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Characterization of Group *B Streptococci* from Rural Women Attending Primary Health Centre

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Abstract

The purpose of this study was to screen both pregnant and non-pregnant women in rural areas for GBS. A total of 30 vaginal samples were collected from patients attending Primary Health Care Centre, Cuddalore. The samples were immediately transported to BHI broth in an ice pack and taken to the laboratory. Samples were inoculated into 5% blood agar plates, incubated at 37˚C in an anaerobic jar for overnight. Both hemolytic and non-hemolytic colonies were identified by standard biochemical tests, Anindita Das *et al.,* (2003). The grouping was done by micro nitrous acid extraction, co-agglutination technique. Antibiogram was performed b y Kirby Bauer Disc Diffusion method. Similarly, 30 cervical were analyzed by direct plating. Out of the 15 samples, from non-pregnant women 26.67% (4) were positive out of 15 samples from pregnant women 13.33% (2) were positive for GBS. 40% and 33.33% were positive for Staphylococcus species and Gram negative rods respectively from non-pregnant women. 46.67% and 40% were positive for Staphylococcus species and Gram-negative rods respectively from pregnant women, Badri *et al.,* (1997). All the six isolates were resistant to antibiotics like Erythromycin, Neomycin and Tetracycline and were sensitive to other antibiotics like Cephotaxime, Penicillin G and Ampicillin. Therefore screening for GBS carriers in non-pregnant women is necessary, Berkowitz *et al.,* (1990).

Keywords: Cervical swabs, Group B *Streptococcus* (GBS), hemolytic and non-hemolytic, Antibiogram, BHI broth.

Introduction

Streptococci are gram positive, spherical or oval cells arranged in pairs or chains of varying each cell is approximately 1 micron in diameter, non-motile, non-sporing and may be capsulated They are widely distributed in nature Some are member of the normal flora (human) others are associated with important human disease attributable in part to infection in *Streptococci,* in part to sensitization to them *Streptococci* elaborate a variety of extra cellular substances and enzymes.

Streptococci are a heterogenous group of bacteria and no one system suffices to classify them the majority are aerobes of facultative anaerobes but there are species that are anaerobic or microaerophilic The aerobes may be divided into those that produce a soluble hemolysin and those that do not Twenty species, including *S.pyogenes* (group A) *S.agalactiae* (group B) and the Enterococci (group D) and characterized by combination of features colony growth characteristics, hemolysis patterns on blood agar [alpha-hemolysis, beta-hemolysis or no hemolysis], antigen composition of group specific cell wall substances and biochemical reactions, *Streptococcus pneumoniae* (*pneumococcus*) types

are further classified by the antigenic composition of the capsular polysaccharides, Kristensen, *et al.*, (1995).

GROUP B STREPTOCOCCUS: [*Streptococcus agalactiae*]

Group B Streptococcus (GBS) is a gram positive encapsulated bacterium possessing an array of immune resistance phenotype and secreted toxins that render it capable of producing serious disease in susceptible host, in particular the human neonate **(**Chris Keenam, 1998), Approximately 20-30% of healthy women are colonized recto-vaginally with GBS, and 50-70% of infants born to these women will themselves become colonized with bacterium**.** These are the important cause of septicemia, pneumonia and meningitis in newborn children as well as cause of serious disease in adults.

i) Physiology and Structure:

Group B *Streptococci* are gram-positive cocci (0.6-1.2 µm) that form short chains in clinical specimens and longer chains in culture, features that make them indistinguishable on gram stain from *S.pyogenes.* The colonies of *S.agalactiae* are buttery with a narrow zone of beta hemolysis on nutritionally enriched media. Some strains (1-2%) are non hemolytic, although their prevalence may be under estimated because non- hemolytic strains are not commonly screened for group B antigen.

Strains of *S.agalactiae* can be subdivided on the basis of three serological markers:

i) The B antigen of group specific cell wall polysaccharide antigen (composed of rhamnose, N-acetyl glucosamine and galactose)

ii) Type specific capsular polysaccharides (Ia, Ib and II to VIII)

iii) The surface protein, C protein.

Eleven immunologically distinct serotypes have been described: Ia, Ia/c, Ic, II, IIc, III, IV, V, VI, VII and VIII. These serotypes are important epidemiological markers with serotypes Ia, III and V being most commonly associated with colonization and disease.

ii) Pathogenesis and Immunity:

The pathogenesis of neonatal GBS infection begins with the asymptomatic colonization of the female genital tract. Pathogenesis will be grouped into thematic categories:

a) Adherence to epithelial surfaces.

b) Penetration of host cellular barriers.

c) Avoidance of immunologic clearance mechanisms and

d) Inflammatory activation.

a) Adherence to epithelial surfaces

GBS adhere to variety of human cells including vaginal epithelium, placental membranes, and respiratory tract epithelium and blood brain barrier endothelium. Maximum adherence occurs at the acidic pH of vaginal mucosa (Finch *et al.*, 1976), which allows vertical transmission to infants. A low affinity GBS interaction with epithelial cells is mediated by its amphiphilic cell wall associated lipoteichoic acid, while higher affinity interactions with host cells are mediated by a series of size variable, pronase-sensitive hydrophobic GBS surface proteins. These proteins are known to interact with host cell-anchored proteins such as integrins and have been demonstrated to mediate adherence of relative gram-positive pathogens (Schwartz- Wibawan, *et al.*, 1995).

b) Penetration of host cellular barriers

While, attachment mechanisms allow GBS to compete with other micro flora for a niche on the gastro intestinal and vaginal mucosa, the ability of the organism to penetrate host cellular barriers is the first distinguishing feature of its pathogenecity. Nevertheless, GBS can traverse placental membrane and weaken their tensile strength, a process that is speculated to involve local generation of oxygen radical and prostaglandin E2.

GBS may access the fetus within the amniotic cavity; include placental membrane rupture or trigger premature delivery. After aspiration of infected amniotic or vaginal fluid, the new born lung is the initial focus of GBS infection, then

rapidly gains access to blood stream and is circulated through other organs and tissues (Lammler *et al.*, 1995).

c) Avoidance of immunologic clearance:

Once the GBS injures or penetrates cellular barriers to reach the blood stream of deeper tissues, an immunologic response is called upon to clear the organism. Central to this response are host phagocytic cells including neutrophils and macrophages. Moreover the effective uptake and killing of GBS by these cells requires opsonization of the bacterium by specific antibodies or serum complement. Neonates are particularly prone to GBS invasive disease because of quantitative or qualitative deficiencies in phagocytic cell function, specific anti GBS immunoglobulin, or the classic and alternate complement pathways.

Almost all GBS associated with human disease are encapsulated, belonging to one of the nine recognized type-specific capsule serotypes: Ia, Ib and II through VIII. The serotype specific epitopes of each polysaccharide are created by different arrangements of four component sugars (glucose, galactose, N-acetylgluocsamine and sialic acid) into a unique repeating unit, but unfailingly these structures contain a terminal sialic acid bound to galactose. The isolated GBS capsule protects GBS by interference with opsono phagocytosis. Another way GBS avoids antimic robial peptide clearance is through the D-alanylation of lipoteichoic acid in the bacterial cell wall**.**

Another factor allowing GBS to survive inside phagocytes is the production of an orange carotenoid pigment, a property unique among hemolytic *Streptococci*. The free radical scavenging properties of the carotenoid neutralize hydrogen peroxide and singlet oxygen, therefore providing a shield against the key elements of phagocyte oxidative burst killing**.**

Materials and Methods

Cervical swabs (Barry Dashefsky 1987) were collected from pregnant women and normal female attending Primary Health Care centre, Pudhucerry.

- *Staphylococcus aureus* ATCC 25923 (beta lysing producing) used for CAMP test.

Serogrouping Antiserum:

- The GBS isolates were confirmed in a reference laboratory at JIPMER, Pudhucerry.

METHODS

Sample collection:

- Cervical swabs were collected as per the standard procedure in **Bailey and Scott's – Diagnostic Microbiology.** Patient's, details were collected as in the proforma sheet.
- Mucus was removed by gently rubbing the area with the cotton ball.
- The swab was then inserted into the cervical canal,
- Rotated moved from side to side for 30 seconds before removal.
- All samples were collected before antibiotic therapy.

(ii) Transportation: (Carol J. Baker *et al.*, 1973)

The collected specimen was immediately transported in BHI broth in ice pack to the laboratory.

↓

The specimens were inoculated into 5% sheep Blood Agar Plate.

↓

Plates were incubated at 37° C in anaerobic jar with 10% CO_2 for 24 hours.

↓

Both hemolytic and non-hemolytic colonies were processed and identified by standard biochemical tests.

iii) Biochemical Tests:

a) CAMP Test:

CAMP test was performed as per the standard procedure given in **Practical Medical Microbiology by Mackie & Mc Cartney**

Beta lysine producing *Staphylococcus* was streaked on 5% Sheep Blood agar.

↓

Then a single streak was made with the *Streptococcus* strain perpendicular to the *Staphylococcal* streak leaving a space of 1 cm between the two organisms.

↓

The plates were incubated anaerobically at 37° C in 10% carbon dioxide for 24 hours.

↓

An arrowhead formation of haemolysis was taken as positive. Control strain was included in each test performed.

b) Hippurate Hydrolysis:

Hippuate hydrolysis was done as per the produce given in **Practical Medical Microbiology** by **Mackie & Mc Cartney**

1% solution of sodium hippurate was prepared and dispensed in 0.4 ml volumes in screw-capped tubes.

↓

A loopful of the isolate was transferred from blood agar plate to the tube containing broth.

↓

Incubated for 2 hours at 37^0C

↓

0.2ml of ninhydrin solution was added to detect the glycine end product (3.5g ninhydrin in100:1 ml mixture of acetone and butanol)

↓

Hydrolysis of hippurate was indicated by deep purple colour and considered positive; negative reactions were colourless.

↓

Control strain was included for each batch.

c) Bacitracin Sensitivity: Chakshu Gupta and Laurence Edward Briski, (2004)

Bacitracin sensitivity test was done as per the procedure of **Myer's and Koshi's Manual of Diagnostic Procedures in Medical Microbiology and Immunology/Serology**

The test organism was lawn cultured into the MHA with sheep blood and 0.04-0.05 IU of bacitracin per disc were applied.

↓

The plates were incubated at 37^0C for 24 hours under anaerobic condition.

↓

After incubation, clearance of zone around the discs was measured.

↓

No zone was seen in GBS (isolated from vaginal specimen)

d) Serogrouping:

The isolates were serogrouped in **Myer's and Kosih's** Manual of diagnostic procedures in Medical Microbiology and Immunology/Serology Micro Nitrous Acid Extraction Method

↓

20µl of 2% sodium nitrite solution was taken in a test tube

↓

3 to 4 colonies of β hemolytic *Streptococci* were inoculated.

↓

3µl of glacial acetic acid was added to the suspension and left at room temperature for 15 minutes.

↓

16 to 24µg of sodium bicarbonate were added for neutralization.

↓

60µl of sterile distilled water was added.

↓

Extract is now ready for co-agglutination (Co-A).

Co-Agglutination Technique (Park, 2002)

1 drop of micro nitrous acid extract was placed into rings/cavities of the slide using Pasteur pipette.

↓

1 drop of sensitized Staphylococcal reagent (A, B, C, and G) was added to each ring (cavity).

↓

Mixed by rotation for about 30 minutes
The mixture was observed for evidence of agglutination against diffused light.

↓

Grade results of agglutination were given as 1+, 2+, 3+, 4+ depending on the degree of clumping and cleaning were observed.

↓

The group reagent, which gives the strongest agglutination, was denoted as the group of *Streptococcal* isolates.

e) Antibiogram: (Stephanie J. Schrg, 2000)
Antibiogram was performed by Kirby Bauer Disc Diffusion method as per the standard procedures. The method was followed as recommended by the **National Committee for Clinical Laboratory Standards (NCCLS 1984, 1987) and the World Health Organization (WHO).**

The test organism was inoculated into BHI broth. Standardization of the inoculums was essential to provide reproducible results. A properly prepared Mc Farland 0.5 turbidity standard (working party report 1991) was helpful in achieving the correct working inoculums of 10^7 CFU/ml.

The test organism was lawn cultured into the Mueller Hinton agar with 5% Sheep blood and antibiotic disc were applied.

↓

The plates were incubated at 37^0 C for 24 hours under anaerobic conditions.

↓

After incubation diameter of zone around the discs were measured.

↓

Control strain was included for each batch of antibiogram performed.

Results and Discussion

The data of samples collected from non-pregnant female18 to 33 ages. Majority of the samples were from the age group 26 to 30 years in total samples of 8. The data of samples collected from pregnant women of different age groups. Maximum numbers of samples were obtained from 26 to 28 years. Total number of pregnancy in trimesters during which the 15 numbers of samples were collected and high percentage of Total isolation of GBS from non-pregnant women (26.67%) than pregnant women (13.33%). All the GBS isolated showed beta hemolysis in 5% Sheep Blood Agar. The results of GBS isolated from both pregnant and non-pregnant women.

The percentage of isolation of Number of positive samples from non pregnant Women (%) 26.67 %(4) GBS and Number of positive sample from pregnant Women (%) 13.33 % (2) GBS, *Staphylococcus* of Number of positive samples from non pregnant Women 40 % (6) and Number of positive sample from pregnant Women *Staphylococcus* species 46.67 % (7) other Gram Negative Rods (GNR) from both non-pregnant 33.33 % (5) and pregnant women 40 % (6).

The sensitivity (David N.Greenberg *et al*., 1995) of GBS to various antibiotics Out of 15 samples, 4 isolates were resistant to Erythromycin, Neomycin and Tetracycline and sensitive to Ampicillin, Penicillin G and Cephotaxime. All the isolates were from the age groups of 18 to 23 years.

The sensitivity of GBS to various antibiotics Out of 15 samples, 2 isolates were resistant to Erythromycin, Neomycin and Tetracycline and sensitive to Ampicillin, Penicillin G and Cephotaxime. All the isolates were from the age groups of 23 to 25 years.

The purpose of this study was to screen both pregnant and non-pregnant women in rural areas for GBS.

A total of 30 vaginal samples were collected from patients attending Primary Health Care Centre, Cuddalore. The samples were immediately transported to BHI broth in an ice pack and taken to the laboratory. Samples were inoculated into 5% blood agar plates, incubated at 37˚C in an anaerobic jar for overnight. Both hemolytic and non-hemolytic colonies were identified by standard biochemical tests. The grouping was done by micro nitrous acid extraction, co-agglutination technique. Antibiogram was performed b y Kirby Bauer Disc Diffusion method.

Similarly, 30 cervical were analyzed by direct plating. Out of the 15 samples, from non-pregnant women 26.67% (4) were positive out of 15 samples from pregnant women 13.33% (2) were positive for GBS. 40% and 33.33% were positive for Staphylococcus species and Gram–negative rods respectively from non-pregnant women.

Table .1
Age wise data of samples from non pregnant women

S.No	Age (years)	No of samples
1	18-20	6
2	21-25	4
3	26-30	8
4	31-33	5
	TOTAL	20

Table 1 shows the data of samples collected from non-pregnant female. Majority of the samples were from the age group 26 to 30 years.

Table.2 Age wise data of samples from preganant female

S.No	Age (years)	No of samples
1	18-21	1
2	22-25	4
3	26-28	7
4	29-32	3
	TOTAL	15

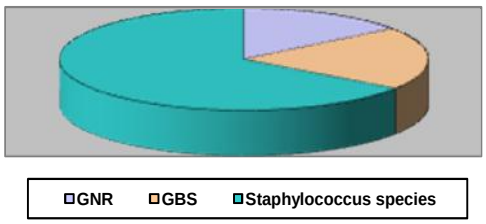
Table no 5.2 shows the data of samples collected from pregnant women of different age groups. Maximum numbers of samples were obtained from 26 to 28 years.

Table .3 Collection of samples at different trimesters of pregnancy

S.No	Duration of pregnancy (Trimesters)	No. Of samples
1	2nd Trimester	7
2	3rd Trimester	8
	TOTAL	15

Table no. 5.3 shows the data of duration of pregnancy in trimesters during which the samples were collected.

FIG.3
ISOLATION OF BACTERIAL FROM CERVICAL SWABS FROM NON PREGNANT WOMEN



Tables .4 percentage of isolation of gbs indifferent age groups from non- preganant and preganant women (in trimesters)

S.No	Samples	Age groups	% of isolation
1	Non-pregnant women	18-23	26.67%(4)
2	Pregnant women (2^{nd} trimester)	23-25	13.33%(2)

Table no. 5.4 shows a high percentage of isolation of GBS from non-pregnant women (26.67%) than pregnant women (13.33%). All the GBS isolated showed beta hemolysis in 5% Sheep Blood Agar.

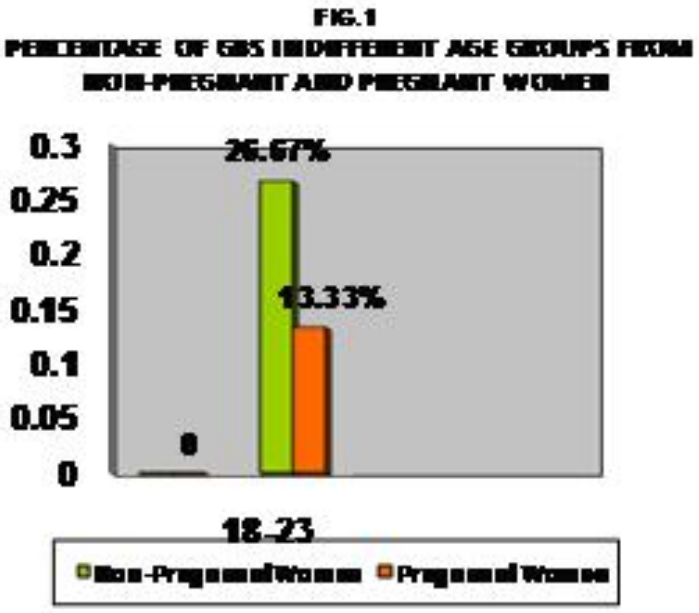


Table 5 identification of GBS

S.NO	TESTS	RESULTS
1	Gram's Staining	Gram Positive cocci in chains
2	Catalase	Negative
3	Hemolytic pattern in Blood agar	Beta hemolysis
4	Hypurrate hydrolysis	Deep purple color formation
5	CAMP	Arrow head formation
6	Serogrouping	Agglutination with group specific antisera

Table .5 shows the results of GBS isolated from both pregnant and non-pregnant women.

Table .6 isolation of bacteria from cervical swabs

S. No	Isolates	No. Of positive samples from non pregnant Women (%)	No. Of positive sample from pregnant Women (%)
1	GBS	26.67 %(4)	13.33 % (2)
2	*Staphylococcus* sp	40 % (6)	46.67 % (7)
3	GNR	33.33 % (5)	40 % (6)

Table no. 5.6 shows the percentage of isolation of GBS, *Staphylococcus* species and other Gram Negative Rods (GNR) from both non-pregnant and pregnant women.

Table .7 Antibiotic sensitivity pattern of GBS isolates from non-perganat women

S.No	Age groups (year)	No. Of samples	No of GBS positive	E	N	Amp	P	Ct	T
1	18-20	5	2	R	R	S	S	S	R
2	21-23	3	2	R	R	S	S	S	R
3	24-26	6	-	-	-	-	-	-	-
4	27-30	1	-	-	-	-	-	-	-

R - Resistant, S- Susceptible, E- Erythromycin (10 mcg/disc), N- Neomycin (30mcg/disc)
Amp – Ampicillin (10 mcg/disc), P- Pencillin G (10 units), Ct - Cephotaxime (30 mcg/disc)
T- Tetracycline (30 mcg/disc)

Table no. 5.8 shows the sensitivity of GBS to various antibiotics. Out of 15 samples, 4 isolates were resistant to Erythromycin, Neomycin and Tetracycline and sensitive to Ampicillin, Penicillin G, and Cephotaxime. All the isolates were from the age groups of 18 to 23 years.

Table .8 antibiotic sensitivity pattern of GBS isolates from pregnant women

S.No	Age groups (year)	No. Of samples	No of GBS positive	E	N	Amp	P	Ct	T
1	20-22	1	-	-	-	-	-	-	-
2	23-25	4	2	R	R	S	S	S	R
3	26-28	7	-	-	-	-	-	-	-
4	29-32	3	-	-	-	-	-	-	-

R-Resistant,S- Susceptible, E- Erythromycin (10 mcg/disc), N- Neomycin (30mcg/disc)
Amp– Ampicillin (10 mcg/disc), P- Pencillin G (10 units), Ct- Cephotaxime (30 mcg/disc)
T- Tetracycline (30 mcg/disc)

Table 8 shows the sensitivity of GBS to various antibiotics. Out of 15 samples, 2 isolates were resistant to Erythromycin, Neomycin and Tetracycline and sensitive to Ampicillin, Penicillin G, and Cephotaxime. All the isolates were from the age groups of 23 to 25 years.

46.67% and 40% were positive for Staphylococcus species and Gram-negative rods respectively from pregnant women.

All the six isolates were resistant to antibiotics like Erythromycin, Neomycin and Tetracycline and were sensitive to other antibiotics like Cephotaxime, Penicillin G and Ampicillin. Therefore screening for GBS carriers in non-pregnant women is necessary.

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