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The Diagnostic value of GeneXpert PCR assay in screening pregnant women for group B *Streptococcus* colonization

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ABSTRACT

Background: Group B *Streptococcus* (GBS) transmitted from colonized pregnant women to the newborns is considered the leading cause of neonatal sepsis with high mortality rate, so screening pregnant women for GBS colonization is important for intrapartum antibiotic prophylaxis to prevent neonatal and maternal infections, this study aimed to detect the prevalence of GBS colonization in pregnant women and to assess the diagnostic value of GeneXpert PCR assay in screening. **Methods:** Rectovaginal swabs from 318 pregnant women with gestational age 35 or more weeks were tested by conventional culture as a standard screening method and by GeneXpert PCR assay as a rapid molecular method. **Results:** The prevalence of GBS colonization in pregnant women was 17% by standard culture method and 22% by the GeneXpert PCR assay with overall 98.2% sensitivity, 93.6% specificity, 94.3% diagnostic accuracy and total turnaround time less than 1 hour. **Conclusion:** The increase in prevalence of GBS colonization of pregnant women raises the concern about importance of the accurate and rapid molecular diagnostic techniques for effective prevention of GBS transmission.

Introduction

Streptococcus agalactiae, named as group B *Streptococcus* (GBS), is considered a common intestinal and genitourinary flora of healthy women, the prevalence rate of pregnant women colonized with group B *Streptococcus* is up to 35% (11-3) and this rate varies with socioeconomic status, ethnic group, marital status, number of deliveries, geographic area and age [1, 2].

The transmission rate of GBS to newborns from colonized women is approximately 50% [3] and GBS is considered the leading cause of early

onset neonatal sepsis and meningitis with incidence rate from 0.5 to 3.0 per 1000 live births and mortality rate from 4.0 to 10 % [4], also GBS can cause maternal chorioamnionitis, preterm labor and stillbirth [5].

The risk factors for early onset neonatal sepsis include GBS infection during pregnancy, preterm labour <37 weeks gestational age and premature rupture of membranes [4], so screening pregnant women for GBS colonization is important for intrapartum antibiotic prophylaxis to control neonatal infection [6].

The gold standard procedure recommended by the Centers for Disease Control and Prevention (CDC) for screening pregnant women for GBS colonization is based on traditional bacteriological culturing of recto-vaginal swabs at 35-37 weeks of pregnancy, the culture is a time consuming method that takes 48 to 72 hours, which is long time for detecting GBS colonization especially for pregnant women who had inadequate antenatal care during current pregnancy or during labour [7].

Antibiotic resistant GBS strains emerged increasingly due to the widespread intrapartum antibiotic prophylaxis for colonized females, so, antibiotics sensitivity tests are essential to choose the proper antibiotics to avoid treatment failures [6].

Nucleic acid amplification techniques including GeneXpert PCR assay, recently used in screening pregnant women for GBS colonization, are rapid and accurate screening techniques with comparable sensitivity and specificity to traditional culturing methods that help to prevent neonatal and maternal GBS infections [5,8,9], we aimed in this study to detect the prevalence of GBS colonization in pregnant women and to assess the diagnostic value of GeneXpert PCR assay in screening pregnant women for GBS colonization.

Patients and methods

Study type and population

A cross sectional study was carried out from October 2023 to December 2023 in Gynecology and Obstetrics and Microbiology Laboratory departments of Najran Armed Forces Hospital, Saudi Arabia and included 318 pregnant women with gestational age 35 or more weeks during their antenatal care visits, pregnant women aged 18-50 years were included and pregnant women who intake antibiotics in the previous period of the visit were excluded from the study, sociodemographics and clinical data as age, occupation, nationality, gravidity, number of antenatal visits, vaginal discharge or history of abortion, preterm labor and stillbirth were obtained.

Sample collection

Two rectovaginal swabs were collected using sterile cotton swabs by a gynecologist from the lower part of the vagina and 2 cm beyond the anal sphincter, according to the CDC and American College of Obstetricians and Gynecologists (ACOG) guidelines [7,10] and sent immediately in amies transport media to microbiology laboratory.

Culture based identification and antibiotics susceptibility testing of GBS

One swab was cultured on 5% sheep blood agar plates and incubated at 36°C in 5% CO₂ for 24 hours and hemolytic colonies were tested by Gram stain and biochemical reactions, then species identification and antimicrobial susceptibility testing was performed using Vitek 2 automated system (bioMerieux, Marcy l'Etoile, France), using colorimetric Gram-positive identification cards (GP ID) and antibiotics susceptibility cards (AST P580) according to the manufacturer's instructions [6,11]

GeneXpert PCR identification of GBS

The second swab was used for molecular screening of GBS using GeneXpert PCR assay, according to manufacturer's instructions, the swab was put and broken at the specified mark of GBS assay cartridge, then the cartridge was loaded into the specific chamber of GeneXpert® system (Cepheid Ltd., Sunnyvale, CA, USA) which is self-contained device integrating automated sample processing and real-time PCR detection of infectious agents, the bacterial cells were lysed, DNA extracted and the eluted DNA was added to PCR reagents which contain specific primers used to amplify GBS *cfb* gene, the GeneXpert system reported within 50 minutes a qualitative result either GBS positive, GBS negative or invalid, invalid results were repeated for confirmation [4].

Statistical analysis

SPSS® version 16.0 was used to perform the statistical analysis; sensitivity, specificity and diagnostic accuracy of GeneXpert PCR assay were calculated with the conventional culture method as a reference test, values were presented as numbers and percentages, *p* value < 0.05 was considered statistically significant

Ethical considerations

The protocol of the study was reviewed and approved by the research ethics committee of Najran Armed Forces Hospital (Code: NAFHREC/2023/LAB/7) (dated 2023) and written informed consent was taken from each participant before specimen collection.

Results

Using the culture method as the gold standard screening test, 54 out of 318 pregnant women were GBS positive by the culture method, estimated about 17 % colonization rate of GBS in the studied pregnant women, the mean age of women in GBS positive group was 25 years while

that of GBS negative group was 24 years with no statistical difference, the highest prevalence of GBS colonization (42.6%) was in women with age group (31-40 years) and the lowest rate (9.3%) was in age group (≤ 20 years), no statistically significant differences between the GBS positive and GBS negative groups regarding occupation, nationality, gravidity, number of antenatal visits, vaginal discharge or history of abortion, preterm labor and stillbirth (p values ≥ 0.05) (**Table 1**).

The patterns of antibiotic sensitivity of the GBS strains isolated from pregnant women showed that GBS isolates had the highest sensitivity for vancomycin and linezolid (100%), followed by ampicillin (98%) and gentamycin (85%) and the

least sensitivity of GBS was for erythromycin (18%) followed by clindamycin (38%) (**Table 2**).

Compared with the conventional culture as standard screening method, the GeneXpert PCR assay within 1 hour identified correctly 53 from the 54 GBS strains isolated by the culture method, also among the 264 GBS culture negative group, 17 were identified as GBS positive estimated about 22 % (70/318) GBS colonization rate with GeneXpert PCR assay (**Table 3**), with overall 98.2% sensitivity(95% CI: 90.1-99.6), 93.6% specificity(95% CI: 89.9-96.2), 75.7% positive predictive value (PPV), 99.6% negative predictive value (NPV) and 94.3% diagnostic accuracy, also Kappa statistic between the two methods was 0.82 indicating almost perfect agreement (**Figure 1**).

Table 1. Sociodemographic and clinical data of GBS culture positive and GBS culture negative groups

Data	GBS culture positive (n=54)	GBS culture negative (n=264)	p-value
Age group (y) ≤ 20	5(9.3%)	45(17.0%)	0.09
21-30	20(37.0%)	97(36.7%)	
31-40	23(42.6%)	102(38.7%)	
> 40	6(11.1%)	20 (7.6%)	
Occupation Housewife	49(90.7%)	235(89.0%)	0.65
Employee	5(9.3%)	29(11.0%)	
Nationality Saudi	48(88.9%)	238(90.2%)	0.5
Non Saudi	6(11.1%)	26(9.8%)	
Gravidity Primigravida	15(27.8%)	92(34.8%)	0.11
Multigravida	39(72.2%)	172(65.2%)	
Antenatal Visits	6(4-7)	6(5-9)	0.91
Vaginal Discharge Yes	15(27.8%)	60(22.7%)	0.12
No	39(72.2%)	204(77.3%)	
Abortion/ Preterm Yes	8(14.8%)	45(17%)	0.18
No	46(85.2%)	219(83%)	

Data are presented as number (percentage), p value < 0.05: Statistically significant

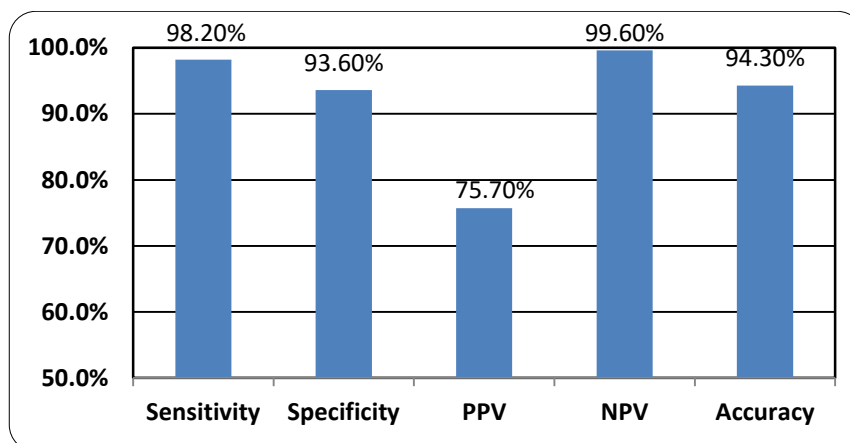
Table 2. Antibiotics susceptibility pattern of GBS isolated from pregnant women.

Antibiotic	Susceptible n(%)	Antibiotic	Susceptible n(%)
Ampicillin	53(98.0)	Levofloxacin	42(77.8)
Clindamycin	21(38.9)	Tetracyclin	29(53.7)
Erythromycin	10(18.5)	Trimethoprim	35(64.8)
Tobramycin	45(83.3)	Vancomycin	54(100)
Gentamycin	46(85.2)	Linezolid	54(100)

Data are presented as number (percentage)

Table 3. Comparison between GeneXpert PCR and culture methods in detecting GBS.

GeneXpert	Culture		Total
	Positive	Negative	
Positive	53	17	70
Negative	1	247	248
Total	54	264	318

Figure 1. Performance data of GeneXpert PCR assay

Discussion

Group B *Streptococcus* colonization of pregnant females is a significant cause of serious infections in neonates like sepsis and meningitis with high mortality in both developing and developed countries [11].

The prevalence of GBS colonization in pregnant women in our study was 17%, this value is nearby the results of **Musleh et al.** and **Khater et al.** who reported that GBS colonization rate were 19% and 16.1% respectively in Saudi pregnant females [5, 12], and also nearby to a study by **Khan et al.** in Makkah city that reported GBS colonization rate was about 16% [13], higher prevalence rates were reported in Saudi studies from Riyadh and Jeddah (27.6% and 31.6%, respectively) [14,15], but lower rates of GBS colonization (2.1% and 7.6% respectively) in Saudi pregnant females were reported in studies by **Ahmed** and **Hussain et al.** [16,17].

Similar studies performed in some Asian countries as in Kuwait and Iran reported that the prevalence rate of GBS colonization was 14.6% and 16%, respectively [18, 19], our finding was higher than that reported in other countries such as Turkey (8%), China (7.1%) and Korea (8.3%) [6], however, our results showed lower rates of GBS colonization compared with other countries such as Taiwan (21.8%) and Brazil (28.4%) [6], a systemic review

performed by **Stoll et al.** on 34 studies from some developing countries showed overall GBS colonization rate was 12.7% [20].

The difference in the GBS colonization rates can be explained by differences in the studied populations according to geographical area, age, parity and socio-economic factors and also can be related to the differences in sample collection and diagnostic methods [21].

In the current study, the patterns of antibiotic sensitivity of the GBS strains isolated from pregnant women showed that GBS isolates had the highest sensitivity for vancomycin, linezolid (100%) and ampicillin (98%) and the least sensitivity was for erythromycin (18%)

These findings are matched with the CDC clinical recommendations for the efficiency of penicillin and ampicillin as the drugs of choice to prevent GBS infections [7], moreover, other studies showed similar findings regarding vancomycin and linezolid and also **Dashtizade et al.** and **Khoshkhoutabar et al.** reported increased rates of resistance to clindamycin (52.9%) and erythromycin (73.6%) [6, 22].

No statistically significant association was detected in our study between GBS positive and negative women regarding different studied sociodemographic and clinical data, this is matched with those results from other studies which did not

report any differences [5, 23], but on the other hand, **Salama et al.** found that GBS colonization rate was significantly higher in the 37th week gestational age and also higher in females who had regular antenatal visits and increased BMI [24], also **El-Kersh et al.** and **Milyani et al.** reported that high GBS colonization 37% and 26%, respectively were present in females with vaginal discharge [14,25].

In our study, the Genexpert PCR assay estimated that prevalence of GBS in pregnant women was 22% compared to the standard culture method that was 17% with overall 98.2% sensitivity, 93.6% specificity, 94.3% diagnostic accuracy and strong agreement between the culture and GeneXpert PCR methods (Kappa= 0.82), in addition the total turnaround time to detect GBS is less than 1 hour

In studies comparing the performance of molecular PCR assays versus culture methods, one study reported GBS carriage rate of pregnant women in Jeddah was 19.7% using culture and 30.5% using a PCR method [15], also other studies showed GBS colonization rates were 21.6% and 20.4% respectively [26], the sensitivity and specificity of PCR assays reported in previous researches ranged from 85.7% to 100% and from 82.6% to 96.6%, respectively [5, 27].

One result was GBS positive by culture but negative by GeneXpert and this can be explained by that the fact that gene mutation in the *cfb* gene may be the cause, this explanation was also reported by **Tickler et al.** who noted four chromosomal deletions in *cfb* gene resulting in false negative results [28], also probably can be resulted from samples with very low bacterial count that is under the detection limit for the molecular assays [29]

One strength of our study is that the GeneXpert assay was performed directly from fresh samples and not from stored frozen ones, also has the strength that few studies were conducted in the southern region of Saudi Arabia to assess the prevalence of GBS among pregnant women

The weakness of our study was that the GBS culture method was performed directly without broth pre-enrichment step as performed in previous studies [4,17,30], also we did not include urine samples from pregnant women as a site of GBS colonization, this was mentioned in a study performed by **Ahmed** who concluded that screening for bacteriuria in pregnancy must be considered in GBS screening [16]

Conclusion

The current study highlights the increase in prevalence of GBS colonization among pregnant women and raises the concern about the clinical importance of the accurate and rapid molecular diagnostic techniques as GeneXpert PCR assay for effective management of GBS and preventing of neonatal infection, further nationwide studies should be conducted to determine the actual prevalence of GBS colonization in pregnant women and to assess the suitable screening method regarding accuracy, timing and cost.

Author contributions

All authors had seen and approved the submission of the manuscript with full responsibility and this research had not been published or under consideration by any other journal.

Conflict of interest

No conflict of interest related to the work was declared.

Financial disclosure

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