

Serotyping of invasive and colonizing group B *Streptococcus* (GBS) isolates at selected hospitals in Sri Lanka: a multicenter study

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Abstract

Background: Group B *Streptococcus* (GBS) causes significant morbidity and mortality in neonates, pregnant women and patients with underlying comorbidities. Intrapartum antibiotic prophylaxis (IAP) is currently the mainstay of prevention and effective vaccine against invasive GBS disease is under clinical trial.

Objectives: To describe the serotype distribution of invasive and colonizing GBS isolates in Sri Lanka.

Methods: Probable GBS isolates from high vaginal swabs (HVS) and sterile body sites were collected from eight selected hospital laboratories. Following confirmation of the identification as group B *Streptococcus* by phenotypic methods including Lancefield grouping test (Plasmatic UK), isolates were tested for serotyping by latex agglutination test kit (STATEN serum institute, Denmark).

Results: Out of the 145 probable GBS isolates only 100 from HVS and 37 from sterile body sites were confirmed as GBS. Serotype III was the most predominant in invasive GBS isolates followed by Ia, Ib, VI, II and V in the descending order of frequency. Serotype VI was the most predominant in HVS isolates followed by serotype III, V, Ia, II, Ib and IV. Difference of GBS serotype distribution between the invasive and HVS group was statistically significant (P value = 0.038)

Conclusion: Serotype distribution pattern of the study isolates was comparable to most of the other developing and developed countries and hence will be beneficial in future vaccine introduction. GBS vaccine which is currently under clinical trial (Ia, Ib and III) is potentially effective for preventing 68% of the early onset disease in neonates in this study setting.

Introduction

Group B *Streptococcus* (GBS), also known as *Streptococcus agalactiae* is recognized as a predominant pathogen causing septicaemia and meningitis in neonates and young infants, and also significant pregnancy related morbidity. It can cause invasive illness in non-pregnant adults with underlying co-morbidities as well. Asymptomatic colonization with GBS may occur in the genital tract and the lower gastrointestinal tract of both pregnant and non-pregnant women at a range of 10%-40%. In men, 10%-25% carriage occurred in the GI tract [1].

Intrapartum antibiotic prophylaxis (IAP) is currently the mainstay of prevention. It helps to reduce the incidence of invasive disease in pregnant women and early onset invasive disease (EOD) of the newborn. Centers for Disease Control and Prevention (CDC) recommends to screen pregnant mothers with lower vaginal and rectal swabs at 35 to 37 weeks of gestation and to treat prophylactically with antibiotics if indicated [2]. However, chemoprophylaxis has several issues and studies are ongoing with immunoprophylaxis as a preventive measure [3].

One major problem that may arise with the use of IAP is the spread of antibiotic resistance over time. Even though it is recommended to screen all pregnant women it may not be feasible in every clinical setting due to many reasons. Screening and treatment are not always possible in resource poor settings in the developing world. Further, the premature deliveries which occurred in less than 35 weeks of gestation may get excluded from the screening process and there may be false negative laboratory results of screening tests as well. Finally, IAP has no significant benefit on late-onset neonatal disease (LOD) at the age of 7 to 89 days [2].

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To overcome the problems associated with IAP, a glyco-conjugate vaccine is under clinical trials and offers the potential for prevention of infections in pregnant women and young infants. Because the invasive disease of the neonates is associated with low concentration of serotype specific maternal antibodies at the time of delivery, this approach will be more appropriate than prophylactic antibiotics, specially for developing countries [3].

Currently, there are 10 serotypes of GBS identified based on 10 immunologically unique capsular polysaccharides (Ia, Ib, II-IX) [4]. An ideal vaccine should have the protection against all 10 existing serotypes. However, currently ongoing clinical trials include trivalent conjugate vaccine which has the protection against serotypes Ia, Ib and III, which are responsible for approximately 80% of the neonatal invasive GBS infection globally [3].

Serotype distribution varies geographically and therefore it is a timely need to know the main circulating serotypes which indicate the specific serotypes which should be included in the vaccine for a given country. This is the first study which describes Group B Streptococcal serotype distribution in Sri Lanka.

Methods

Ethics approval and consent to participate

This study was approved by the ethics review committee at Medical Research Institute, Sri Lanka and written informed consents were retrospectively obtained from patients or guardians of patients from whom the isolates were collected.

Study setting

GBS isolates were collected from Microbiology laboratories of National Hospital of Sri Lanka (NHSL), Colombo South Teaching Hospital (CSTH), Lady Ridgeway Hospital (LRH), Castle Street Hospital for Women (CSHW), De Zoysa Maternity Hospital (DMH), Sri Jayawardenapura General Hospital (SJGH), Teaching Hospital Karapitiya (THK) and Teaching Hospital Anuradhapura (THA).

Laboratory tests were carried out at the department of Bacteriology, Medical Research Institute (MRI) Sri Lanka.

Study sample

Isolates which were identified as GBS from clinical samples which had been sent from the patients to hospital microbiology laboratories were included in the study. Isolates which were recovered from high vaginal swabs were considered as colonizing isolates and those recovered from sterile body sites were considered as invasive isolates.

Study duration

Invasive isolates which have been identified as GBS were collected from all selected laboratories during the period commencing from obtaining ethical clearance up to the end of study period (From 19th May 2016 to 31st March 2017). Isolates which have been identified as GBS from HVS of pregnant women were collected from the CSHW and DMH during the 4 month study period (from 1st of December 2016 to 31st of March 2017).

Study design

Descriptive cross sectional study.

Inclusion criteria

All invasive isolates which have been identified as GBS by the selected centers during the study period and all colonizing isolates from HVS which have been identified as GBS by the selected centers during the study period were included in the study.

Exclusion criteria

After subculturing the isolates, identification was confirmed as GBS by routine microbiological test methods and those isolates which were negative for group B by the confirmatory Lancefield grouping test were excluded.

Once identified as GBS in hospital laboratories, the isolates were preserved in blood glycerol broth containing cryo vials at - 20°C until dispatch to MRI. Upon receiving, cryo vials were stored at - 70°C until the tests were performed.

Bacterial isolates in blood glycerol broth were subcultured on sheep blood agar (MAST[®]) and MacConkey agar (MAST[®]) and incubated at 35°C overnight. Grayish white colonies of 3-4 mm size with narrow zone of beta haemolysis on sheep blood agar and with growth in MacConkey agar were preliminarily identified as *Streptococcus* species and further confirmed with Gram positive cocci in Gram stain and negative catalase test. Lancefield grouping test was done for all beta haemolytic *Streptococcus* isolates using Plasmatec streptococcal grouping kit according to manufacturer's instructions. Confirmed GBS isolates were tested for serotyping by latex agglutination test with type specific antisera of Denmark STATEN[®] serum institute according to the manufacturer's instructions.

Data analysis

Data were entered and analysed by the SPSS 21st version and results were described by using frequency, percentage and chi square. Invasive and colonizing isolates were separately analysed where appropriate.

Results

*Descriptive statistics***Table 1. Distribution of probable GBS isolates in HVS and invasive samples**

<i>Hospital</i>	<i>HVS</i>		<i>Invasive</i>		<i>Total</i>
	<i>Number</i>	<i>%</i>	<i>Number</i>	<i>%</i>	
NHSL	0	0	7	17.94	7
CSTH	0	0	11	28.20	11
LRH	0	0	5	12.82	5
THK	0	0	5	12.82	23
SJGH	0	0	4	10.25	4
CSHW	38	35.8	4	10.25	34
DMH	68	64.2	1	2.56	59
THA	0	0	2	5.12	2
Total	106	100	39	100	145

Total number of 145 isolates which have been identified as GBS by selected hospital laboratories were collected. Hundred and six isolates were from HVS and 39 were from the sterile sites. Of the 106 HVS isolates, 100 were confirmed as GBS while 37 were confirmed as GBS from 39 sterile culture isolates.

Blood cultures were the most predominant invasive specimen type with 35 (94.59%) out of 37 invasive samples in which the confirmed GBS were isolated. There were 2 (5.4%) GBS isolates from pus cultures.

Of the 37 total confirmed GBS isolates 22 (59.5%) were from the neonates with EOD. Eight were from the adults < 65 years and three were from the elderly patients > 65 years of age. Two isolates were from the neonates with LOD and infants > 90 days.

Table 2. Distribution of GBS serotypes in invasive and HVS isolates

<i>Serotype</i>	<i>Invasive</i>		<i>HVS</i>		<i>Total</i>
	<i>Frequency</i>	<i>%</i>	<i>Frequency</i>	<i>%</i>	
Ia	9	24.3	10	10	19
Ib	5	13.5	6	6	11
II	4	10.8	9	9	13
III	13	35.1	28	28	41
IV	0	0	1	1	1
V	1	2.7	12	12	13
VI	5	13.5	34	34	39
VII	0	0	0	0	0
VIII	0	0	0	0	0
IX	0	0	0	0	0
Total	37	100	100	100	137

Serotype III (35.13%) was the most predominant among invasive isolates followed by serotype Ia (24.32%), Ib (13.5%), VI (13.5%), II (10.81%) and V (2.7%) in the descending order of frequency. Serotype VI (34%) was the most predominant among HVS isolates followed by III (28%), V (12%), Ia (10%), II (9%), Ib (6%) and IV (1%).

Table 3. Significance of serotype distribution between invasive and HVS isolates

<i>Serotype</i>	<i>Chi square value</i>	<i>P value</i>	<i>Significance</i>
Ia + III	5.052	0.024	significant
III	0.655	0.418	not significant
Ia	4.639	0.031	significant
VI	5.566	0.018	significant

HVS and invasive GBS isolates were compared in relation to the serotype distribution.

After considering the two most predominant serotypes of invasive GBS isolates together (III+Ia), and the other invasive GBS serotypes collectively (Ib +II+IV+V) a significant difference of (III+Ia) was observed between HVS and invasive groups. (p=0.024)

However serotype III (most predominant in invasive isolates) when compared with all other serotypes collectively (Ia+Ib+II+IV+V+VI), there was no significant difference of serotype III distribution between invasive and HVS groups. (p=0.418)

Serotype Ia (second commonest in invasive isolates) was compared with the other serotypes collectively (Ib+II+III+IV+V+VI), and a significant difference was observed in the serotype Ia distribution between invasive and HVS groups. (p=0.031)

Serotype VI (most predominant in HVS isolates) when compared to other serotypes collectively (Ia+Ib+II+III+IV+V), there was a significant difference of distribution between invasive and HVS groups. (p=0.018)

Table 4. GBS serotypes distribution according to age group

<i>Age group</i>	<i>Serotype</i>										<i>Total</i>
	<i>Ia</i>	<i>Ib</i>	<i>II</i>	<i>III</i>	<i>IV</i>	<i>V</i>	<i>VI</i>	<i>VII</i>	<i>VIII</i>	<i>IX</i>	
EOD (1-6 days)	5	2	4	8	0	0	3	0	0	0	22
LOD (7- 90 days)	0	0	0	2	0	0	0	0	0	0	2
Infants > 90 days	2	0	0	0	0	0	0	0	0	0	2
Children 1-5 years	0	0	0	0	0	0	0	0	0	0	0
Children 5-18 years	0	0	0	0	0	0	0	0	0	0	0
Adults < 65 years	2	1	0	2	0	1	2	0	0	0	8
Elderly > 65 years	0	2	0	1	0	0	0	0	0	0	3
Total	9	5	4	13	0	1	5	0	0	0	37

Of the 22 GBS isolates with EOD, majority (8, 36.3%) were caused by serotype III followed by Ia (22.7%), II (18.2%), VI (13.6%) and Ib (9%). Both isolates from LOD were of serotype III.

Two isolates in the age category of children > 90 days up to 1year were due to serotype Ia.

Of the eight GBS isolates in the adults < 65 years, two each represented serotype Ia, III and VI. Other two were serotype Ib and V.

Two of the three GBS isolates from the elderly age group (> 65 years) showed serotype Ib and the remaining isolate was of serotype III.

Table 5. Age group distribution according to the serotype

Age group	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	Total
Early onset disease	5	2	4	8	0	0	3	0	0	0	22
Late onset disease	0	0	0	2	0	0	0	0	0	0	2
Infants > 90 days to 18 years	2	0	0	0	0	0	0	0	0	0	2
Adults < 65 years	2	1	0	2	0	1	2	0	0	0	8
Elderly > 65 years	0	2	0	1	0	0	0	0	0	0	3
Total	9	5	4	13	0	1	5	0	0	0	37

Of the nine serotype Ia isolates five (55.5%) were received from the patients with EOD. Two each of the serotype Ia was distributed among children > 90 days up to 18 years group and adults < 65 years.

All four of the serotype II were isolated from the patients with EOD.

Of the thirteen serotype III isolates eight (61.53%) were from the patients with EOD. Two each of the serotype III were from the patients with LOD and one was from an elderly (> 65 years) patient.

The single isolate represented serotype V was received from an adult (65 years) patient.

Three of the five serotype VI (60%) isolates were from the patients with EOD and the remaining two isolates were from the adults < 65 years.

Discussion

Considering the lower prevalence of invasive GBS disease, several collection centers and longer time period was identified to collect invasive GBS isolates, whereas only two collection centers (DMH and CSHW) for shorter period was identified for HVS isolates due to higher prevalence of maternal GBS in pregnant women.

This is the first study done in Sri Lanka to describe the circulating GBS serotypes. Out of the 37 invasive GBS isolates serotype III was the most predominant followed by Ia, Ib, VI, II and V in the descending order of frequency. This pattern of prevalence is closely similar to that described in a systematic review and meta-analysis including 19 studies [5].

Above serotypes except serotype V in varying percentages caused EOD in the current study. Serotype III (36.3%) was the predominant and followed by Ia (22.7%), II (18.2%), VI (13.6%) and Ib (9%) in EOD. Two cases of LOD were due to serotype III. Our findings were comparable to that of South Africa where they have observed type III as the commonest in EOD and LOD [6]. In both studies serotype III was predominated in LOD than EOD.

Two cases of infants more than 90 days were caused by serotype Ia. Accordingly Serotype III, Ia and II were responsible for more than 80% of the invasive disease of infants in this study population. Serotype Ia, Ib and III were responsible for 90% of the early onset and late onset GBS diseases in a multi-country observational study [7]. Previous studies done in United States also described serotypes III and Ia as the most common serotypes causing invasive disease of the infants [8].

Serotypes Ia, Ib and III which is included in the trivalent GBS vaccine which was undergone several clinical trials in humans, were present in 73% of the infants whereas serotypes Ia, Ib, II, III, IV and V which has been described in the latest hexavalent vaccine trials in animals, were present among 84.6% of the infants in our study population [9].

Presence of serotype VI in invasive GBS isolates in our study population is an uncommon finding compared to most of the studies done elsewhere.

Serotype distribution of adults <65 years is variable in our study population. Seventy five percent of the invasive GBS infection in adults were due to serotype Ia, III and VI whereas others were caused by Ib and V.

However, the results cannot be generalized because of the low number of cases. Serotype Ia, III and V were predominated in reproductive age women and pregnant women in North America and China respectively [4,10]. In Canada, serotype V was the most predominant in adult invasive disease [11].

Comparable to the study done by Madzivhandila et al. (2011) where he has described a significant difference of serotype III between invasive and colonizing GBS isolates, a significant difference of serotype distribution between invasive and HVS groups was observed with serotype III+Ia combination (predominant invasive serotypes) and serotype Ia (second commonest invasive serotype) in our study population. However, there was no significant difference of serotype III between the two groups of current study.

Data were not analyzed based on the gender and age because of the low number of cases in the study population.

Colonizing GBS isolates in HVS

GBS serotype VI was the most predominant in colonizing HVS isolates followed by III, V, Ia, II, Ib and IV in the descending order of frequency. Serotype VI, III and V were responsible for 74% of the colonizing GBS isolates. Compared to this 81% of the colonizing GBS isolates were due to serotype III, V and Ib in Central Africa [12].

Although the majority of EOD and all of the LOD were caused by serotype III, serotype VI was predominated in HVS of pregnant women in our study population and difference of serotype VI distribution between the invasive and colonizing GBS is significant. However serotype III which was predominated in EOD and LOD was the second commonest HVS serotype in the present study.

Even though serotype VI which was the commonest in colonizing isolates in this study, it is less common elsewhere and was the second commonest colonizing isolate in Japan [13]. However serotype VIII which is the most predominant in Japan was not present in our study population.

Except serotype IV which was only present in colonizing isolates, other serotypes were distributed in both groups in different percentages in present study.

Conclusion

Present study indicates the common serotypes that lead to invasive disease and colonizing HVS in the study setting. In the current era of ongoing GBS vaccine trials, this pattern of local serotype distribution will probably indicate the serotypes that should be included in the vaccine for our country.

Serotype III and VI are the most predominant GBS types in invasive and HVS isolates respectively in this

study population. Serotype distribution in invasive GBS isolates in the current study is comparable to most of the other developing and developed countries and will be beneficial in future vaccine introduction. GBS vaccine currently under human clinical trials (Ia, Ib and III) is potentially effective for preventing 73% of invasive GBS disease of infants and 68% of EOD in neonates in the study population.

Authors' contributions

RC, SP and NC designed the study and took part in developing the protocol and coordinated hospitals. RC collected isolates, performed the laboratory tests including serotyping, drafted the initial version and did statistical analysis. SP and NC provided critical comments on the draft and managed subsequent revisions. All authors reviewed and approved the final version for submission.

Conflicts of Interest

We declare that we have no conflicts of interest.

Acknowledgement

We acknowledge all the patients from whom the isolates were collected.

Ethics approval

This study was approved by the Ethics Review Committee at Medical Research Institute, Sri Lanka (Project No: 14/2016) and all the participant hospitals.

Patient consent (for case reports where the material is identifiable)

Informed consent was obtained.

Source of funding (if relevant)

This study was supported by grants from Medical Research Institute, Sri Lanka. The funding supported for consumables and reagents for conducting microbiological tests.

Abbreviations (where relevant)

CDC - Center for Disease Control and Prevention

EOD - Early onset disease

GBS - Group B *Streptococcus*

HVS - High vaginal swab

IAP - Intrapartum antibiotic prophylaxis

LOD - Late onset disease

MRI - Medical Research Institute

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