone of beta hemolysis, Gram-positive cocci, catalase negative, resistance to bacitracin, positive sodium Hippurate hydrolysis, and positive CAMP test.

DNA extraction and polymerase chain reaction assay

Positive sample of GBS in culture method were carried out for molecular detection after DNA extracted by using presto TM mini g DNA, bacterial kit (Catalog No.GBB 35-1, Geneaid Biotech Ltd, Taiwan).

The primers and PCR conditions used to amplify genes encoding virulence factors with PCR are listed in **Table (1)**. The primers include *scpB* and *cfb* gene. Each 25µl of PCR reaction contained 1µl of each forward and reverse primers, 16 µl of nuclease free water, 2 µl of DNA extract lyophilized b master mix (Catalog No. PRA7505, Promega, USA) The PCR amplification product was visualized by electrophoresis on 2% agarose gel.

Statistical analysis

Quantitative variables were expressed as a mean ± standard deviation (SD) and analyzed to student t-test, while binomial variables were expressed as frequency and percentage and analyzed by Chi-squared test whenever possible. The statistically significant was set at ***p*** value <0.05.

Results

Distribution of *streptococcal* colonization according to the age of patients and maternal medical history

Two hundred pregnant female were recruited in this study after meeting the inclusion criteria with age ranged from (15-39 years). Results of vaginal colonization showed that the majority were from age ranged (18-36 years), 20 (20.6%) of them with a history of abortion, females with positive vaginal colonization indicated that they had a history of rupture membrane only in 10 (9.9%) with prolonged rupture membrane >18hr in 3(30%) as illustrated in **Table (2)**.

Distribution of *S. agalactiae* infection according to age of pregnant women

The pregnant women in current study were divided into five groups according to their ages; the highest incidence of *S. agalactiae* infection was in age (20-25) followed by the (26-30) as shown in **Table (3)**.

Detection of specific virulence genes in the group B *streptococcus* isolates by PCR

Identification of C5a peptidase (*scpB*) gene

PCR analysis revealed that out of 36 bacterial isolates by culture dependent method, *scpB* gene was detected in 17 (47%) with (255 bp) amplification fragment in GBS as shown in **Figure (1)**.

The relation between *scpB* gene with maternal outcomes

There were 17 (47%) patients with positive result for *scpB* gene, in those patients there were 11 (11.3%) from all patients presented with abortion, with no statistically significant ***p***-value <0.05 (0.162), the most common maternal outcomes reported in current study in association with *scpB* gene positivity were rupture of membrane ***p***-value (0.001) as shown in **Table (4)**.

Identification of *Cfb* gene

The specific primers for *Cfb* gene encodes CAMP factor of GBS were selected. PCR analysis revealed that out of 36 bacterial isolates by culture dependent method , *Cfb* gene was detected in 31 (86%) with specific bands of approximately (153 bp) in size as shown in **Figure (2)**.

The relation between *cfb* gene with maternal outcomes

There were 31 (86%) patients with positive result for *Cfb* gene, in those patients there are 17(17.5%) from all patients presented with abortion, with no statistically significant ***p***-value <0.05 (0.442), rupture membrane which was presented as maternal outcomes reported in 8 (7.9%) in association with *Cfb* gene ***p***-value (0.003) as demonstrated in **Table (5)**.

Table 1. The primers sequence for scpB and cfb genes and PCR program steps [18].

Genes	Primer sequence (5′-3′)	The amplicon size (bp)	PCR condition
scpB gene	5'-ACAATGGAAGGCTCTACTGTTC-3' 5'-ACCTGGTGTTTGACCTGAACTA-3'	255	94ºC 3min 1cycle
			94ºC 45 sec 57ºC 45 sec 72ºC 1min 33cycle
Cfb gene	F 5'-TTTCACCAGCTGTATTAGAAGTA-3' R 5'- GTTCCCTGAACATTATCTTTGAT-3'	155	
			72ºC 7min 1cycle

Table 2. Frequency distribution of vaginal colonization according to age and maternal medical history.

		Vaginal colonization		*p*-value
		Positive	Negative	
Age (mean) yrs. Range (15 – 39 yrs) Std. Deviation		26.11 (18-36 yrs) 4.646	27.49 (15 - 39 yrs) 4.940	
History of abortion	With	20 (20.6%)	77 (79.4%)	0.350
	Without	16 (15.5%)	87 (84.5%)	
History of rupture membrane	With	10 (9.9%)	91(90.1%)	0.003
	Without	26 (26.3%)	73 (73.3%)	
Prolonged rupture membrane	>18hr	3	23	0.001
	<18hr	7	68	

Table 3. Distribution of *S. agalactiae* infection according to age groups.

Age groups (years)	No. of sample	Positive results	Negative results
< 20	16 (8.0%)	2 (12.5%)	14 (87.5%)
20 – 25	64 (32.0%)	18(28.1%)	46 (71.9%)
26 – 30	86(43.0%)	13 (15.1%)	73 (84.9)
31 – 35	31(15.5%)	3 (9.7%)	28 (90.3%)
36 – 40	3 (1.5%)	0	3 (1.5%)
Total	200	36 (18%)	164 (82%)

Table 4. The relationships between *scpB* gene and maternal outcomes.

	***scpB* gene**		**p-value**	**Odds ratio**
	Positive	**Negative**		
With abortion	11 (11.3%)	86 (88.7%)	0.162	2.068
Without abortion	6 (5.8%)	97 (94.2%)		
Rupture membranes	2 (2%)	99 (98%)	0.001	0.113
Non-rupture	15 (15.2%)	84 (84.8%)		

Table 5. The relation between *cfb* gene and maternal outcomes.

	***Cfb* gene**		***p*-value**	**Odds ratio**
	Positive	**Negative**		
With abortion	17(17.5%)	80 (82.5%)	0.442	1.351
Without abortion	14 (13.6%)	89 (89%)		
Rupture membrane	8 (7.9%)	93 (92.1%)	0.003	0.284
Non-rupture membrane	23 (23.2%)	76 (76.8%)		

Figure 1. 2% Agarose gel electrophoresis at 70 volt for 30 min for *scpB* PCR products visualized under U.V light at 280 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (1 - 8) were positive for this gene, the size of product is 255 bp.

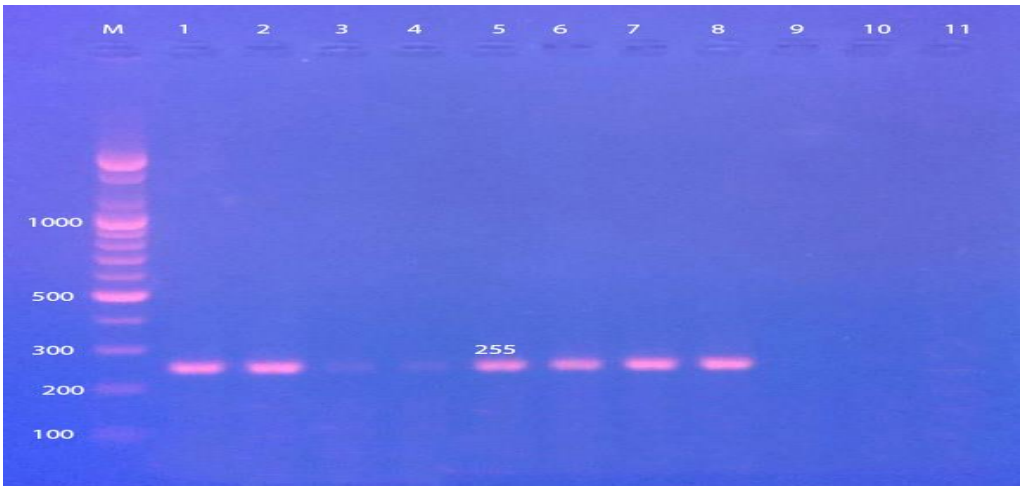
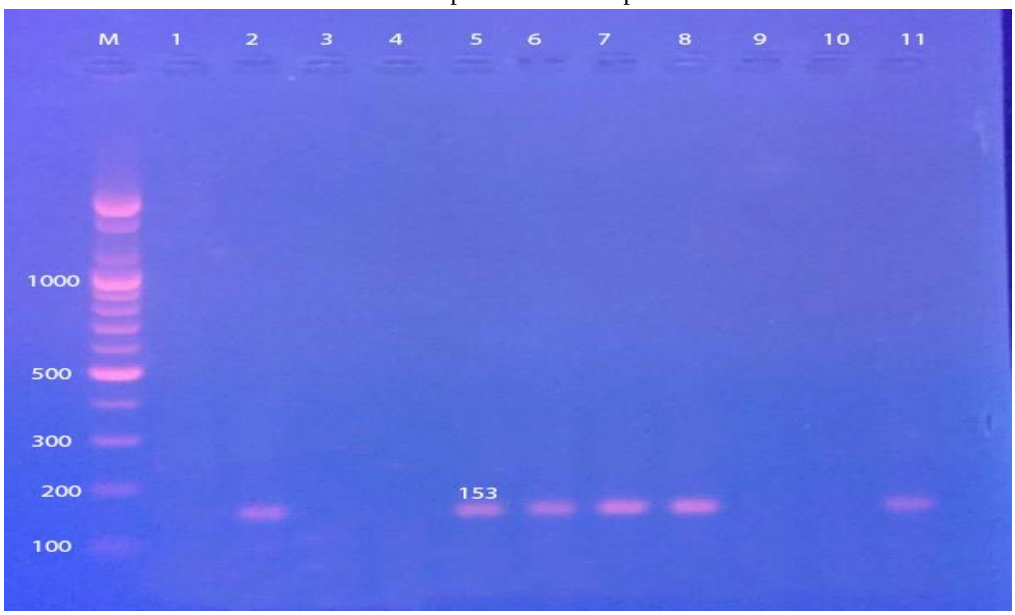


Figure 2. 2% Agarose gel electrophoresis at 70 volt for 30 min for *cfb* PCR products visualized under U.V light at 280 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (2,5-8) was positive for this gene, the size of product is 153 bp.



Discussion

Based on data obtained from current study, the prevalence of group B *streptococcus* among pregnant women was 36 (18%) out of 200 samples, this was in disagreement with a previous study conducted in Iraq by **Taiseer *et al.*** [19] who found that (10%) of pregnant women were positive for vaginal colonization. Another study conducted by **Hamid *et al.*** [20] stated that carriers of group B *streptococci* among pregnant women was (24.3%). However, a study conducted in Turkey by **Alp *et al.*** [21] reported that colonization rate for GBS was (9.8%) out of 215 pregnant women, while in Iran **Mashouf *et al.*** recorded the prevalence of GBS vaginal colonization in pregnant women was 7.39% out of 203 obtained specimens [22].

The observed variations in study outcomes can be attributed to differences in several factors related to the study methodologies involving pregnant women. Discrepancies arise from variations in specimen numbers, methodologies employed for sampling from the vaginal mucosa, swab timing, the presence of intrauterine devices, and the types of transport media used. These methodological disparities significantly influence

Group B *Streptococcus* cultivation outcomes. Furthermore, differences in exclusion and inclusion criteria across studies contribute to the observed heterogenesity in results, emphasizing the importance of standardized protocols in research involving pregnant populations.

Current investigation reported that there was no statistically significant relationship between GBS carriage, age and history of abortion. Such results are similar to **Karadag *et al.*** [23]. This study identified GBS colonization in 10 (9.9%) women who had premature rupture of membranes (PROM) demonstrating a statistically significant relationship between GBS positivity and history of PROM. This result comes compatible with study by **Alp and Findik** [21].

Premature rupture of membranes (PROM) one of the most culprit in maternal morbidity and mortality and also leads to fetal death as a result of sepsis, asphyxia, and pulmonary hyperplasia [24]. Many studies have indicated a close relationship between women with intrauterine bacterial infection such as GBS with rupture of membranes and infants born for those women with a mortality rate four times higher than those without [25,26].

In this study, results showed the highest incidence of *S. agalactiae* infection was in age (20-25) followed by the (26-30). This shows that the active age group was at extra risk of being infected with *S. agalactiae*, this result was in harmony with many studies in this field which found that the most affected age women was ≥ 30 [19,27].

Streptococcus agalactiae has fourteen unique islands that contain virulence genes that encode all surface proteins as well as mobile elements genes, for this reason these islands are considered as pathogenicity islands, which play critical role in genetic diversity as well as acquisitions of virulence in GBS [28,29]. Data in this study reported that *scpB* gene was detected in 17 (47%) out of 36 bacterial isolates, while *cfb* gene was detected in 31 (86%). These results contradicted with recent study conducted in Iraq by **Tiasire *et al.*** [19] and in Argentina by **Laczeski** [30] who stated that *scpB* gene was present in percentage of (100%), while **Shabayek et al.** reported that *scpB* and *cfb* genes were found in 30 %, 30.6% respectively [27].

It is important to note that the discrimination in such results may be due to regional or strain variations which affect gene expressions or as a result of mutations in such gene.

Reichenberger *et al.* reported that bacterial mutations may occur spontaneously or induced by a mutagen in the environment. However, it has also been hypothesized that bacteria might probably be able to selectively increase mutation rates when they are presented to certain “stressful” or growth-limiting condition. It is conceivable to demonstrate that ecological elements drive changes in nucleotide content, not only between highly diverged environment types, but also between specimens obtained from the distinctive human [31].

Our data demonstrated that there were no correlations between repeated abortions and the presence of *C5a* peptidase and *cfb* genes as virulence factors in GBS, while there were statistically significant correlations between the presences of these virulence genes and membranes rupture. The implicit pathogenesis of PROM is believed to be because of a blend of physical stresses and biochemical debilitatinn of the chorioamnion. In relations with infection, an examination of PROM principally centered on the catabolic degradation of collagen mediated by matrix metalloproteinases (MMP) with certain investigations of different pathways including apoptosis and oxidative stress [32].

The chorioamnion of fetus is made out of two membranes considered the amnion and chorion that encase the amniotic cavity. Inflammation of the chorioamnion and within the amniotic fluid is thought to assume a critical role in the pathogenesis of premature rupture untimely in preterm delivery. Infection-related preterm labor is commonly characterized by raised amniotic fluid cytokine levels in pregnant women[33].

Vanderhoeven *et al.* hypothesized that premature membrane rupture may be due to ascending infection during pregnancy that may increase risks of chorioamnionitis, in addition to mediating inflammation. Cytokines have additionally been related with increased collagen remodeling and, ultimately, biophysical weakening of the fetal membranes or chorioamnion in vitro [34].

The data in this study aligns with Surve et al.'s findings in a rat model. **Surve et al**. showed that Group B Streptococcus (GBS) produces membrane vesicles, which cause chorioamnionitis and damage to the rupture of membranes. This leads to notable increases in amniotic fluid cytokines such as TNF-

α, IL-8, IL-1β, and IL-6, which are activated by GBS's virulence factors [35].

Conclusion

Our data suggest that GBS is highly prevalent among pregnant women. There was a high degree of correspondence between vaginal carriage for GBS and premature rupture of membrane (PROM) which is the most adverse pregnancy outcome associated with GBS carriage.

Conflict of interest

The authors declared no conflict of interest**.**

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