



## DETECTION OF VAGINAL COLONISATION OF GROUP B STREPTOCOCCI IN PREGNANT WOMEN BY CULTURE METHODS IN A TERTIARY CARE CENTRE.

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### Abstract:

**Background & Objectives:** *Streptococcus agalactiae* or Group B *Streptococcus* is an important cause of maternal complications in pregnancy and neonatal infections leading to potentially severe ailments due to transmission during delivery. This study aimed to detect *Streptococcus agalactiae* in pregnant women by culture for early detection and prevention of infection.

**Methods:** A total of 90 vaginal swab samples were collected from Index hospital attached to Index Medical College Hospital & Research Centre and were subjected to culture, Gram staining, biochemical reactions, antibiotic susceptibility testing.

**Results:** 5 out of 90 (5.6%) samples showed growth of Group B *Streptococcus* by culture. All the positive isolates were subjected to antimicrobial susceptibility testing by disc diffusion. It was found that all isolates were susceptible to Penicillin (100%) and Vancomycin (100%). Maximum resistance was seen with Tetracycline (25%) followed by Erythromycin (20.83%), Linezolid (12.50%) and Clindamycin (8.33%).

**Interpretation:** Similar rates of colonization are seen in different parts of the country when culture is the primary method of detection.

**Conclusion:** Timely detection of GBS is pivotal in preventing neonatal morbidity and eventually, mortality owing to neonatal meningitis. Antenatal screening for GBS at 35-37 weeks of gestation is recommended. Although culture is the gold standard method for detection, the colonization rates may be under-recognized or under-documented. Penicillin allergy can lead to serious anaphylaxis. Hence, it is important to know the antimicrobial susceptibility pattern of the pregnant women.

**KEYWORDS:** *Streptococcus agalactiae*, vaginal swabs, neonatal infections, antimicrobial susceptibility.

### Introduction:

*Streptococcus agalactiae* is a bacterium which presides in lower part the genital tract of women. It is more commonly known as Group B *Streptococcus* and often, it is present in pregnant women as a colonizer seldom showing symptoms. The anatomical presence of this bacterium is due to migration of GBS from the gastrointestinal region to the genital tract. <sup>(1)</sup>

GBS is a Gram positive cocci. It exhibits beta hemolysis, which causes complete zone of clearing which can be appreciated in media like blood agar plates. GBS also generates antigens that are made up of type specific polysaccharide surrounding the bacterium, with all the capsular polysaccharides

bearing a terminal chain; a side chain with sialic acids as their primary antigenic determinant. GBS occurs as a part of normal vaginal microflora and typically do not cause any symptoms. <sup>(2)</sup>

Albeit frequently asymptomatic, GBS has the potential to cause infections like urinary tract infections chorioamnionitis, postpartum endometritis, febrile illness, and in rare cases, endocarditis in pregnant mothers as well. The colonization in childbearing women with GBS imposes a threat for the newborn babies, neonates and infants. Pregnant mothers may also deliver prematurely owing to GBS leading to complications in the newborn. <sup>(3)</sup>

Approximately half of the GBS infections in newborns manifest within the first 7 days of life, known as Early Onset Disease (EOD) with majority of cases presenting within hours of birth, leading to severe condition such as pneumonia, shock, sepsis, stillbirth and also a perinatal mortality rate of 10 to 20%. If GBS infection occurs after 7 days of life or specifically, between 7-90days after birth, it is known as Late Onset Disease (LOD). A landmark achievement to the perinatal health community will be the recognition of *Streptococcus agalactiae* infection in mother's genital tract as the prime root of genesis for neonatal morbidity and mortality. <sup>(4)</sup> Studies and research bear citations and documentation that transmission rates from mothers colonized with GBS to their infants are 29 times greater than those from noncolonized mothers. Depending on cross ethnic groups and geographical locations, prevalence of GBS vaginal and/or rectum colonization varies significantly, typically falling between approximately 10% and 40%. <sup>(5)</sup> Colonization rates can be detected in pregnancy by appropriate screening of the pregnant women. Screening for Group B *Streptococcus* (GBS) can be approached in mainly two ways :– Screening based on selective risk factors and antibiotic prophylaxis or antenatal screening all pregnant women universally at 35-37 weeks gestation and antibiotic prophylaxis.<sup>(6)</sup> Group B  $\beta$ -hemolytic *Streptococcus* or *Streptococcus agalactiae* often abbreviated as GBS are distinguished by the presence of a Lancefield-grouping antigen, which is a cell surface polysaccharide that is type-specific and crucial for the bacterium's classification. This Lancefield-grouping antigen aids in distinguishing GBS from other bacterial groups. Additionally, GBS also contains various protein antigens on its cell surface that serves to its immunological profile, playing a significant role in the identification, study, and understanding of the bacterial reactions with the host immune system and its general pathogenicity <sup>(7)</sup> Group B *Streptococcus* (GBS) is classified into 10 distinct serotypes on the basis of its distinct capsular polysaccharide (CPS) antigens. These capsular polysaccharides are critical virulence factors for GBS isolates serving antiphagocytic functions, aiding the bacteria in evading the host's immune defences. Due to their significant role in bacterial virulence, CPS antigens in bacterial virulence has led to their use as key components in new multivalent GBS vaccines development. These vaccines aim to provide comprehensive protection by targeting multiple GBS serotypes simultaneously, leveraging the antiphagocytic properties of the CPS antigens to enhance the host's immune response against GBS infections. <sup>(8)</sup> Vertical transmission, occurring through recto-vaginal maternal colonization is widely recognized as the most critical determinant of neonatal infection. This mode of transmission significantly escalates the risk of infection for neonates, who are exposed to the bacteria during the birthing process. Specifically, neonates born to women colonized with GBS face an alarming increase in risk, being more likely to acquire this potentially serious infection compared to their counterparts born to women who are not colonized. This stark disparity in infection risk highlights the imperative of vigilantly monitoring and managing GBS colonization in expectant mothers to safeguard the health and well-being of their newborns reinforcing the importance of targeted interventions and preventive strategies in obstetric care.<sup>(9)</sup> GBS colonization rates exhibit notable variability on a global scale influenced by diverse culture methods which encompass variations in the number and types of anatomical sites sampled and the specific growth media utilized for detection. Nevertheless, regardless of these dissimilarities, substantial and genuine regional variations in GBS colonization persist, highlighting differences in local epidemiological dynamics, healthcare practices and diagnostic methods. These methodological nuances play an important role for some of the observed disparities in colonization rates across different regions. <sup>(10)</sup> In India, determining the prevalence rate during pregnancy can provide crucial and invaluable information, given the fact that at least 1-2% of childbirth from mothers with GBS

during delivery go on to manifest in to early-onset GBS disease that can lead to severe complications in newborns. This information is essential for understanding the potential burden of neonatal GBS disease in the Indian context and for informing targeted public health strategies aimed at preventing and managing it. Spontaneous deliveries also occur in many cases. Furthermore, with evidence and data as such, it can be used to guide the development of national guidelines and protocols for GBS screening and management, implement appropriate interventions, such as intrapartum antibiotic prophylaxis. <sup>(11)</sup> The impact of neonatal infections on the global disease burden is substantial. In nations where economy lags behind the rest of the world or developing nations, the cases of neonatal infections are approximately 6.9 million every year. This high incidence is accountable for at least half a million deaths in the world as reported in 2012. Among the various pathogens responsible for these infections, Group B Streptococcus (GBS) stands out as a leading cause, having been identified for over five decades. Its role is also well known in causing invasive GBS (iGBS) diseases in young infants. <sup>(12)</sup> When early-onset disease (EOD) is established as invasive Group B *Streptococcus* (GBS) disease occurring in infants from the day of birth to 6 days after birth and late-onset disease (LOD) as occurring in infants a week or 7 days after to 89 days of birth, a review will be done comprehensively citing that EOD is roughly twice as common as LOD. However, this prevalence does not hold true for all regions, with certain Asian locations, such as Hong Kong, India, and Thailand, reporting EOD rates up to six times greater than the prevalence of LOD. <sup>(13)</sup> Over the past thirty years, reducing the vertical transmission of Group B *Streptococcus* (GBS) to newborns has been a major priority. The most effective and efficient method involves routine checkup or screening tests for pregnant women to detect GBS colonization followed by administering intrapartum antibiotics. <sup>(14)</sup> In countries that are equipped with better infrastructure and developed economically, routine screening for pregnant women and the implementation of intra-partum antibiotic prophylaxis (IAP) have been broadly adopted and have significantly decreased the incidence of morbidity and mortality in neonates due to GBS. Conversely, in low-income regions, despite similar rates of recto-vaginal colonization with *S. agalactiae* among pregnant women, screening and IAP to avert invasive disease are largely not practiced because of constraints in health resources, lack of knowledge and inaccessibility. <sup>(15)</sup> Early recognition of Group B *Streptococcus* (GBS) colonization in pregnant mothers is crucial for timely interventions. These timely identifications can help prevent neonatal disease by facilitating antibiotic prophylaxis. This proactive approach not only reduces the risk of transmission during childbirth but also important in comprehensive screening and management. <sup>(16)</sup> Efforts have been initiated to evolve and effectuate a rapid enrichment combined with antigen detection test designed to swiftly detect Group B *Streptococcus* (GBS) colonization in pregnancy within less than 8 hours. This innovative diagnostic approach can help reducing the cases of neonatal septicemia by early diagnosis and therapeutic management. <sup>(17)</sup> There are a few of studies from India and Pakistan that enumerates *Streptococcus agalactiae* as the primary or one of the leading organisms behind cases of sepsis of neonates <sup>(18)</sup> But only a handful of evidence from research articles have been documented till date from India and as such our knowledge regarding the true prevalence of GBS is not reliable and remains unknown. The lack of appropriate, timely screening during pregnancy In short, published data regarding the prevalence of *Group B Streptococci* in pregnant women are scanty from Indian subcontinent. is one of the prime causes. It is safe to say that GBS is very likely under reported from India. <sup>(19)</sup> The present study aims to detect the presence of Group B streptococci from vaginal swabs of pregnant woman attending a tertiary care center, in central India.

### Materials & Method:

After obtaining approval and clearance from the institutional ethics committee, the patients fulfilling the inclusion criteria will be enrolled for the study after obtaining informed consent.

### Sample collection

Vaginal swabs from pregnant women consenting to the study will be collected maintaining aseptic precautions and immediately transported to the laboratory. The swabs used will be Sterile Flocked

Nylon Swab with break point (HiMedia, Mumbai). Excessive vaginal secretions or discharge will be wiped off and the swab will be inserted into vagina, about 2cm inside the vaginal introitus. <sup>(20)</sup>

### Sample transport

Swabs will be transported to laboratory within 4 hours and will be placed into enrichment media, Todd-Hewitt broth supplemented with Colistin (10µg/ml) and Nalidixic acid (15µg/ml) (known as Lim broth).

### Processing of sample

Swabs placed in the in-enrichment broth medium will be incubated at 37°C for 18-24 hr and enriched broth will be observed for turbidity. If no turbidity will be seen, broth medium will be incubated for another 24 hours. A loop full of turbid broth will be plated on to 5% Sheep Blood Agar plates and incubated at 37°C under microaerophilic condition i.e. in candle jar containing 5-10% CO<sub>2</sub> for a period of 24-48 hours. The 5% Sheep Blood Agar plates will be examined at 24 and 48 hours of incubation. Group B *Streptococcus* will be identified based on β-hemolysis on 5% Sheep blood agar, colony morphology, Gram staining, Hippurate hydrolysis, CAMP factor. All the suspected *Streptococci agalactiae* colonies will be also identified using standard laboratory protocol and automated culture method by VITEK-2 system, automated instrument for ID/AST testing, BioMerieux Diagnostics. <sup>(21-23)</sup>. Further confirmation of the organism was done by Hippurate hydrolysis and CAMP test.

### Antibiotic Sensitivity Testing

Antimicrobial susceptibility testing (AST) will be done by Kirby Bauer disc diffusion method on Mueller-Hinton Agar (MHA) with 5% sheep blood. Colonies from overnight culture on sheep blood agar plate will be suspended in Mueller Hinton or 0.9% saline to a density equivalent to the turbidity of a 0.5 McFarland Standard. Into the suspension made, a sterile swab will be dipped and after removing excess inoculum by rolling the swab firmly against the side of the tube, we used the swab to streak the MHA with 5% Sheep blood agar plate. The required specific antibiotic discs will be pressed on to the agar to firmly place them on the media with the help of a sterile forceps under aseptic precautions. The plates will be incubated at 37°C for 24 hours. Isolates will be interpreted as resistant, intermediate and sensitive as per the latest CLSI M100, 34<sup>th</sup> Edition, 2024 guidelines (109). The drugs tested will be Penicillin(10µg), Erythromycin (15 µg), Clindamycin (2 µg), Tetracycline (30 µg) and Vancomycin (30 µg) as per CLSI M100, 34<sup>th</sup> Edition, 2024. <sup>(24)</sup>

### RESULTS:

A total of 90 vaginal swabs from pregnant women attending Index Medical College Hospital & Research Centre, Indore at gestational age 35-37 weeks was collected from January 2022 to January 2025.

### Age distribution of the patients

The age of the pregnant women included in the study ranged from 19 to 40 years of age.

**Table 1: Age distribution of the study population**

Age of the pregnant woman	Number of pregnant women	Percentage
<20 years	1	1.1%
21-30 years	81	90%
31-40 years	8	8.9%
>40 years	0	0

**Table 2: Age group of pregnant women with GBS.**

Age of the pregnant woman	Number of GBS positive women	Percentage
<20 years	0	0
21-30 years	24	100%
31-40 years	0	0
>40 years	0	0

It was observed that all the GBS samples were isolated from women belonging to the age group 21-30 years.

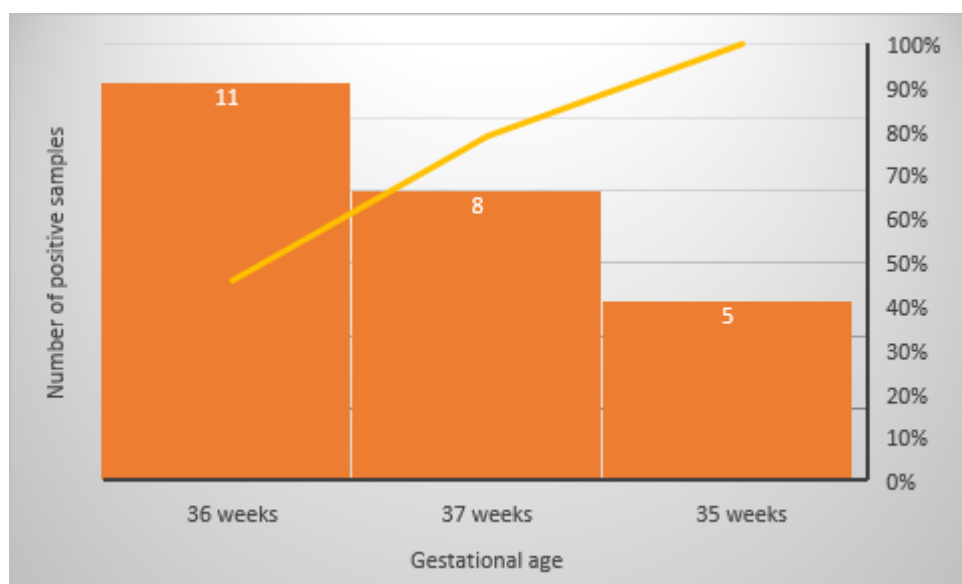
**Table 3: Gravida status of the total pregnant women**

OBSTETRICAL CODE	NUMBER OF WOMEN	PERCENTAGE
PRIMIGRAVIDA	51	57%
MULTIGRAVIDA	39	43%

In our study, 51 of the total women were primigravidae, while 39 of them were multigravida. Among the total 24 positive samples, 14 of these women were primigravida i.e., their first pregnancy. While 10 of the women had previously been pregnant and were multigravida women.

### Gestational age:

The study population chosen for our study was pregnant women in gestational age 35-37 weeks. We found that majority of pregnant women were in the gestational age of 37 weeks followed by 36 and 35 weeks.



**Fig 1: Gestational age of the women with GBS organism**

Overall, GBS positivity was higher from pregnant women at gestational age 36weeks. 11 of the total women with GBS were in the gestational age of 36 weeks. 8 women were in 37 weeks and 5 in 35 weeks.

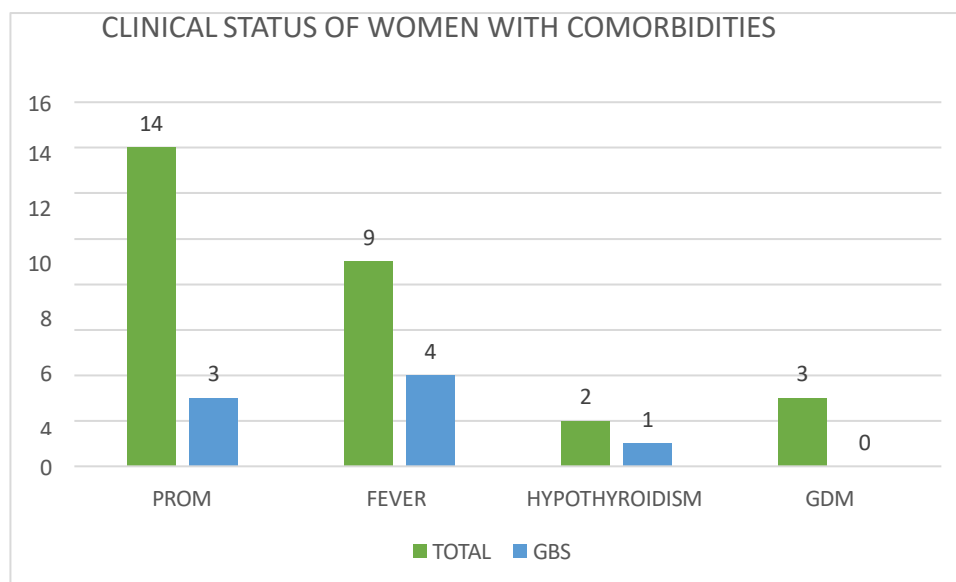
### Risk Factors and Comorbidities:

In this study, premature rupture of membranes (PROM) was present in 14 i.e., 15.5% of total pregnant

women. During the time of delivery, 9 pregnant women had presented with fever.

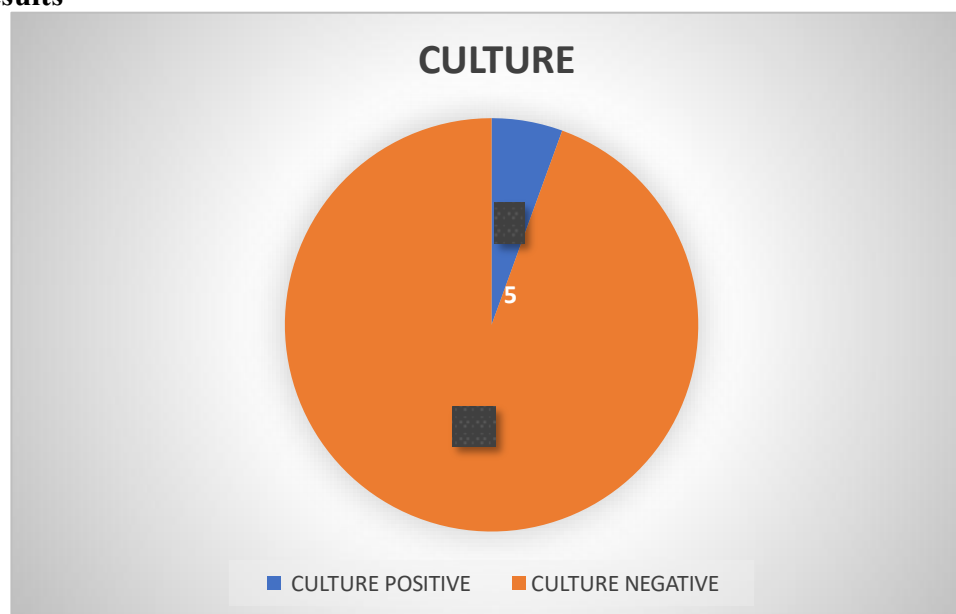
Other co-morbidities like Gestational Diabetes Mellitus (GDM) were present in 3 mothers. 2 women had past history of Hypothyroidism (HYPOTHYROIDISM).

Out of 14 women who presented with premature rupture of membranes, 3 women with PROM were positive for GBS. 4 women with fever were positive for GBS. 1 out of 2 women with past history of Hypothyroidism was identified to be GBS positive while pregnant women with GDM had negative culture or PCR results for GBS.



**Fig 2: Bar diagram showing women with GBS and their clinical risk factors**

### Culture Results



**Fig 3: Number of culture positive Group B *Streptococcus* isolates**

A total of 90 vaginal swab samples were collected from pregnant women at gestational age 35-37 weeks. 5 of the total 90 vaginal swabs showed growth on 5% Sheep blood agar plate culture that was confirmed with biochemical reactions and automated Vitek-2(BioMérieux) compact system. This yielded a positivity percentage of 5.6%.

### Antimicrobial Susceptibility Testing

The antibiotic susceptibility pattern of the isolates was identified by Disc diffusion method using drugs specific for Group B Beta hemolytic streptococci based on latest CLSI guidelines. The vaginal isolates were analysed for sensitivity to Penicillin, Erythromycin, Clindamycin, Tetracycline, Vancomycin, Linezolid. It was found that all the GBS positive isolates were sensitive to Penicillin (100%) and Vancomycin (100%). Maximum resistance was seen to Tetracycline (25%) followed by Erythromycin (20.83%), Linezolid (12.50%) and Clindamycin (8.3%).

**Table 4: AST results of the GBS isolates**

Drugs	Total Isolates	Sensitive (S)	Resistance (R)	Sensitive %	Resistance %
Penicillin	24	24	0	100	0
Erythromycin	24	19	5	79.17	20.83
Clindamycin	24	22	2	91.67	8.33
Tetracycline	24	18	6	75	25
Vancomycin	24	24	0	100	0
Linezolid	24	21	3	87.50	12.50

### DISCUSSION

In the present study, 90 vaginal swabs sample were obtained from pregnant women. Vaginal colonization in the women with Group B *Streptococcus* was determined by culture, and biochemical reactions, and antibiotic sensitivity of the isolates was performed.

Age distribution of the samples showed that most of the pregnant women screened were in the range 19-40 years of age. Group B *Streptococcus* was isolated by culture from 5 women in the age group 26-30 years. This is similar to a study conducted by Assefa et al., and Alshahrani et al., where the age group of total population was 18-39 years with higher frequency of isolation from age group 20-29 (72.9%) and 18-46 years with maximum cases from age group 18-39 years respectively. (25,26)

This age range also concurs with the study by Hariharan et al., at a tertiary care hospital in Chennai, India where the pregnant women that were included in the study were in the age range 19-35 years of age and higher frequency of GBS isolated from pregnant women ranged from ages 23-28 years. (27) In another study by Khan et al., it was found that the majority of the population of pregnant women was in the ages between 17-47 years. They further observed that the rate of colonization increased with age. (28)

**Table 5: Comparison of age distribution among various studies**

AUTHORS	AGE DISTRIBUTION OF STUDY POPULATION
Present study	19-40
Assefa et al	18-39
Alshahrani et al	18-46
Hariharan et al	19-35
Khan et al	17-47

In the present study, 51 women were primigravida and 39 were multigravida. This is similar to a study by Petca et al., where 50.7% women were primigravida and 49.3% were multigravida. (29) while the data found in the present study is in contrast to Laycock et al., where 56.9% were multigravida and 43.1% were primigravida. (30) Ali et al., in their study included 280 pregnant women and observed that the percentage of primigravida women was 53.6% and 46.4% women in the study were multigravidas. (31) In a study by Balan K et al., which included only 100 women also found that majority of GBS isolated was from primigravida women followed by second

gravida and multigravida. GBS was isolated from 4 primigravida women i.e, 12.5%.(32)

**Table 6: Comparison of gravida status among various studies**

AUTHOR	PRIMIGRAVIDA	MULTIGRAVIDA
Present study	57%	43%
Petca et al	50.7%	49.3%
Ali et al	53.6%	46.4%

In our present study, Group B *Streptococcus* was found in 5 out of 90 pregnant women by culture yielding a culture positivity rate of 5.6%. This is very similar to the data by Gurudas et al, in Kerala where GBS was isolated by culture from 4.8% of the mothers screened during pregnancy for *Streptococcus agalactiae*.(33) The studies conducted in South East Asian countries and primarily India, show similar rates, some studies in India have documented even lesser rates while studies in Africa show higher positivity rates. There is also difference in prevalence rates within different states of India itself due to various factors.

Present study rates concurs with Arif et al., who in their study in Mumbai, India that included 100 pregnant women isolated GBS from 4 women. The culture positivity rate was 4%. (34)

Goel et al., in their study amongst 450 women found the vaginal colonization rate to be 3.3% by culture. (9) This data aligns with the current study rates. In another study by Vinod et al., GBS isolation by culture on 5% Sheep blood agar from 126 pregnant women identified similar rates with current study. 5.5% rectal swabs and 3.17% swabs from vagina were positive for Group B *Streptococcus*.(31) In a study conducted in CMC Vellore, India by Santhanam et al, conducted an observational study to find the GBS colonization rate in pregnant area. It was observed that when vaginal samples were cultured primarily into blood agar plates, the colonization rate was 2.6% while using an enrichment medium increased the rate to 7.6%, which closely resembles the rate of present study.(14) Higher rates of isolation were also seen from a few studies in India that showed a prevalence rate of 15% and 14.3% (11,121) while studies showing lower rates than detected in present studies have also been documented where the culture positivity rates were 2%.(2) All the isolates that were found to have Group B *Streptococcus* by culture and PCR were subjected to antibiotic sensitivity pattern by Kirby- Bauer Disc diffusion method as per latest CLSI guidelines. All the isolates showed sensitivity to Penicillin and Vancomycin. Maximum resistance was seen for Tetracycline and subsequently by Erythromycin, Linezolid and Clindamycin. This aligns with a study by Sharmila et al., in South India. All the GBS colonized pregnant women were sensitive to Penicillin as well as Clindamycin while 14.3% were resistant to erythromycin and 71.4% were resistant to tetracycline. In a study by Balan et al., similar results to antibiotics was observed where all the isolates were sensitive to Penicillin, Vancomycin, Clindamycin.(32) Another study by R et al., in a tertiary level hospital in India found the maximum amount of resistance to Tetracycline and all the isolates were sensitive to Penicillin, Vancomycin.(31) Our results concur with a study by Van du et al., which revealed high resistance for tetracyclines. All the test isolates were sensitive to Penicillin and Vancomycin (100%) but highest resistance was detected for Tetracyclines followed by Erythromycin (89.66%) and Clindamycin (76.23%).(100) A study in Egypt by Abdullah et al., revealed 100% sensitivity to Penicillin while 76% and 61% resistance was depicted for Clindamycin and Erythromycin.(35) Berg et al., in their study documented that all the isolates were sensitive to Penicillin and Vancomycin while resistance was seen for Erythromycin (45.2%) and Clindamycin (28.7%).(36)

## Conclusion:

*Streptococcus agalactiae* or Group B *Streptococcus* (GBS) infection affects pregnant women and their newborns during childbirth causing potentially severe infections like neonatal sepsis, meningitis and preterm births. Prompt diagnosis of GBS during pregnancy relies on effective antenatal screening of women and necessary intrapartum antibiotic prophylaxis.

There is a lack of appropriate guidelines for antenatal screening of pregnant women for GBS in our



country and thus, a majority of neonatal deaths that unfortunately occurs within first seven days of life after birth are due to GBS which was not detected or identified during early pregnancy. Regular screening for this bacterium is necessary to avoid the risk of infection and subsequent morbidities to the mother and child.

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