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Оригинальное исследование
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Выявление *Streptococcus agalactiae* молекулярными методами у беременных женщин и частота вертикальной передачи новорождённым в провинции Вавилон

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Аннотация

Введение. *Streptococcus agalactiae* — грамположительные, неподвижные и инкапсулированные кокки. На кровяном агаре они образуют узкую зону бета-гемолиза. Этот возбудитель при передаче от инфицированных матерей вызывает инвазивные бактериальные заболевания у новорождённых, в том числе сепсис, менингит, септицемию и пневмонию. *Streptococcus agalactiae* является патогеном, вызывающим первоочередную озабоченность общественного здравоохранения.

Цель работы — провести объективное исследование по выделению и молекулярному выявлению гена вирулентности стрептококка группы В (СГВ) и оценить частоту передачи инфекции от матери новорождённому.

Материалы и методы. В проспективное когортное исследование вошли 300 беременных женщин со сроком беременности более 35 нед. У всех участниц исследования собирали вагинальные мазки, всех женщин с диагностированным СГВ обследовали после родов, чтобы взять мазки у их новорождённых. Для оценки выделенных бактерий использовали традиционные микробиологические и молекулярные подходы.

Результаты. В исследовании приняли участие 60 (20%) из 300 беременных женщин и 16 (26,6%) их новорождённых. СГВ был обнаружен с помощью культуральных методов и подтверждён с помощью ПЦР с праймерами для выявления гена *atr* (гена домашнего хозяйства). Положительные изоляты были на 100% чувствительны к антибиотикам, таким как цефтриаксон, пенициллин и ванкомицин, 93% были чувствительны к хлорамфениколу, 83% — к эритромицину, 13% — к тетрациклину.

Заключение. Наши данные показали высокую частоту инфицирования СГВ у беременных женщин и их новорождённых. Необходимо проводить обязательный скрининг и профилактическое лечение, чтобы свести к минимуму потенциально смертельные последствия этого заболевания.

Ключевые слова: *стрептококки группы В, молекулярное обнаружение, колонизация беременных женщин, колонизация новорождённых, чувствительность к противомикробным препаратам*

Этическое утверждение. Перед участием все беременные женщины добровольно подписывали форму информированного согласия. Протокол исследования одобрен Этическим комитетом Вавилонского технического института Технического университета Аль-Фурат Аль-Аусат (протокол № 8643/27/7 от 24.09.2023).

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Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Original Study Article
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Molecular detection of *Streptococcus agalactiae* in pregnant women and percentage of vertical transmission to their neonates in Babylon province

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Abstract

Introduction. *Streptococcus agalactiae* are gram-positive, non-motile and encapsulated cocci. On blood agar, they produce an narrow zone of beta-haemolysis. This pathogen causes invasive bacterial diseases in newborns, including sepsis, meningitis, septicaemia, and pneumonia, when transmitted from infected mothers. Since *S. agalactiae* is a pathogen of primary concern for public health, this research has been conducted on it.

The **objective** of the study is the isolation and molecular detection of virulence gene of *S. agalactiae* group B (GBS), and evaluation of the percentage of mother-to-child transmission of the pathogen.

Materials and methods. A prospective cohort study was designed that included 300 pregnant women who were at more than 35 weeks of pregnancy. The gynaecologist collected 300 vaginal swabs from all participants in this study and followed up on all GBS-positive pregnant women after delivery to take swabs from their neonates. Traditional microbiological and molecular approaches were used to study isolated bacteria.

Result. Sixty (20%) of three hundred pregnant women and 16 (26.6%) of their newborns were enrolled in this study. GBS was detected via culture methods and was confirmed by PCR with primers employed for the detection of *atr* gene (housekeeping gene). Positive isolates were 100% susceptible to antibiotics such as ceftriaxone, penicillin, and vancomycin, 93% were sensitive to chloramphenicol, 83% to erythromycin, and only 13% to tetracycline.

Conclusion. Our data showed a high frequency of GBS infection in pregnant women and their newborns. A mandatory screening test and preventative medicine should be adopted to minimize the potentially fatal repercussions of this sickness.

Keywords: *Streptococcus agalactiae group B, molecular detection, pregnant women colonization, neonatal colonization, antimicrobial susceptibility*

Ethics approval. Prior to participation, all pregnant women signed a consent form detailing the study's objectives and their willingness to participate. The research protocol was approved by the Ethics Committee of the Babylon Technical Institute, Al Furat Al-Awsat Technical University (protocol No. 8643/27/7, September 24, 2023).

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Introduction

Group B *Streptococcus agalactiae* (GBS) are normal inhabitants of the human gastrointestinal and genitourinary systems. They are the primary cause of serious bacterial infections like neonatal meningitis, symptoms-free bacteriuria, Urinary tract infections (UTIs), bladder infection, inflammation of kidneys and pelvis, intra-amniotic infection (IAI), postpartum endometritis and pre- and postpartum bacteraemia. It is a gram-positive, opportunistic, beta-haemolytic bacteria with possible complications. It also causes infections of surgical wounds in pregnant women [1, 2]. By maternal rectovaginal colonization, GBS causes a variety of prenatal and maternal illnesses, consisting of infections in the mother, stillbirths, premature births, as well as early and late-onset sepsis in infants [3–6]. GBS-colonized mothers run the risk of vertical transmission of these bacteria to the newborn. It is one of the risk factors for early-onset sepsis in newborns [7]. This dynamic colonization constitutes the highest infectious disease risk for newborns. Notably, reports from international literature show that the rates of maternal GBS colonization were 6.5–36% in Europe [5, 6], 10–30% in North America [7, 8], 16.5–31.6% in African nations [9], and 1.4–36.7% in South America, which includes Brazil [10, 11], Chile[1], Peru [12], and Argentina [13].

[1] WHO. Number of infant deaths (between birth and 11 months); 2022. URL: https://who.int/data/gho/data/indicators/indicator-details/GHO/number-of-infant-deaths

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The first recommendations for women's intrapartum antibiotic treatment to prevent GBS infection were established in 1966 [12]. Following the introduction of these treatments, the incidence of newborn GBS disease was reduced by 80% in America [14]. According to recommendations from the CDC (Centres for Disease Control and Prevention)[2], GBS can be diagnosed at 35–37 weeks of gestation using selective enrichment broth culture; however, this is not always possible in poor-income regions [5].

Maternal colonization with GBS is the greatest significant threat for newborn early-onset disease (EOD) (from 0 to 6 days) [15]. In 1973, a single infant was born to a GBS-infected woman from a group of 46 pregnant mothers, and EOD was first recorded. Among the neonates born to women with GBS infection, 2.17% were at risk for EOD at the time intrapartum antibiotic prophylaxis (IAP) has been suggested for both microbiological and risk-based screening in the United States [8]. According to the data from the World Health Organization (WHO), there were 1601 new-born fatalities in Sri Lanka in 2017, of which 0.04% were related to sepsis and other infectious diseases [11]. The convenient use of antibiotics and the detection of pathogens can further lower mortality rates in this population [15]. Consequently, monitoring maternal pathogen colonization is a crucial safeguard against infant infection. In 1980, the global prevalence of maternal GBS colonization was 18%, the Caribbean had a higher prevalence (34%), while Melanesia had the lowest (2%). Similar colonization rates (23%) were observed in North America, Europe, and Australia; Comparatively, the incidence was slightly higher in South Africa than in the Western nations, although it was lower in East Asia (9%), South and Southeast of Asia together was observed around (14%), West Africa (13%), and Central America (10%) [10]. The lack of publications in East Asia, South Asia, Southeast Asia, Western Africa and Central America may be the cause of the subpar prevalence estimates from these locations [10].

Polymerase chain reaction (PCR) assays provide an additional option for the quick identification of GBS colonization [11]. The **aim** of our study was the isolation of GBS strains and detection of the virulence gene by molecular method from pregnant mothers and their neonates, evaluation of the ratio of vertical transmission from infected women to their neonates, and reduction of the mortality and morbidity rates associated with GBS infection by using appropriate prophylactic antibiotics in Iraq, where neither screening for GBS nor an IAP protocol existed until the beginning of this study.

[2] Verani J.R., McGee L., Schrag S.J. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC; 2010. URL: https://cdc.gov/Mmwr/preview/mmwrhtml/rr5910a1.htm

Materials and methods

Study Design

A prospective cohort study was designed that involved 300 pregnant women who were at 35 weeks or more of gestation. Three hundred vaginal swabs were taken before labor from all expectant mothers by the gynaecologist and 60 swabs from neonates born to GBS-positive women were taken shortly (to avoid contamination from other sources) after birth in the delivery room, including neonatal swabs from three sites (oral cavity, ear, and umbilicus). These neonates were born healthy.

Inclusion and Exclusion Criteria

Pregnant women who were in the final weeks of gestation (≥ 35 weeks) and were attending the Al-Zahraa Hospital of Obstetrics in Babylon (Iraq) were included in this study. This study excluded pregnant women who took antibiotics within 10 days of delivery and those who underwent caesarean surgery.

Bacteriological Identification of Isolates

All swab isolates from pregnant women and their neonates were screened for the period from November 2021 to June 2022. It was done by incubating specimens directly in the Todd-Hewitt Broth selective media overnight at 37ºC and subsequently subculturing them on blood agar to select the proper colony. The colonies were inspected and identified using the following criteria: a narrow beta haemolysis zone, gram positive cocci, bacitracin resistance, catalase negativity, sodium hippurate hydrolysis positivity, and CAMP positivity, in order to determine whether or not the plates contained GBS organisms.

Molecular identification of isolates

Samples that were positive for GBS in the culture method were sent for molecular detection after DNA

Table 1. Primer sequence for *atr* genes [16]

Gene	Primer Sequence (5'–3')	Size, bp
Atr	F-5'CAACGATTCTCTCAGCTTTGTTAA3' R-5'TAAGAAATCTCTTGTGCGGATTTC3'	780

Table 2. PCR programme

Steps	Temperature, °C	Time, min	Cycles
Initial denaturation	94	1.00	1
Denaturation	94	1.00	
Annealing	55	0.75	30
Elongation	72	1.00	
Final extension	72	10.00	1
Hold	4	7.00	1

extraction using the bacterial DNA extraction kit Geneaid (Taiwan). The primers and PCR steps used in the experiment to amplify *atr* housekeeping gene are listed in **Table 1** and **Table 2**. 1 µl of each upstream and downstream primer, 16 µl of nuclease-free water and 2 µl of extracted DNA were added to Master Mix to total volume of 25 µl in each reaction. Electrophoresis in a 2% agarose gel was used for detection of the PCR amplification product.

Antibiotic susceptibility test

The CLSI-recommended Kirby–Bauer disc diffusion technique with modifications on Muller Hinton agar and 5% blood was used to conduct the antibiotic sensitivity test [12]. In this method, antibiotics such as ciprofloxacin, ampicillin, penicillin, chloramphenicol, erythromycin, levofloxacin, vancomycin, tetracycline and levofloxacin were used.

Statistical Analysis

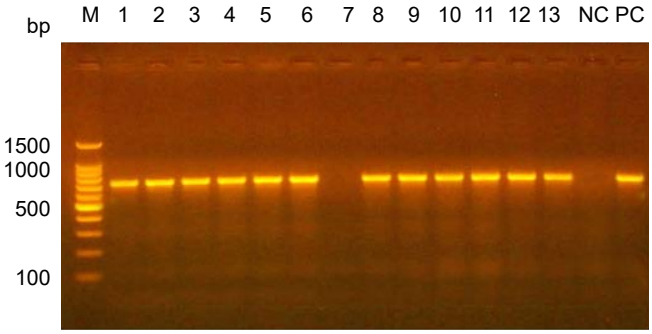
Student t-test was employed for quantitative variables and Chi-squared test was performed whenever available for binomial variables. *P* values less than 0.05 were considered to be statistically significant.

Table 3. GBS Isolates from Women and Their Newborns

Isolates	Frequency	Percentage	Total
Pregnant women	60	20.0	300
Newborns	16	26.6	60

Results

The GBS colonization rate was 20% (60/300) among three hundred pregnant women and 26.6% (16/60) in neonates born of GBS-positive mothers (**Table 3**). In this study, the youngest woman was 15 years old, and the oldest woman was 44 years old, with the average age of participants being 28 years. The various sociodemographic variables are shown in **Table 4** and **Table 5**. On comparing the variations in GBS colonization rates between rural and urban residents, a statistically significant difference was found for GBS colonization among GBS-positive women (78.3% in rural residents versus 21.7% in urban residents; $p = 0.015$).



Electrophoresis of PCR product for *atr* gene on agarose gel. Lane M represents 100 bp DNA ladder, every lane (1–13) except lane 7 represented positive results. Lanes NC and PC represented negative and positive controls respectively.

Table 4. Age, residence and parity of patients

Variables		Culture result		Total	*p*
		negative (*n* = 240)	positive (*n* = 60)		
Age (mean ± SD), years Range		28.44 ± 8.20 (15–44)	29.92 ± 8.60 (15–41)	28.60 ± 8.20 (15–44)	0.3
Residence	Urban *n* (%)	92 (28.4)	13 (21.7)	105 (100.0)	0.015*
	Rural *n* (%)	148 (61.6)	47 (78.3)	195 (100.0)	
Parity	1 *n* (%)	26 (10.8)	7 (11.6)	33 (100.0)	0.2856
	2 *n* (%)	64 (26.6)	15 (25.0)	79 (100.0)	
	> 3 *n* (%)	150 (61.6)	38 (63.3)	188 (100.0)	

Note. *Represent a significant difference at $p \leq 0.05$.

Table 5. Age groups of female participants

Age group, years	Culture		Total	*p*
	negative, *n* (%)	positive, *n* (%)		
15–24	68 (28.4)	15 (20.0)	83 (27.7)	0.784
25–34	148 (61.6)	37 (61.1)	185 (61.6)	
35–44	24 (10.0)	8 (18.9)	32 (10.7)	
Total	240 (100.0)	60 (100.0)	300 (100.0)	

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Table 6. Antimicrobial sensitivity of maternal and neonate isolates

Antimicrobial agent	Sensitivity of GBS Isolates	
	mothers (*n* = 60), *n* (%)	neonates (*n* = 16), *n* (%)
Penicillin	60 (100.0)	16 (100.0)
Ampicillin	60 (100.0)	16 (100.0)
Levofloxacin	55 (91.6)	15 (93.7)
Vancomycin	60 (100.0)	16 (100.0)
Tetracycline	8 (13.3)	4 (25.0)
Ceftriaxone	60 (100.0)	16 (100.0)
Erythromycin	50 (83.3)	13 (81.2)
Chloramphenicol	56 (93.3)	15 (93.7)
Ciprofloxacin	58 (96.6)	16 (100.0)

Molecular detection of all GBS isolates

Conventional PCR was used to detect a specific gene (*atr*) to confirm the bacteriological identification of *Streptococcus agalactiae*. This gene had a molecular size of 780 bp in gel electrophoresis as shown in **Figure**.

Antimicrobial sensitivity

The susceptibility of the isolates towards antibiotics is shown in **Table 6**. Based on the results of antimicrobial sensitivity, it was found that there is more than one antibiotic capable of inhibiting the growth of this bacteria, so they can be used as alternatives in the event of a drug allergy in the patient.

Discussion

Due to the possibility of transmission from the mother to the fetus throughout the pregnancy and the postpartum period, resulting in serious illness or death, research into maternal GBS colonization is crucial. The effect of GBS infection is not restricted to childhood only but may continue to adulthood and may lead to dangerous neurological disorders. The prevalence of GBS maternal colonization worldwide is variable because this prevalence depends on many variable factors like hygienic conditions, socio demographic conditions, sample population, diagnostic techniques, and others. Our research is a conformational vertical transmission study conducted in Babylon (Iraq). This is also the first GBS cohort study that has been done in Babylon. 20% of pre-delivery mothers and 26.6% of neonates born of GBS-positive mothers were found to be colonized with GBS. The colonization rate in mothers observed in our study was comparable to the global data [8, 11].

The percentage of vertical transmission of GBS from infected mothers to neonates in the present study (26.6%) falls within the global report ranges. Compared to other global publications, however, this fraction of vertical transmission is lower than in studies conducted in Kuwait (35.5%), Bangladesh (38.0%) [17], China (7.6–16.7%) [19–21], the United States (53.8%) [22], and Eastern Ethiopia (53.8%) [23]. Other studies, such as those conducted by A. Joachim *et al*. in 2009 in Dar es Salaam, Tanzania (8.9%) [24] and by M. Gizachew *et al.* in Northwest Ethiopia was 10.4% [25], have shown results lower than that in the current study. Our explanation for this variation can be attributed to many reasons such as sample size, techniques used in diagnosis, period of stay in the delivery canal, premature rupture of membrane, and prophylaxis treatment by mothers.

The high vertical transmission rate of GBS contributes to substantial newborn and maternal morbidity and fatality. Vertical transmission of GBS can be prevented, hence healthcare practitioners and government officials must take this into account when developing initiatives to reduce maternal and newborn mortality [26]. Vertical transmission of GBS from a colonized woman to her neonate has not been explored properly, particularly in low income countries. A study investigating the risk variables that could be linked to vertical transmission would therefore help with the formulation of prevention measures. In the present study, we found that three maternal risk factors, mother work and antenatal care follow-up, were strongly associated with the vertical transmission of GBS from asymptomatic colonized mothers to their newborns. Women who had 4–5 ANC visits throughout their current pregnancy had a 20.9% lower risk of vertical GBS transmission to their neonates [17]. According to several studies, GBS colonization in pregnancy may be linked to a variety of factors including education, parity, mother's age, status of marriage, occupation, and an elevated body mass index [27, 28]. A substantial threat for morbidity and death rate in neonates with early-onset GBS illness has been linked to the colonization of the mother's birth canal.

This study showed statistically significant differences between GBS colonization in women in rural areas compared to those in urban areas (78.3% for rural versus 21.7% for urban; $p = 0.015$). This data disagrees with a study conducted in China by S. Li *et al.* in 2018, which showed no significant difference between women residing in rural and urban areas [29]. The plausible explanation for this difference is a lot of variables that make it easier for GBS to spread in rural regions such as low educational status, lack of health care facilities, and contamination from water and other sources. Furthermore, in this study, the neonatal samples were taken from three sites to increase the chance of GBS detection. The sample locations may influence vertical transmission rates. According to a study conducted in Pakistan [30], the risk of acquiring newborn GBS infection was much higher in sites like abdominal skin (53%) than in ear canals (18%). Furthermore, two Turkish studies published in the same year found that the rate of vertical transmission was 54.2% for three sites (throat, ear canal, and umbilicus) and 15.2% for

two sites (throat and umbilicus) [31, 32]. In terms of parity, the prevalence of GBS colonization in our study was greater in more than three parities; the colonization rate of GBS in relation to parity was the highest during the reproductive years [33, 34]. The actual causes of such variable colonization are unknown and require additional research [35]. Thus, further research should be conducted to analyze the association of parity of women with GBS colonization.

The majority (61.6%) of participants in this study were between the ages of 25 and 34 years and had been pregnant at least once. This data differed from a similar study conducted by C. Turner et al. in 2012 on a population of refugees along the Thailand-Myanmar border in Southeast Asia, in which, most of the carriers were in their 20s [36]. These outcomes are similar to those of C. Adware *et al.* from Cameroon in 2008 [37], P. Foumane *et al.* in 2002 in Cameroon [38], A. Mengist *et al.* in 2016 in Ethiopia [39], and N.M. Nkembe *et al.* in 2018 [40], who reported the values to be 75%, 60%, 64%, and 65%, respectively. Our explanations for this age range consist of two reasons, firstly, some women delay pregnancy due to the presence of health issues that prevent pregnancy at the start of a marriage, and secondly, some married couples delay childbearing in the initial years of marriage. In particular, each and every GBS strain was susceptible to penicillin, ampicillin, vancomycin, and ceftriaxone indicating that these antibiotics could be used for preventative purposes. The majority of isolates obtained from mothers were sensitive to ciprofloxacin (96.6%), chloramphenicol (93.3%), levofloxacin (91.6%), and erythromycin (83.3%). In 87% (50/60) of isolates, tetracycline resistance was observed. In other nations such as Tunisia (97.3%) [41] and Iran (96%) [42], tetracycline resistance was extremely prevalent. Its use is currently restricted since the emergence of resistance appears to be linked to the extensive administration of antibiotics [43] and efficient plasmid transfer [43]. Penicillin is the drug of choice for treatment. In the case of penicillin allergy and anaphylaxis, ampicillin or vancomycin might be used as an alternative for treatment.

Nonetheless, the use of PCR to identify GBS and other pathogenic bacteria is crucial. This technique is considered a crucial method in the medical field, it is utilized in a number of medical fields to identify clinical diseases [44–48] and other dangerous genetic diseases such as cancers [49–64].

Conclusion

According to our findings which have demonstrated a high frequency of GBS infection in pregnant women and their newborns, a mandatory screening test for all pregnant women should be implemented, as well as preventive medication should be provided, to avoid the potentially fatal effects of this illness.

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