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Role of PCR in Rapid Detection Group B *Streptococcus* in Pregnant Females in Al-Quwayiyah General Hospital, Riyadh, Saudi Arabia

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Authors' contributions

This study was carried out by two authors. Author ESK choose the study topic, planned the study, carried out the practical laboratory work, interpreted the results and revised the manuscript. Author ASAM shared in the designation of the study, obtaining the samples, collected the clinical and author demographic data, shared in the interpretation of the results. The final manuscript was edited and approved by both authors.

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ABSTRACT

Background: Group B streptococcus is one of the most common causes of severe neonatal infections.
Aim: To detect the prevalence of group B. Streptococcus and their antimicrobial susceptibility and to assess the role of PCR as a rapid method of its detection.
Place and Duration of the Study: A cross sectional and prospective cohort study was carried out from September 2019 to February 2020 in Gynaecology and Obstetrics OPD and inpatient units in Al- Quwayiyah General hospital, Riyadh, Saudi Arabia
Methodology: Paired rectal/vaginal specimens were collected from 540 pregnant females with gestational age 35 or more, Each swab was inoculated into selective medium, Todd Hewitt, One swab is streaked onto blood agar plates incubated in 5% CO_2 for 24h at 36ºC. β-hemolytic colonies growth is identified by Gram's stain, colony morphology and CAMP test. The confirmation and antimicrobial susceptibility were done by Vitek II machine, The second swab was used for PCR to identify *atr* gene.

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Results: Out of 540 pregnant women 87 (16.1%) were colonized with GBS isolates. The positive GBS women aged 25 (22-34) and negative GBS women aged 23 (24-35) with no statistical difference. Patients aged more than 35 years old has the higher rate of positive GBS, 46.2%. No significant association detected between GBS and gestational age at delivery, antenatal visits, BMI and gravidity. The GBS strains isolated from pregnant women was 100% susceptible to linezolid and vancomycin followed by ampicillin (93.1%) and tobramicin (83.9%) then gentamicin (81.6%) and levofloxacin(78.2%) and showed least antibiotic susceptibility to erythromycin (26.4%). The PCR was positive in 145 (26.9%). Using culture as gold method, PCR sensitivity was 100% (95% CI: 91.62-100), while specificity was 83.2% (95% CI: 82.61-91.02). Negative and positive predictive values were 100% and 61% respectively. Kappa between the two methods was 0.71, which indicate major agreement.

Conclusion: The GBS prevalence among the pregnant females in Al Quwayiyah General Hospital was 16.1%. Detection of GBS using new PCR technique was found to have high sensitivity and faster results, allowing efficient management of GBS and reduction in newborn morbidity and mortality however the cost is high for some laboratories. Further studies should be assessed to be both low cost and accurate rapid screening.

Keywords: Group B Streptococcus; screening; vaginal carriage; PCR.

1. INTRODUCTION

Streptococcus agalactiae, now named as Group B Streptococcus (GBS), present in the gastrointestinal tract of healthy adults as a common normal flora. GBS continues to be the world's most common etiology of neonatal sepsis and meningitis, occurring in 0.5 to 3 newborns per 1000 live births [1]. Moreover Group B Streptococcus also induces maternal chorioamnionitis, preterm labor or even stillbirth [2].

Pregnant women are estimated to be colonized with GBS at a rate of 11 to 35%, and socioeconomic status have a direct effect on the rate of carriers among population [3]. Despite the fact that early neonatal onset of disease has been decreased owing to screening programs and intrapartum antibiotic prophylaxis, this pathogen still the leading cause of infections in neonates in the developing countries [4].

CDC's current gold standard procedure for detecting GBS recto-vaginal carriage at 35-37 weeks of pregnancy is based on bacteriological tests, which involves culturing recto-vaginal swabs into selective broth and later streaking on bacteriological medium. The results of culture, take at least 48-72 hours to be completed, which is unacceptable long time for GBS detection [5].

Accurate and rapid screening of GBS carriage in pregnant females, especially those who have got inadequate antenatal care during pregnancy will allow to effectively prevent GBS infections in neonates and mothers [6]. Polymerase chain reaction is a rapid technique with comparable sensitivity and specificity to traditional methods based on culture. Unlike culture-based approaches, this technique can also detect non-hemolytic GBS, which is present in 1 to 4% of pregnant women [7].

The aim of the study:

1. To identify the prevalence of Group B *Streptococcus* and their antimicrobial susceptibility.
2. To evaluate the role of PCR as a rapid technique for Group B *Streptococcus* detection.

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional and prospective cohort study was carried out from September 2019 to February 2020 in Gynecology and Obstetrics OPD and inpatient units in Al- Quwayiyah General hospital. 540 pregnant females with gestational age 35 or more weeks, aged 18-48 years were included in the study. Pregnant women who administered antibiotics in the previous week, patients undergoing elective caesarean delivery, and women declined to be enrolled in the study were excluded. Data for demographics age, gestational age at delivery, body mass, antenatal visits, gravidity and antibiotics administration was collected after patient permission.

2.2 Samples Collection

The CDC recommended that combined rectal/vaginal specimens should be obtained. Paired rectal/vaginal specimens were obtained and transported in amies transport media from each pregnant female visited Gynecology and obstetrics OPD or admitted to the obstetric unit [5]. The GBS culture was done with one swab, and the PCR testing was done with the other. Swabs for PCR test were obtained and preserved at 2–8°C and -80°C until used.

2.3 Culture and Identification of GBS

Each swab was inoculated into selective medium, Todd Hewitt (Becton Dickinson, USA) that contain nalidixic acid and gentamicin (8 µg/mL) (15 µg/mL), followed by incubation in 5% CO2 for 24h at 36ºC, then streaked onto blood agar medium (BioMérieux, Marcy l'Étoile, France) and kept in 5% CO_2 for 24h at 36ºC. β-hemolytic colonies growth was picked up to be identified, in absence of β-hemolytic colonies, plates should be re-incubated for 24h. Identification of β-hemolytic colonies by morphology, Gram's stain and CAMP test. Further confirmation was done by Vitek II machine.

2.4 Testing of GBS Isolates for Antibiotics Susceptibility

Each GBS isolate was tested for antimicrobial susceptibility using Vitek II's gram-positive susceptibility AST-586 cards, which include penicillin, clindamycin, erythromycin, levofloxacin, linezolid, tetracycline, gentamicin, tobramycin, trimethoprim/ sulfamethoxazole and vancomycin as proposed by Clinical and Laboratory Standard Institute guidlines in 2017 [8].

2.5 Polymerase Chain Reaction (PCR) [9]

2.5.1 DNA extraction

Todd Hewitt selective medium was used to incubate the recto-vaginal swabs for 24 hours. The precipitate obtained after broth centrifugation was washed with 1X PBS solution and re-suspended in 500 l of 10 mM Tris-EDTA, pH 7.4 for DNA extraction by using a QIAamp DNA mini kit (Qiagen, USA) as the manufacturer's guidelines.

2.5.2 Atr gene amplification by Polymerase Chain Reaction (PCR)

CAA CGA TTC TCT CAG CTT TGT TAA and TAA GAA ATC TCT TGT GCG GAT TTC were used, respectively as forward and reverse primer sequences, to amplify a 779bp sequence of the *atr* gene, which encodes the *S. agalactiae* glutamine transporter protein (gbs05380. To verify the isolates, a 780 bp *S. agalactiae* specific *atr* gene (GenBank accession number: AF15135) was used as an internal positive control for the PCR assay.

one *µL* of bacterial DNA, 0.5 *µ*L of forward primer, 0.5 *µ*L of reverse primer, 12.5 *µL* of 2x Taq Premix-Master mix, and 10 *µL* of sterile double distilled water, were used to form the PCR reaction volume which was equal to 25 *µ*L. Thermal cycles were amplified as follows: a 5-minute denaturation stage at 94°C, followed by 35 cycles of 94°C for 30 seconds, 55°C for 55 seconds, and 72°C for 1 minute, and finally extension step at 72°C for 10 min using Proflex, Thermofisher, USA.

The amplified products were identified by electrophoresis technique by adding 10 µL of the amplified reaction mixture to agarose gel 2%, which contained ethidium bromide 0.4 mg/mL. An ultraviolet light transilluminator was used for visualization. The PCR products were analyzed using a 100-bp molecular weight marker (Invitrogen®, Calbad, USA) and a positive control of *Strep. agalactiae*. GBS positivity depended on the presence of a 780-bp amplicon in the samples.

2.6 Statistical Analysis

SPSS® version 14.0 was used to perform the statistical analysis. Z ˝ test was used for comparing 2 variables and ˝ χ2 _(Chi square) test for more than two variables. P value of <0.05 was considered statistically significant. The PCR technique's sensitivity, specificity, PPV, and NPV were analyzed considering culture as the gold standard. The Kappa coefficient was used to assess the correlation between assays.

3. RESULTS

Out of 540 pregnant women 87 (16.1%) were colonized with GBS isolates, their age were 26 (24-33) years old. The positive GBS women aged 25 (22-34) and negative GBS women aged 23 (24-35) with no statistical difference. Patients aged more than 35 years old has the higher rate

of positive GBS, 46.2%. No significant association detected between GBS and gestational age at delivery, antenatal visits, BMI and gravidity.

The GBS strains isolated from pregnant women was 100% susceptible to linezolid and vancomycin followed by ampicillin (93.1%) and tobramicin (83.9%) then gentamicin (81.6%) and levofloxacin(78.2%) and showed least antibiotic susceptibility to erythromycin (26.4%) as shown in Table 2.

Table 3 showed that out of 540 specimens the culture method were GBS positive in 87 (16.1%) specimens, but the PCR positivity was determined in 145 (26.9%). Using culture as gold method, PCR sensitivity was 100% (95% CI: 91.62-100), all positive culture results were also PCR positive, while of the 453 culture-negative samples for GBS, 58 were PCR positive while 395 showed negativity with the two methods, that revealed a specificity of 83.2% (95% CI: 82.61-91.02) of the PCR method. Negative and positive predictive values were 100% and 61% respectively. Kappa between the two methods was 0.71, which indicate major agreement.

Table 1. Demographic and clinical data of the positive GBS and negative GBS groups

Ch.ch.	Positive GBS (n=87)	Negative GBS (n=453)	P-value
Age, median	25 (22-34)	23 (24-35)	0.83
16-25 years	25(28.7%)	123 (27.2%)	0.010
26-35years	22 (25.3%)	224 (49.5%)	
> 35 years	40 (46%)	106 (23.3%)	
Gestational age per weeks, median	38 (37-40)	37 (36-41)	0.56
prenatal visits, median	10 (8-11)	10 (9-13)	0.47
Pre-pregnancy BMI, median	24.7 (21.5-29.4)	23.8 (21.4-27.9)	0.41
Gravidity, median	3 (2-4)	3 (2-5)	0.52

Table 2. Group B Streptococcus antibiotics susceptibility pattern among studied pregnant women

Antibiotics	Susceptible strains		Resistant strains	
	No	%	No	%
Linezolid	87	100%	0	0%
Vancomycin	87	100%	0	0%
Clindamycin	41	47.1%	46	52.9%
Erythromycin	23	26.4%	64	73.6%
Ampicillin	81	93.1%	6	6.9%
Tetracycline	50	57.5%	37	42.5%
Levofloxacin	68	78.2%	19	21.8%
Gentamicin	71	81.6%	16	18.4%
Trimethoprim	58	66.7%	29	33.3%
Tobramycin	73	83.9%	14	16.1%

Table 3. Comparison between PCR and culture in detecting GBS

PCR	Todd Hewitt Culture		Total
	Pos.	Neg.	
Pos.	87	58	145
Neg.	0	395	395
Total	87	453	540

PCR sensitivity was 100% (95% CI: 91.62-100)
PCR specificity of 83.2% (95% CI: 82.61-91.02)
negative predictive value was 100%
positive predictive value was 61%.

4. DISCUSSION

GBS vagino-rectal colonization in the mother is a significant risk factor for invasive neonatal infections. In both developed and developing countries, GBS infection is a major cause of infections in neonates, with high mortality and serious conditions like sepsis and meningitis [5].

In this study the prevalence of GBS carriage in pregnant females was 87 (16.1%). this results matched with those of Musleh et al.,2018 [10] who found that GBS colonization rate in Saudi females attended in labor to the King Fahd University Hospital was 19%. a study in Makkah was done by Khan et al.,2015 [11] reported positive GBS equal to 16.3% Similar results also reported that the rate of Group B Streptococcus colonization in Kuwait and Iran (14.6% and 16%, respectively) [12,13]. Higher prevalence rates were obtained in the Saudi Arabian from Riyadh and Jeddah (27.6% and 31.6%, respectively) [14,15]. The differences in the GBS colonization prevalence was explained by the wide variation according to geographical area, age, parity and socio-economic factors [16].

The positive GBS women aged 25 (22-34) years and negative GBS women aged 23 (24-35) years old, the highest rate were in women older than 35 years 46.2%. which was similar to the previous studies performed [17,18]. Joachim et al.,2009 [19] reported in a study done in Tanzania that GBS was more generally isolated from females in the 30–34 year old age group (32.1 %). Other studies have shown that as people get older, their GBS positivity rises [20,21]. In this study no significant association detected between GBS and gestational age at delivery, antenatal visits, BMI and gravidity. These findings were in agreement with those from other studies [17,21,22] which reported that when socio-demographic and medical obstetric characteristics, such as risk factors for the newborn to acquire infections, were considered, there was no significant differences in GBS carriage rates were found. Salama et al. [23], on the other hand, revealed that GBS carriage was significantly greater among pregnant females in their 37th week of pregnancy who had regular antenatal visits, increased BMI and using the condom for contraception.

Chemoprophylaxis still the most effective ways for prevention of GBS infections in mothers and neonates [24]. in the current work the GBS strains isolated from pregnant women was 100% susceptible to linezolid and vancomycin followed by ampicillin (93.1%) and tobramicin (83.9%) then gentamicin (81.6%) and levofloxacin(78.2%) and showed least antibiotic susceptibility to erythromycin (26.4%). These findings are matched with the CDC clinical recommendations for the efficiency of penicillin and ampicillin as the drugs of choice to prevent and treat the infections related to GBS [5]. Moreover, other studies with increased rates of susceptibility of GBS strains to amoxicillin, linezolid, and vancomycin showed similar findings. [25] it was noticed in this study that there were increased rates of resistance to clindamycin (52.9%) and erythromycin (73.6%), Dashtizade et al. 2020 [24] stated also that Erythromycin and clindamycin resistance rates were 73.7% and 52.6%, respectively. In other published literatures, resistance to erythromycin and clindamycin ranges from 4 to 58.3 percent and 2.3% to 57.9%, respectively [26,27]. Consequently, erythromycin was excluded from the list of alternative antibiotics recommended by the CDC in 2010 [27].

In the current study PCR technique showed positivity in 145 (26.6%). a study in a Jeddah hospital, GBS carriage rates were 19.7% using culture technique while it was 30.5% when using PCR method [15]. The other studies which used PCR technique to detect GBS strains also showed 21.6% and 20.4% carriage rates [28]. In Brazil, some studies found that the prevalence of GBS ranged from 15.9% to 22.5% by using the culture technique [29,30], and from 26.9% to35.9% by using the PCR method [31,32]. These are quite close to the rates found in this study.

Although the CDC's guidelines recommend that culture to be used to screen pregnant women for GBS, many medical conditions necessitate a quicker and more effective methods [30]. The use of *atr* gene as amplification target by PCR method in this study had high sensitivity and high NPP which recommend it as a good screening method. In the current study PCR sensitivity was 100% (95% CI: 91.62-100), while specificity was 83.2% (95% CI: 82.61-91.02) of the PCR method. Negative and positive predictive values were 100% and 61% respectively. Di Renzo et al., 2015 [33] on comparing GBS cultures and PCR, he also revealed better sensitivity and specificity of PCR, ranged from 84.6–100% and 62.5–100%, respectively [33]. The sensitivity of PCR in studies conducted by Gavino & Wang, 2007 [34] and Alfa [35], sensitivity was 95.8%

and 90.5%, respectively, while in this work it was 100%, this could be explained by the use of selective and enriched broth prior to performing PCR in this study that increased the test sensitivity. Regarding the specificity Gavino & Wang, 2007 [34] reported that specificity of PCR test was 86.88% which is a quite similar to the results obtained in this study, 83.2%.

In this study using PCR even with selective broth incubation, needed 24 hours to submit the final report, on the other hand, the culture is a time-consuming process that requires at least 48-72 hours to completely identify GBS. Therefore, GBS PCR is considered as a rapid screening reliable test for detecting GBS in pregnant women [34].

5. CONCLUSION

The GBS prevalence among the pregnant females in Al Quwayiyah General Hospital was 16.1%. Detection of GBS using new PCR technique was found to have high sensitivity and faster results, allowing efficient management of GBS and reduction in newborn morbidity and mortality however the cost is high for some laboratories. Further studies should be assessed to be both low cost and accurate rapid screening.

ETHICAL APPROVAL AND CONSENT

As per international standard or university standard guideline patients consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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