Screening Arab Israeli Pregnant Women for Group B Streptococcus by the AmpliVue GBS Assay: Are the Rates Higher than the National Average?

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ABSTRACT: **Background:** The recommendation of the U.S. Centers for Disease Control and Prevention regarding universal screening for Group B *Streptococcus* (GBS) at 35–37 weeks gestational age in pregnancy is not accepted in Israel. The National Council for Obstetrics, Neonatology and Genetics recommends intrapartum prophylaxis, mainly based on risk factors, to prevent early neonatal GBS infection. This policy is based on past studies demonstrating low colonization rates of the bacteria in Israeli pregnant women and very low neonatal sepsis rates.

Objectives: To determine the applicability of the high-risk group prophylaxis policy for Arab Israeli pregnant women.

Methods: Vaginorectal swabs from Arab Israeli pregnant women who attended the labor ward between October 2015 and February 2016, were obtained before any pelvic examination for GBS identification using Quidel's AmpliVue® GBS assay. Women who tested positive received intrapartum antibiotic prophylaxis to prevent neonatal infection. Obstetric data were collected from each woman from a standardized questionnaire. Data regarding the delivery and neonates were collected as well.

Results: The study comprised 188 Arab pregnant women who met the inclusion criteria and signed a consent form to participate in the study. Of these, 59 had positive tests, and a carriage rate of 31%. No neonatal colonization of GBS was found.

Conclusions: The carrier rate in Arab pregnant women in northern Israel is higher than the national average, at least partially due to the more sensitive method of GBS detection used in the present study.

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KEY WORDS: Group B *Streptococcus* (GBS), prevention strategies, neonatal sepsis, GBS carriage rate, AmpliVue® Group B *Streptococcus* assay

Group B *Streptococcus* (GBS) infection is a leading cause of neonatal morbidity and mortality in the United States [1,2]. Surviving infants may suffer from development disabilities, mental retardation, and hearing or vision loss [3]. Maternal GBS carriage state is a crucial factor as it is estimated that approximately 50% of infants delivered from colonized women become colonized at birth in the absence of antibiotic prophylaxis. The U.S. Centers for Disease Control and Prevention issued guidelines for decreasing the incidence of perinatal GBS infection [4]. These guidelines recommend prophylactic intrapartum antibiotic for mothers found positive by a universal GBS culture screening at 35–37 weeks gestation or based on a risk factor profile for those not screened. The risk factor profile includes premature labor before 37 completed weeks, maternal fever (≥ 38.0°C) during labor, prolonged rupture of membranes (≥ 18 hours), previous delivery of an infant with GBS disease, and GBS urinary tract infection during pregnancy. The use of intrapartum prophylaxis has led to a 70% decline in the incidence of GBS disease in the past decade [4]. In addition, the CDC guidelines include suggestions for improving the sensitivity of cultures, such as utilizing selective pigmented broths, DNA probes, or nucleic acid amplification tests (NAATs) such as the AmpliVue® GBS Assay (Quidel Corporation, Athens, Ohio, USA), but these remain optional [5].

In Israel, however, universal screening is not routine. Maternal GBS carriage rates in Israeli women vary according to different studies. Eidelman and colleagues [6] reported maternal GBS carriage rate ranging between 3.5 and 11% during the years 1984 and 1997. Drai-Hasid and co-authors [7] revealed a GBS carriage rate of 17.7%, a significantly higher rate than past findings. In addition, the authors found a significantly higher carriage rate in Orthodox Jewish women compared to secular Jewish women and suggested an association between ritual immersion in a mikveh and GBS carriage [7]. A 2006 prospective study, including 700 pregnant women conducted at a hospital in Nahariya, investigated GBS colonization in Arab women from northern Israel. The study reported a significant increase in the incidence of GBS colonization in pregnant women in northern Israel in general (16.4% vs. 11.8% in the 1970s) and specifically an increased rate of GBS carriage rate in Arab women (19%) relative to Jewish women (13.7%), $P = 0.038$ [8]. All of these studies have used the culture-based screening method to detect GBS.

The recommendations of the National Council for Obstetrics, Neonatology and Genetics advocate for the administration of prophylactic antibiotics during labor for pregnant women with the risk factors mentioned earlier. These recommendations were published by the Israeli Ministry of Health and have been clinical guidelines since July 2005 [9].

The current study was conducted to address the continuously higher incidence of early neonatal GBS infection at the Nazareth Hospital, in the years 2006 to 2012, compared to the national average, as reported by the Israeli Ministry of Health. The incidence of neonatal GBS infection in the Nazareth Hospital was 1.75 per 1000 births compared to 0.26 per 1000 births, which was the national average in Israel between 2010 and 2012 [10]. Initially, we thought that at-risk pregnant women were probably missed and therefore did not receive intrapartum antibiotic prophylaxis per the guidelines. However, after reviewing all of the positive neonatal GBS cases, it became obvious that the majority of the neonates who developed GBS infection were delivered to mothers who did not belong to any of the high-risk groups. Thus, the objective of this study is to address the question of whether the GBS carriage rate among pregnant women in the Arab sector, which comprises most of the women admitted to the Nazareth Hospital, is higher than the general carriage rate in Israel, and to determine whether the policy of intrapartum antibiotic prophylaxis, as is the case in Israel, is applicable to this ethnic group.

PATIENTS AND METHODS

This prospective study included 188 Arab Israeli pregnant women who attended the labor suite at the Nazareth Hospital in Nazareth between 1 October 2015 and 28 February 2016 for any reason other than active labor. The institutional review board at the Nazareth Hospital approved the study and the clinical study was registered at ClinicalTrials.gov (registration no. NCT02528890). All women were recruited after giving written informed consent. The following parameters were recorded: maternal obstetric characteristics, parity, adequacy of prenatal care, gestational age at the time of screening, presence or absence of previous screening for GBS, previous infant with GBS disease, and detection of GBS bacteriuria. The obstetric data of the women are presented in Table 1. Vaginal and anal swabs were obtained at admission before any pelvic examination and were sent to the microbiology laboratory of the hospital for GBS detection. Women with positive GBS were tracked and given prophylactic antibiotics when they returned for delivery. The neonates of these GBS positive women were cultured for GBS in case the delivery occurred less than 4 hours from the time of antibiotic administration to their mothers, per the guidelines of the ward, and the following parameters were collected: GBS carriage status of the mother, maternal perinatal infection, Apgar scores, neonatal GBS colonization, and presence or absence of neonatal disease.

Table 1. Demographic characteristics of the women by carriage status

	Entire population	GBS carrier (n=59)	Non-carrier (n=129)	*P*
Maternal age, years (range)	27.4 ± 5.1 (19–40)	28.8 ± 5.4 (19–40)	26.8 ± 4.8 (19–40)	0.02
Parity (range)	2.6 ± 1.4 (1–7)	2.9 ± 1.6 (1–7)	2.4 ± 1.3 (1–7)	0.03
Gestational age, weeks (range)	37.5 ± 1.6 (34–40)	37.4 ± 1.6 (34–40)	37.5 ± 1.6 (34–40)	0.82
Maternal age › 35 years, n (%)	17 (9.0)	10 (16.9)	7 (5.4)	0.01
Nulliparous, n (%)	51 (27.1)	12 (20.3)	39 (30.2)	0.16
Multiparous (› 5) , n (%)	8 (4.3)	5 (8.5)	3 (2.3)	0.11
Twin pregnancy, n (%)	4 (2.1)	1 (1.7)	3 (2.3)	› 0.99

SWAB COLLECTION AND PROCESSING

One vaginorectal swab was collected from each pregnant woman. The swabs were placed in Amies transport medium (Becton, Dickinson and company, New Jersey, USA) and sent to the laboratory to be stored up to 24 hours at 4°C before processing. Swabs from the transport medium were inoculated directly to Lim Broth (Becton, Dickinson and company, New Jersey, USA) for selective enrichment of group B streptococci and incubated with loosened caps at 35°C in an aerobic atmosphere for 18–24 hrs.

GBS DETECTION

The U.S. Food and Drug Administration (FDA)-approved AmpliVue® GBS Assay (Quidel Corporation, Athens, Ohio, USA) was used for GBS detection. This assay combines simple sample processing, an isothermal amplification technology known as Helicase Dependent Amplification (HDA), and a self-contained disposable amplicon detection device for the detection of Group B Streptococcus. The sensitivity and specificity of the AmpliVue GBS assay is 99.5% and 92.7%, respectively, when compared to bacterial cultures as reported previously in 908 samples [11].

A 50 µl of cultured specimen was added to a dilution tube; 50 µl of the diluted sample culture was then transferred into a lysis tube and the cells were lysed by simple heat treatment for 10 minutes at 95°C. An additional 50 µl of the lysed sample was transferred to a reaction tube containing a lyophilized mix of HDA reagents, and specific primers for the amplification of the thiolase acetyltransferase (atoB) gene, which is a conserved gene in GBS, were applied. The tube was heated for 60 minutes at 64°C. After completion of the HDA reaction, the reaction tube was transferred to a cassette for rapid detection of the result, which is displayed as "test" and/or "control" lines in the window of the cassette.

STATISTICAL ANALYSIS

To ascertain whether there were demographic or clinical differences between GBS carriers and non-carriers, two sample *t*-tests were performed for the continuous variables, and chi-square test

and Fisher's exact test were performed for the categorical variables. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 21 (SPSS, IBM Corp, Armonk, NY, USA). Significance was considered as $P < 0.05$.

RESULTS

Of the 188 Arab women sampled (mean age 27.5 years, range 19–40), 59 had GBS positive tests, a carriage rate of 31.4% (95%CI 25.2–38.3). GBS positive women were statistically significantly older ($P < 0.02$) and had higher parity compared to GBS negative women ($P < 0.03$); however, there was no statistically significant difference in the percent of nulliparous ($P > 0.16$) or multiparous women ($P > 0.11$).

Table 2 presents the neonatal outcomes. There were no cases of neonatal infection in either group. In addition, there was no statistically significant difference in Apgar score at 1 or 5 minutes ($P > 0.95$, $P > 0.39$, respectively) or in the percentage of neonates with Apgar < 7 at 1 minute (0.0 vs. 0.8%, $P > 0.99$).

Of the 59 neonates delivered to GBS positive women, 22 were cultured for GBS and none of them were found to be positive. The rest were not cultured for GBS since the delivery occurred more than 4 hours from the time of antibiotic prophylaxis administration, when the antibiotic becomes effective and the cultures become negative.

DISCUSSION

Our research was motivated by the consistently higher rate of early neonatal infection occurring in the Nazareth Hospital between the years 2006 and 2012, as has been reported by the Israeli Ministry of Health. To this end, the study aimed to evaluate the GBS colonization rate in the Arab Israeli population using an FDA-approved molecular GBS assay.

Previous studies on GBS colonization rates in pregnant women in Israel, as determined by cultures from vaginorectal swabs, have reported carriage rates between 13.7% and 19.3% [6,7,8,12,13]. Specifically, a study conducted at the Western Galilee Medical Center and published in 2006 [8] reported an increased rate of GBS carriage in Arab women (19%) compared to Jewish women (13.7%), $P = 0.038$. In our study, we reported a maternal GBS colonization rate of 31% in Arab pregnant women attending the Nazareth Hospital. Unlike previous studies, we used the AmpliVue® GBS assay (Quidel Corporation, Athens, Ohio, USA), which uses HDA for the amplification of a highly conserved fragment of the thiolase (atoB) gene sequence with GBS identification sensitivity of 99.5% [11]. Nucleic acid amplification tests (NAAT), which target specific DNA regions unique to GBS, have been intensely studied during the past 20 years to improve speed and accuracy of GBS detection. In fact, one study [5] evaluated the performance of three molecular GBS assays: the AmpliVue GBS assay used in our study, the BD Max GBS assay (BD Diagnostics, Quebec, Canada), and the illumigene group B streptococcus assay (Meridian Bioscience, Cincinnati, Ohio, USA), and compared them to the Lim-broth-enriched culture as the gold standard in 200 samples. All three NAATs were more sensitive (sensitivity 90.9–100%) than culture (sensitivity 53.6%). Sensitivity was calculated for the illumigene assay as 90.9% (95% confidence interval [95%CI] 79.3–96.6%), for the BD Max GBS assay as 100% (95%CI 91.9–100%), and for the AmpliVue assay as 96.4% (95%CI, 86.6–99.3%). Specificities were 97.9% (95%CI 93.6–99.5%), 95.8% (95%CI 90.8–98.3%), and 95.8% (95%CI 90.8–98.3%), respectively. No significant difference in sensitivity or specificity between the three NAATs was found (McNemar's chi-square test, $P > 0.5$).

The choice of an FDA-approved NAAT test, despite its high price, was intended to verify that we were measuring the real rate of GBS (sensitivity > 96%). Culture-based screening would have missed some cases of GBS as a result of the high rate of false negatives. To the best of our knowledge, our study is the first time that molecular diagnostics was used for GBS identification in Israel. The higher rate achieved in our study may be due to the more sensitive detection method used.

There are a few limitations to our study. First, the limited number of participants in the study and the fact that all of them were of Arabs ethnicity hindered our ability to compare with other ethnicities and determine whether the rate of GBS is higher in Arabs or the true national average is, in fact, higher. In addition, we underestimated the importance of demographic data and did not collect data such as religion, economic status, place of birth, and place of living. These data could be helpful in investigating the existence of more reliable risk factors and suggesting opportunities to further define those at risk of early-onset infection.

One assumption is that the higher rate of GBS colonization in this ethnic group is attributable to the low socioeconomic status of Arabs. The relation between socioeconomic status and carriage rate of GBS has been suggested by Orrett [14] who found up to 37.3% GBS carriage rate in pregnant women who had low socioeconomic status in the West Indies. Other studies conducted in Israel found that other groups in the Israeli population have a higher carriage rate, such as women from the former Soviet Union [12] and from North America [13].

In this study, 22 neonates were tested for GBS colonization, per the department's policy (delivery in less than 4 hours

Table 2. Neonatal outcome by maternal GBS carriage status

	Entire population	GBS carrier (n=59)	Non-carrier (n=129)	*P*
Apgar at 1 minute (range)	9.0 ± 0.3 (6–9)	9.0 ± 0.3 (6–9)	9.0 ± 0.3 (6–9)	0.95
Apgar at 5 minutes (range)	10.0 ± 0.3 (7–10)	10.0 (10)	10.0 ± 0.3 (7–10)	0.39
Apgar < 7 at 1 minute, n (%)	1 (0.5)	0 (0.0)	1 (0.8)	> 0.99
Neonatal sepsis, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	–

from antibiotic prophylaxis administration) and none of them were found to be positive for GBS, perhaps because of the low number of participants, or attributed to the use of antibiotic prophylaxis in the study group.

The results of this study can also explain the higher rate of early neonatal infection in the Nazareth Hospital during the years 2006–2012, as reported by the Israeli Ministry of Health. During the time in which the study was conducted, starting in October 2015 and ending in September 2016, five neonates were born with early-onset GBS infection out of 2014 deliveries, a neonatal infection rate of 2.5/1000 live births, 10 times higher than the incidence rate of early onset GBS in 2008–2009 in Israel based on data from the Israel Center for Disease Control and the Ministry of Health [15]. However, on review of these cases, it turned out that only one of the five mothers was considered in the high-risk criteria and she received prophylactic antibiotics. The other four neonates were delivered to mothers who were not in any of the risk groups.

From a cost perspective, implementation of universal GBS screening is associated with significant higher costs [15]. To investigate this aspect, Ginsberg and colleagues [15] conducted a cost-utility analysis that integrated epidemiological, clinical, and economic data. The authors found that even though applying universal screening would increase screening costs and penicillin costs in approximately 5.6 times, 93% of this increment would be offset by decreased treatment costs and work productivity gains as a result of a decrease in neurological sequelae from GBS caused meningitis. In addition, the culture-based screening would reduce the burden of disease by 12.6 discounted Quality Adjusted Life Years (QALY), giving a very cost effective baseline incremental cost per QALY of $3000 per QALY. For summary, Ginsberg and co-workers [15] recommended that Israel adopt universal culture-based GBS to screen all pregnant women at 35–37 weeks gestation. Strickland and colleagues [16] and Boyer [17] investigated the cost-benefit of the treatment protocols for neonatal GBS prevention. Both research groups suggested that screening and treatment were justified from a medical and economic standpoint because of the high costs of caring for neonates with early-onset disease. However, based on their calculations, universal screening programs are not cost-efficient in geographic areas where the maternal GBS colonization rate is less than 10%.

Two studies from Israel advocated for the screening approach in certain ethnic groups, the first from the south [12] and the second from the Jerusalem area [7]. They highlight the question of whether the current prevention strategy is appropriate for the total population of Israel or needs reassessment for some ethnic groups.

In this study, using an FDA-approved NAAT test with sensitivity and specificity higher than the culture method, we showed that approximately one-third of the Arab pregnant women tested in our study were GBS carriers. Further studies using a larger cohort of patients, from different ethnicities and using a highly sensitive NAAT method in addition to the culture method, are needed to investigate the existence of more reliable risk factors, confirm the reproducibility and consistency of the GBS rate and if relevant, consider general screening of pregnant women in addition to the antibiotic prophylactic recommendations to prevent GBS neonatal sepsis.

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