

Serotype Identification of *Group B Streptococci* isolated from Iraqi pregnant women

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INTRODUCTION:

Group B streptococcus (GBS) or *Streptococcus agalactiae* one of the important microbiotas found in genitourinary tract, as well as the gastrointestinal tract in healthy individual, these bacteria have catastrophic impact, particularly in pregnant women because its leading causes of in early-onset infections (1).

These bacteria colonized the vaginal mucosa through transmitted from the gastrointestinal tract, during pregnancy mainly in 34–37 weeks of gestation can spread into utero by ascending infection or through delivery when the infected fluid from amniotic or vaginal aspirated by the neonates cause of neonatal sepsis. (2). Vertical transmission to the infant occurs in (50%) of deliveries involving colonized women, and (1–3%) of colonized neonates go on to develop invasive disease (3).

Serotyping is a compelling epidemiological method for identification of GBS. There are 10 particular GBS serotypes dependent on capsular polysaccharide antigen, specifically Ia, Ib, II, III, IV, V, VI, VII, VIII and IX (3).

Capsular polysaccharide has been recognized as a noteworthy harmfulness factor of GBS and comprises of the primary synthesis of GBS vaccines (4). The antigenicity and immunogenicity of capsular antigens contrast, among stereotypes and is related to the obtrusiveness of GBS illnesses, the predominant of GBS serotypes changes after some time and shifts with geographic area and populace (5)

The most well-known serotypes among GBS infective disengages are

Ia, Ib, II, III, and V, despite the fact that there are some topographical and authentic varieties (6) For example, the recurrence of type V has expanded over the most few years, though serotype IV has as of late developed as a reason for grown-up and neonatal invasive sickness (7).

The goal of this study was to determine the serotype distribution of group B streptococcus Bacteria isolated from pregnant women.

SUBJECTS AND METHODS

A cross-sectional study included 200 pregnant women at 34–37 weeks of gestation, 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 include; pregnant women who were on antibiotic treatment two weeks prior to recruitment, women with vaginal bleeding. The current research authorized by the ethical committee College of Medicine Al-Nahrain University and its conducted in the department of the medical Microbiology College of Medicine Al-Nahrain University.

Specimens collection and processing
A total of two hundred vaginal swabs was 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 status and specula was not used during swab collection. Swabs inoculated in Todd-Hewitt broth

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media containing 10 µg/ml colistin and 15 µg/ml nalidixic acid, and aerobically incubated at 37°C overnight.

GBS isolated bacteria was diagnosis two approach. The first based on isolated of GBS by culture media, which included Todd-Hewitt broth as an enrichment and selective media for group B streptococcus then sub-cultured on blood agar to select the appropriate colony, while the second method including Gram staining, catalase, Bacitracin sensitivity, Hippurate and Lancefield group to confirm group B Streptococci isolates.

Latex agglutination test for serotyping:

Bacterial isolates of group B streptococcus (GBS) that have been confirmed previously were subcultured in Todd-Hewitt broth for 24 hr at 37 °C, centrifuged for 1 minute at 14xg then 10 µL from this sediment was blended with equal volume from latex suspension in which each vial contain antisera related to serotypes Ia, Ib, and II_IX specific to capsular polysaccharide antigen(Immulex™ strep-B Diagnostica Denmark) The result of the agglutination is then read 30 within seconds.

RESULTS AND DISCUSSION

Culture and biochemical dependent detection of GBS:

Out of 200 samples were cultivated by culture methods, 36 (18%) of bacterial isolates were Beta hemolysis (β -hemolysis) on blood agar, the suggestive colonies for GBS were subjected to farther detection methods, including Hippurate hydrolysis, Bacitracin sensitivity and Lancefield grouping, all isolates were Hippurate hydrolysis positive and Bacitracin resistance. Furthermore, Lancefield grouping showed 36 isolates belong Group B *streptococcus* remainder belong other groups. Table (1).

Table (1): Diagnostic methods of *S. agalactiae*.

No. of swab samples	On culture method	
	Positive results	Negative results
200 samples	36 (18%)	164 (82%)
Hippurate hydrolysis	36 (18%)	164 (82%)
Lancefield grouping	36 (18%)	164 (82%)
Bacitracin sensitivity	36 (18%) R	164 (82%) S

R: Resistance to Bacitracin. S: Sensitive to Bacitracin.

Accurate identification of group B streptococcus colonization is an essential element of laboratory screening of pregnant women to decide eligibility for intrapartum antibiotic prophylaxis and treatment. Consequently, a top-quality screening test ought to show off high reliable detection GBS in clinical samples. GBS-like colonies that develop on Blood agar should always be confirmed as GBS using additional tests to avoid false-positive results. For this reason, in the current study, identification of GBS accomplished with a variety of microbiological tests, including Hippurate hydrolysis, Bacitracin sensitivity and Lancefield grouping. These tests showed a significant correlation in the results with each other's, which enhances the final diagnosis of group B streptococcus in this investigation. Finding in the current study in harmony with the studies conducted in 2017 (8).

Distribution of pregnant women with vaginal colonization, according to gestational age

Out of 200 pregnant women who enrolled in the current study, Women of age 35-36weeks had the highest proportion of GBS infection table (2)

Table (2): Percentage of GBS vaginal colonization in relation to gestational age

	Vaginal colonization	
	Positive	Negative
35 weeks	12 (33.3%)	59 (35.9)
36 weeks	17 (47.2%)	52 (31.7%)
37 weeks	7 (19.4%)	53 (32.3%)
Total	36 (100%)	164 (100%)

In this study, Women of age 35-36 weeks had the highest proportion of GBS infection, this finding is consistent with many background data and research who revealed that bacterial vaginosis more contracted during 35-36 weeks of gestational age. (9,10).

Bacterial vaginosis is a polymicrobial synergistic infection and several factors can be attributable outcome of this infection, one of the most important factors are disequilibrium in the vaginal microbiota which influences by hormonal of human pregnancy such as Oxytocin levels which raise gradually during third trimester.

Serotypes distribution of group B streptococcus based on Capsular polysaccharide Antigen:

Current study showed that among 36 GBS isolates, serotype IV (30.5%) was the most frequent followed by serotype Ia(22.2%), V(19.4%),Ib(11.1%),II(8.3%),II(5.5%),VI(2.7%) respectively. While serotypes (VII, VIII, IX) are not detected, nontypeable strain in this study table (3).

Serotype category of GBS based totally on a capsular polysaccharide, which is one of the critical virulence factors in this bacterium which effect the acquired immune responses by anti-phagocytic character or inhibiting complement-mediated opsonophagocytic killing, such serotype-specific immune

effects may have added to the rise of new container types. (11)

Table (3): Serotype and percentage of Group B streptococcus isolates

Number of GBS isolates (n=36)	Capsular serotype	%
11	IV	30.5
8	Ia	22.2
7	V	19.4
4	Ib	11.1
3	III	8.3
2	II	5.5
1	VI	2.7

Serotype study of GBS has an important role in epidemiological studies as well as in the synthesis of broadly protective vaccines containing capsular polysaccharides or polysaccharides conjugated to protein (12).

Distribution of GBS based on serotyping through the world is distinct from one area to another, and its change from time to time, this variation may be due to ethnic factors, and the time the study was conducted (13.)

Current study, revealed that the overall prevalent GBS serotypes were serotypes IV (30.5%),Ia(22.2%),V(19.4%),Ib(11.1%), III(8.3%) followed by serotypes II(5.5%) and VI(2.7%).

Fabbrini, et al Reported that Serotypes Ia, Ib, II, III, and V are prevalent colonizers in the United States and Europe (14).

While a study of Andrade et al., 2017 found that the most prevalent GBS serotype was Ia followed by Ib, II, III and VI (15). (Dutra et al., 2014) demonstrated GBS serotype geographic differences in Brazilian pregnant women (16). A study conducted in United Arab Emirates and Egypt, showed that serotypes IV and V predominate in the United Arab Emirates and Egypt, respectively (Amin et al., 2002; Shabayek

et al., 2014)(17,18) In addition, another studies, found that a wide range serotype distribution in GBS isolates from clinical cases in the USA, Europe and Australia (Corrêa et al.,(2011) ((Zhao et al., 2008)((Zeng et al., 2006)(19-21).

Th severity and types of invasive human disease varies according to GBS serotypes, the majority of early and late neonatal sepsis is caused by serotype III GBS, and this serotype responsible for most neonatal GBS meningitis and overall the types Ia, Ib, II, III, and V are implicates as the most GBS serotypes that responsible for invasive human GBS diseases.(22).

In conclusion, the results in this study highlight the difference GBS serotype compared with global study, which can assess the health risks resulting from this difference and can facilitate the identification of the type of vaccine and guideline drug of choice.

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Ethical Clearance:

This study authorized by Institutional Review Board (IRB) in the College of Medicine /AL-Nahrain University, and conducted in the Microbiology Department on the College of Medicine Al-Nahrain University.

Conflict of Interest

The author declares that they have no competing interests.

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