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Original article

Identification of Streptococcus Agalactiae and Antibacterial Resistance in Women and Neonates

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Abstract
Streptococcus caucus agalactiae or Group B Streptococci (GBS) is a Gram-positive, that produces β hemolysis on blood agar. It is the most common in the gut of humans and the lower genital tract in women, and remains an important cause of neonatal diseases (sepsis, pneumonia, meningitis). It is the leading cause of early and late-onset neonatal sepsis in the world. The purpose of this study is to isolate specifically from cervical samples in women and neonates to determine the most common and to determine the appropriate antibiotic. In this study, the collection of samples was from Al Zawia and Tripoli areas, during the period from February to April. It includes 125 samples taken from the cervix of women and from different areas of neonates by using medical swabs, where we used different diagnostic techniques to detect Streptococcus (culture test, biochemical test, BD PHOENIX M50 system), and then antibiotic sensitivity testing. The results showed that 26% of the total samples are positive for Streptococcus Agalactiae bacteria. The study concluded that Streptococcus Agalactiae is mostly prevalent among women, while other bacteria are the least prevalent. Moreover, it was demonstrated that bacteria have resistance to Tetracycline (76.92%), Clindamycin (57.69%), Erythromycin (61.54%), Gentamicin (26.92%), Amoxicillin (30.77%), Cefotaxime (53.85%); while it showed sensitivity to penicillin. Therefore, the study recommends that all pregnant women should be screened for GBS with vaginal swabs or urine samples culture during the pregnancy and treated with antibiotics if the culture is positive, and then retested to check for and prevent complications for both the mother and the fetus.
Keywords. Group B Streptococcus (GBS), Pregnant Woman, Neonatal.

Introduction

Streptococcus agalactiae, also known as group B *Streptococcus* (GBS), was first differentiated from other *streptococci* by Rebecca Lancefield in the 1930s after it was isolated from milk and cows with bovine mastitis [1]. Lancefield described GBS colonization of the vaginal tract of asymptomatic women. GBS is a part of the normal gastrointestinal and genitourinary flora of healthy adults and acts as an opportunistic pathogen. It develops from asymptomatic carriage to non-invasive or invasive disease. GBS causes a range of maternal-fetal illnesses during pregnancy and post-partum, from mild urinary tract infections to chorioamnionitis and sepsis in pregnant women to severe neonatal invasive disease such as meningitis or sepsis. Approximately 20 million pregnant women worldwide were colonized by the microorganism in 2020 and nearly 400,000 children suffered from early-onset *S. agalactiae* disease (EOD, 0 to 6 days after birth) or late-onset disease (LOD, 7 to 89 days after birth). In addition, there were 90,000 child deaths, almost half of which were in Sub-Saharan Africa. Approximately 46,000 stillbirths resulting from *in utero S. agalactiae* infection and more than 500,000 preterm births may have been associated with *S. agalactiae* colonization in 2020 [2].

Results collected from different regions indicated inconsistent prevalence. According to the reports from Western countries, especially the United States, 5-35% of pregnant women are carriers of GBS, and 29-70% of newborns are colonized by this bacterium, which predisposes the neonates to infections such as septicemia, meningitis, and pneumonia [3, 4].

The frequency of maternal carriage in vagina have been reported from some developing countries, including Kuwait (14.6%), India (5.8%), Libya (5%), Saudi Arabia (13.9%), Brazil (26%), Nigeria (19.5%) [5] and Ivory Coast (19.3%) [6]. This study aims to isolate and identify GBS in colonized women and neonatal and also to evaluate the antibiotic resistance patterns of GBS isolates. The results of the study will enhance the overall understanding of the prevalence and health impact of GBS infection.

Methods

Sample Collection

The researchers collected 125 samples, 100 of which were vaginal swabs, and 25 samples were Neonatal during February to April, 2024. These samples were collected from different regions. There were 76 samples of vaginal swabs collected from Zawia, 24 vaginal swabs collected from Tripoli, and 25 samples of Neonatal swabs collected from Tripoli.

Culture Media

The cultivation was carried out in the Blood Agar media, prepared 500g in 1 Liter of distilled water. It was brought to a boil to dissolve completely. It was sterilized by autoclaving at 121℃ for 15 minutes. For blood

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agar, it was cooled to 45˚- 50˚C and aseptically added 6% (5-10% is typically) of sterile defibrinated blood. Then, the culture on blood agar media was put in the dish in the incubator for 24 hours at a temperature of 37˚C.

Gram Stain

Normal saline was put on a slide, and from the colony by sterile loop, and mixed, then fiction by heat. The Grain Strain has to go in specific steps. First, a primary stain (crystal violet) was put for one minute. Then, a mordant (Iodine solution) was added for one minute. Next, rapid decolorization was performed using ethanol. The fourth step is counterstaining with safranin for one minute. The last step is to keep the slide dry and to put it under the microscope for examination.

Catalase test

This test demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate those bacteria that produce an enzyme catalase, such as *staphylococci*, from non-catalase-producing bacteria such as *streptococci*. Normally 3% H_2O_2 is used for the routine culture, while 15% H_2O_2 is used for the detection of catalase in anaerobes.

CAMP Test

The CAMP test is a test to identify group B β-hemolytic streptococci (*Streptococcus agalactiae)* based on their formation of a substance (CAMP factor) that enlarges the area of hemolysis formed by the β-hemolysin elaborated from Staphylococcus aureus.

Antibiotic tests

All serogrouping-confirmed specimens underwent the disk diffusion antimicrobial susceptibility test; the tested antibiotics were Tetracycline (10 µg), Gentamicin (10 µg), Amoxicillin (10 µg), Erythromycin (15 µg), Clindamycin (2µg), Cefotaxime (30 µg), Penicillin (0.125µg)

BD Phoenix M50 System

The BD Phoenix M50 Automated Microbiology System is an advanced microbiology instrument designed to streamline microbial identification and antimicrobial susceptibility testing. It uses a combination of broth microdilution and automated reading and interpretation technology to provide accurate and reliable results for a wide range of microbial species.

The BD Phoenix M50 is a versatile system, with the ability to analyze multiple antimicrobial agents simultaneously, and can process up to 50 panels at a time. The system's efficient workflow, user-friendly interface, and connectivity to laboratory information systems make it an essential tool for clinical microbiology laboratories, helping clinicians to diagnose and treat infections with precision and confidence. The BD Phoenix™ M50 system tool provides identification-only panels and combination ID/AST panels, using 51 wells to identify substrates. This system has the feature of offering quick identification of most clinically significant Gram-negative and Gram-positive bacteria.

The BD Phoenix™ automated identification and susceptibility testing system provides accurate and reliable detection of known and emerging antimicrobial resistance.

Results

Sample collection

From the 125 analyzed samples, 26 presented group B *Streptococcus Agalactiae*, which represents a 20.8% colonization prevalence among the studied pregnant women and neonatal.

We collected 25 samples from neonatal aged from 1 day to 5 days, 1 presented group B *Streptococcus Agalactiae,* which represents 4%. 100 samples from women aged from 16 years to 62 years, 25 presented group B Streptococcus *Agalactiae,* which represents 25%.

Table 1. GBS distribution

Sample from	Sample number	Positive group B *Streptococcus agalactiae*	Percent %
Neonatal	25	1	4%
Woman	100	25	25%

The provided data presents the distribution of a sample of GBS-positive women across the four age groups. The first group, with 25-34 years old, represents 40% of GBS-positive women. The second group, between 35 and 45 years old, represents 44% of GBS-positive women. The third group, with 46-55 years old, constitutes 16% of GBS-positive women. However, there is no data regarding women over 55 years old. This indicates that the distribution of GBS-positive women is relatively even between the 35-45 and 25-34 age groups. A lower percentage of GBS-positive women is observed in the group of 46-55 years old.

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Table 2. Age distribution

Age	Percent
25 – 34	40%
35 – 45	44%
46 – 55	16%
Total	100%

As for the weight of 25 women, it was found that 40% of women are in the 60-80 kg weight range, while 60% of women are in the 80-100 kg weight range, as shown in Figure 16.

Table 3. Weight distribution

Weight	Number of pregnant women with GBS	Percent
60 – 80 Kg	10	40 %
80 – 100 Kg	15	60 %

It was observed that in a sample of 25 women with *Streptococcus agalactiae*, 15 (60 %) had urinary tract infection (UTI). This indicates that the proportion of individuals with UTI who have bacteria is higher than the proportion of individuals in the general population who have bacteria.

Table 4. UTI distribution

Number of women with GBS (n=25)	Percentage
15 with UTI	60 %
10 without UTI	40 %

Using blood agar media, there was 26 sample positive for group B *streptococcus* (GBS) (20.8%), 30 sample *klebsiella* (24%), 40 sample *Escherichia coli (*32%), 10 *staphylococcus aureus* (8%), 17 Gram negative *bacilli* (13.6%), 2 samples *Enterococcus faecalis* (1.6%).

Table 5. Comparison between types of bacteria on blood agar media

Types of Bacteria	No. of Bacteria	Percentage (%)
Streptococcus agalactiae	26	20.8%
Klebsiella	30	24%
Escherichia coli	40	32%
Staphylococcus aureus	10	8%
Gram-negative bacilli	17	13.6%
Enterococcus faecalis	2	1.6%

The results of culture media revealed β-hemolysis overnight. As for Gram stain, the group B *Streptococcus Agalactiae* can be seen under the microscope clearly. However, the results of the catalase test were negative as there is no air bubble was found.

Antimicrobial testing

All the samples that were identified as positive for *Streptococcus agalactiae,* used the test on some antibiotics as Penicillin (0.125µg/ml = 10 units), Tetracycline (10 µg), Gentamicin (10 µg), Amoxicillin (10 µg), Erythromycin (15 µg), Clindamycin (2µg), and Cefotaxime (30 µg). The sensitivity profile showed that all the *Streptococcus Agalactiae* were more sensitive to penicillin, clindamycin, and amoxicillin (59.09 %). However, there was resistance to tetracycline, erythromycin, clindamycin (50.98%), and the intermediate gentamicin, amoxicillin, and cefotaxime (100%).

Table 6. The Effectiveness of antibiotics

Antimicrobial	Sensitive (S)	Intermediate (I)	Resistant (R)
Penicillin	23	3	
Tetracycline	2	4	20
Gentamicin	7	12	7
Amoxicillin	10	8	8
Erythromycin	10		16
Clindamycin	11		15
Cefotaxime	6	6	14

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Discussion

The Group *B Streptococcus Agalactiae*_(GBS) colonization prevalence in this study was 20.8%. This percentage is also befitting with the data from the *Center for Disease Control and Prevention* (CDC) [7] that point towards a colonization prevalence from 10% to 30%. Moreover, the prevalence described in this study correlates to the data observed in Taiwan [8] (21.8%), Italy [9] (25.5%), Ethiopia [10] (19.0%), and Pakistan [11]. (17.0%). According to a latest systematic review, it is predicted that GBS will colonize the maternal around the world at a rate of 18% with variations by region from 11% to 35% [12].

However, pregnant women in the studied area were not underwent GBS screening during the prenatal evaluation. The high incidence of GBS found in the current study may thus have a vital role in developing the GBS invasive infection among newborns as it was reported that 1% and 2% of the newborns got infected from colonized mothers [7]. Following CDC recommendations, the CAMP test should be used to identify the GBS biological samples after performing microbial cultivation in 5% blood agar and GBS isolation and identification in selective enrichment broths (e.g. Thioglycolate broth) [7].

In this study, among 26 samples, the distribution of GBS-positive women is relatively even between the 35-45- and 25-34-years age groups, and the lower percentage of GBS-positive women was observed in the 46-55-year age group. As for the weight, it was found that women with 60-80 kg are 40%, and women who weigh 80-100 kg represent 60%.

Furthermore, the CAMP test revealed that serogrouping was 100% sensitive. Although the CAMP test used as a presumptive method, it should not be used as a confirmatory method to identify GBS. In addition to GBS, the CAMP factor is also found in other kinds of bacteria, including *Listeria monocytogenes strains* and streptococci of serogroups A, C, F, and G [13]. It was also demonstrated that the CAMP test should be carried out in a very comprehensive manner, through a standard *Staphylococcus aureus* strain that have not been frozen for a long time. Hence, in order to achieve a high degree of accuracy, the blood agar has to be purchased commercially. Commercial kits are not recommended to be used because they have a short shelf life and the hemolysin strips dry out quickly after the label of the kit is opened.

GBS is one of the pathogens causing UTI [14]. It was observed that in a woman with *Streptococcus agalagtia*, there were 15 (60%) had urinary tract infection (UTI). This indicates that the proportion of individuals with UTI who have bacteria is higher than the proportion of individuals in the general population who have bacteria. Comparing the diagnostic methods as well as the susceptibility profile of the GBS strains circulating in the area of this study was both assessed. This evaluation is very necessary in order to provide effective antibiotic prophylaxis and lower the incidence of neonatal group B streptococcal infection.

The resistance rates of the erythromycin (61.54%) and clindamycin (57.69%) in this study were found to be higher than in other studies, including the studies conducted by [15], [16], and [17]. However, in South Africa, it was reported that the resistance rates of clarithromycin and erythromycin were 17.2% and 21.1% respectively [18]. In Ethiopia, the resistance rates were 3.2% for the clarithromycin and 6.5% for erythromycin [8]; while in Switzerland, the rates were 8.2% for the clarithromycin and 14.5% for erythromycin [19]. Moreover, Pinheiro et al demonstrated that clarithromycin and erythromycin were resistant at the rates of 9.6% and 15% respectively. These results recommended that using clarithromycin and erythromycin as an alternative for pregnant women with penicillin allergy and higher anaphylaxis risk should be complemented with susceptibility tests to choose the most suitable therapy for each case. In this context, it is very crucial to emphasize that pregnant women with penicillin allergy are not very common. As a result, the therapeutic alternatives for the antimicrobial prophylaxis are limited by the significant incidence of antimicrobial GBS strains. It is suggested that the use of broad-spectrum antibiotics is essential; it leads to selection pressure across other microorganisms, resulting in growing antimicrobial resistance [7, 15, 9, 20, and 21]. Most of the recent studies revealed that GBS susceptibility to penicillin is consistent; however, other studies reported that the GBS susceptibility has been reduced [22, 23, and 24]. Therefore, this study did not aim to examine any GBS isolate that shows resistance or reduction of penicillin sensitivity.

Conclusion

Understanding *Streptococcus agalactiae* and its antibacterial resistance is critical in managing infections effectively. Ongoing surveillance, research, and prevention of this pathogen, and ensuring effective treatment options remain available. The findings of this research require more studies, such as assessing the rates of transmission of *Streptococcus agalactiae* from mother to neonates and the detection of serotypes of *Streptococcus agalactiae* with more samples at different areas among Libyan pregnant women and neonates. There should be an increase in the level of awareness and education among healthcare providers and pregnant women about the risks of GBS infection during pregnancy and the importance of screening and antibiotic prophylaxis. Besides, surveillance and reporting systems for GBS infections should be strengthened in order to improve data collection and analysis.

Conflict of interest. Nil

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References

1. Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. J Exp Med. 1933;57(4):571-95.
2. *Curr Opin Pediatr. 2023;35:223-30. doi: 10.1097/MOP.0000000000001223.
3. Edwards MS, Baker CJ. Streptococcus agalactiae (group B Streptococcus). In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th ed. Philadelphia, PA: Saunders; 2015. p. 2340-8.
4. World Health Organization. 2023.
5. Alhhazmi A, Hurteau D, Tyrrell GJ. Epidemiology of invasive group B streptococcal disease in Alberta, Canada, from 2003 to 2013. J Clin Microbiol. 2016;54(7):1774-81.
6. Chaiwarith R, Jullaket W, Bunchoo M, Nuntachit N, Sirisanthana T, Supparatpinyo K. Streptococcus agalactiae in adults at Chiang Mai University Hospital: a retrospective study. BMC Infect Dis. 2011;11:149.
7. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59(RR-10):1-36.
8. Lee WT, Lai MC. High prevalence of Streptococcus agalactiae from vaginas of women in Taiwan and its mechanisms of macrolide and quinolone resistance. J Microbiol Immunol Infect. 2015;48(5):510-6.
9. Matani C, Trezzi M, Matteini A, Catalani C, Messeri D, Catalani C. Streptococcus agalactiae: prevalence of antimicrobial resistance in vaginal and rectal swabs in Italian pregnant women. Infez Med. 2016;24(3):217-21.
10. Mengist A, Kannan H, Abdissa A. Prevalence and antimicrobial susceptibility pattern of anorectal and vaginal group B Streptococci isolates among pregnant women in Jimma, Ethiopia. BMC Res Notes. 2016;9:351.
11. Najmi N, Jehan I, Sikandar R, Zuberi NF. Maternal genital tract colonization by group-B streptococcus: a hospital based study. J Pak Med Assoc. 2013;63(9):1103-7.
12. Russell NJ, Seale AC, O'Driscoll M, O'Sullivan C, Bianchi-Jassir F, Gonzalez-Guarin J, et al. Maternal colonization with group B Streptococcus and serotype distribution worldwide: systematic review and meta-analyses. Clin Infect Dis. 2017;65(Suppl 2):S100-S111.
13. Hardie JM, Whiley RA. Classification and overview of the genera Streptococcus and Enterococcus. J Appl Microbiol. 1997;83(Suppl 1):1S-11S.
14. Rajagopal L. Understanding the regulation of Group B Streptococcal virulence factors. Future Microbiol. 2009;4(2):201-21.
15. Melo SC, Santos NC, Oliveira M, Scodro RB, Cardoso RF, Pádua RA, et al. Antimicrobial susceptibility of Streptococcus agalactiae isolated from pregnant women. Rev Inst Med Trop Sao Paulo. 2016;58:83.
16. Dutra VG, Alves VM, Olendzki AN, Dias CA, de Bastos AF, Santos GO, et al. Streptococcus agalactiae in Brazil: serotype distribution, virulence determinants and antimicrobial susceptibility. BMC Infect Dis. 2014;14:323.
17. Borger IL, D'Oliveira REC, Castro ACD, Mondino SSB. Streptococcus agalactiae em gestantes: prevalência de colonização e avaliação de suscetibilidade aos antimicrobianos. Rev Bras Ginecol Obstet. 2005;27(9):575-9.
18. Bolukaoto JY, Monyama CM, Chukwu MO, Lekala SM, Nchabeleng M, Maloba MR, et al. Antibiotic resistance of Streptococcus agalactiae isolated from pregnant women in Garankuwa, South Africa. BMC Res Notes. 2015;8:364.
19. Lerner PI, Gopalakrishna KV, Wolinsky E, McHenry MC, Tan JS, Rosenthal M. Group B Streptococcus (S. agalactiae) bacteremia in adults: analysis of 32 cases and review of the literature. Medicine (Baltimore). 1977;56(6):457-74.
20. Pinheiro S, Radhouani H, Coelho C, Gonçalves A, Carvalho E, Carvalho JA, et al. Prevalence and mechanisms of erythromycin resistance in Streptococcus agalactiae from healthy pregnant women. Microb Drug Resist. 2009;15(2):121-4.
21. Mousavi SM, Hosseini SM, Mashouf RY, Arabestani MR. Identification of group B streptococci using 16S rRNA, cfb, scpB, and atr genes in pregnant women by PCR. Acta Med Iran. 2016;54(11):765-70.
22. Kimura K, Suzuki S, Yamamoto K, et al. Reduced susceptibility of Streptococcus agalactiae to penicillin. Emerg Infect Dis. 2008;14(9):1423-5. doi:10.3201/eid1409.080079.
23. Cooper K, Abbott F, Gould IM. Reduced penicillin susceptibility of group B Streptococcus: an assessment of emergence in Grampian, Scotland. Br J Biomed Sci. 2016;73(1):25-7.
24. Dahesh S, Hensler ME, Van Sorge NM, Gertz RE Jr, Schrag S, Nizet V, Beall BW. Point mutation in the group B streptococcal pbp2x gene conferring decreased susceptibility to β-lactam antibiotics. Antimicrob Agents Chemother. 2008;52(8):2915-8.

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