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REVIEW ARTICLE



Group B *Streptococcus* colonisation and their antimicrobial susceptibility among pregnant women attending antenatal clinics in tertiary care hospitals in the Western Province of Sri Lanka

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ABSTRACT

The proportion of Group B Streptococcus (GBS) colonisation in pregnant women >35 weeks of gestation was 18% and 49% by culture and real-time PCR respectively in selected hospitals from the Western Province of Sri Lanka. A Descriptive cross-sectional study was conducted from January to April 2019. Two low vaginal and rectal swabs were collected from 100 pregnant women. Identification of GBS was done by culture and real-time PCR. GBS isolates were found to be sensitive to penicillin, ampicillin, cefotaxime, vancomycin, while 5 and 4 isolates out of 18 were resistant to erythromycin and clindamycin, respectively. Further, there was a significant association between GBS colonisation and a history of vaginal discharge and unemployment.

IMPACT STATEMENT

- What is already known on this subject? Prevalence of GBS colonisation in the vagina and rectum of pregnant women in developing countries ranges from 8.5% to 22%. The Conventional method of culture has been considered the gold standard for diagnosis, however, the culture method does not give positive results for all cases of GBS. Polymerase chain reaction (PCR) has been found to be more sensitive for the detection of GBS than culture. In Sri Lanka, ante-natal screening for GBS is not practiced as the prevalence of GBS is still unlcear due to non-availably of data. Only a few scattered studies have been conducted using culture in Sri Lanka. Thus there is an urgent need to determine the magnitude of the GBS colonisers of ante-natal women in order to set up guidelines for screening and management of GBS.
- What do the results of this study add? In this study, the overall GBS colonisation rate which was detected using both culture and PCR was 50% in Western Province of Sri Lanka. That was a high figure when compared to the figures which were detected previously in Sri Lanka using only conventional culture methods. The risk factors for GBS colonisation were found to have a significant relationship with the history of abnormal vaginal discharge. Further, it was found that when Candida species coexisted with GBS, the existence of GBS was enhanced. Penicillin remains the antibiotic of choice for GBS.
- What are the implications of these findings for clinical practice and/or further research? This study emphasises the importance of establishing national policies for screening of pregnant women of >35 weeks of gestation to reduce the risk of neonatal infection. Further, it gives an insight into the options of antibiotics that can be used for treatment of these GBS colonisers from Sri Lanka.

KEYWORDS

GBS colonisation; real-time PCR; pregnant women; antibiotic resistance; risk factors

Introduction

Group B Streptococcus (GBS) is a major cause of sepsis in newborns in developed countries and a causative agent of disease in the elderly and in immunocompromised. It is also a coloniser of the colon and found in genitourinary tract and throat. Approximately, 10%–40% of pregnant women are known to carry this organism in their rectum or vagina (Wernecke et al. 2009). Prevalence of GBS colonisation in vagina and rectum of pregnant women in developing countries ranges from 8.5% to 22% (Munir et al. 2016). Carriers of

GBS are at a risk of colonising their newborns by vertical transmission; thereby increasing susceptibility to neonatal infections. Prolonged rupture of membrane, infection of intra-amniotic fluid, pregnancy at young age are risk factors predisposing to neonatal infections by GBS (Kim et al. 2011).

Approximately, 2200 early-onset GBS infections are detected yearly in the USA (American College of Obstetricians and Gynecologists Committee on Obstetric Practice 2011). A study conducted in 2006 at the Colombo South Teaching

Hospital Sri Lanka found a colonisation rate of 26.7% (Fernandopulle et al. 2006) and another study conducted by Dissanayake et al. (2015) concluded the rate of vaginal and rectal carriage as 30% (Dissanayake et al. 2015). The prevalence of GBS in Sri Lanka is still not identified by multi-centre studies and remains unknown.

The drug of choice for GBS infection is penicillin; ampicillin, erythromycin and clindamycin are frequently used as alternative drugs. Previous studies which were conducted both locally and internationally have demonstrated that some GBS isolates showed intermediate or decreased sensitivity, in vitro, to penicillin and ampicillin (Dissanayake et al. 2015; Goudarzi et al. 2015). This decreasing sensitivity to penicillin needs to be monitored due to possible development of resistance in the near future (Silbert et al. 2016). It is estimated that 0.7-4% of patients develop allergic reactions to penicillin (Spong et al. 2012). In clinical practice, broth culture in selective medium and subsequent subculture are the gold standard methods for identification of GBS colonisation (Verani et al. 2010). However, novel rapid methods such as polymerase chain reaction (PCR) are used in some countries (Wernecke et al. 2009; Alfa et al. 2010). A recent study has demonstrated that the culture method does not give positive results for all GBS. PCR targeting genes of Streptococcus agalactiae are 16S rRNA, cfb (CAMP factor), scpB (S. agalactiae C5a peptidase) and atr. Out of them, cfb gene is the most important gene that encodes the S. agalactiae CAMP factor which presents in all GBS universally (Clarke et al. 2016).

The current study was conducted with a view to determine the proportion of pregnant mothers colonised with GBS in the local situation using conventional culture as well as a novel PCR technique and to detect the antibiotic susceptibility. The data derived from this study would envisage the usefulness of prophylaxis to GBS positive pregnant mothers in Sri Lanka.

Materials and methods

A descriptive cross-sectional study was conducted from January to April 2019 in four teaching hospitals in Western Province, Sri Lanka. One hundred pregnant women of more than 35 weeks of gestation were enrolled after obtaining informed written consent. Two low vaginal and rectal swabs were collected. One set of swabs was collected separately in Todd-Hewitt broth supplemented with gentamicin (8 μg/ml) and nalidixic acid (15 µg/ml) for culture and the other was in phosphate-buffered saline for molecular studies. Swabs for culture were processed according to standard methods.

Identification of GBS was done by observing characteristic colonies with beta haemolysis, being Gram-positive cocci in chains, positive catalase test, negative reaction in bile aesculin agar, positive by Christie-Atkins-Munch-Petersen (CAMP) test and detection of capsular polysaccharide antigen by latex agglutination (Streptococcal Lanced field grouping kit, Oxoid, UK).

Isolated GBS were subjected to antibiotic susceptibility testing (ABST) for penicillin (10 units), ampicillin (10 μg), clindamycin (2 μg), cefotaxime (30 μg), erythromycin (15 μg) and vancomycin (30 µg) according to Clinical and Laboratory Standard Institute (CLSI) guideline 2018 (CLSI 2018). The organism was tested for inducible clindamycin resistance and the minimum inhibitory concentration (MIC) was detected for penicillin, clindamycin and erythromycin by epsilometer (E-Strip, Oxoid, UK) test method.

GBS DNA from rectal and vaginal swabs were detected using a commercial real-time PCR (RT PCR) TagMan chemistry kit (quantification of *S. agalactiae*, Primer DesignTM Ltd., UK) equipped with an internal control and a specific DNA primer/ probe mix (FAM labelled, BHQ quenched) which is designed to detect *cfb* gene.

A positive control (DNA from GBS provided by the manufacturers), standard GBS ATCC (12386) culture and a negative control (a sample without template) were used as controls. RT PCR was optimised with known isolates of GBS according to the manufacturer's instructions and the cut-off values were established by using serial dilutions of positive control with the Bio-Rad CFX 96 PCR machine.

Pregnant women of less than 35 weeks of gestation and pregnant women who have taken antibiotics within one month were excluded.

Associated factor for GBS colonisation was assessed using a questionnaire.

Data were analysed by Statistical Package for the Social Sciences (SPSS) version 20.

Ethical clearance was obtained from the Ethics Review Committees of University of Sri Jayewardenepura (number: 89/17) and the relevant hospitals.

Results

Culture and RT PCR

The proportion of GBS vaginal colonisation in the 100 specimens was 18% (18 vaginal and 0 rectal) by culture method and 49% (37 vaginal and 27 rectal) by RT PCR. Use of PCR detected a higher positive number (49) of samples as opposed to use of culture (18) only (Table 1).

One specimen (vaginal) which was positive by culture was negative by PCR, and 32 specimens (vaginal and rectal) which were positive by RT PCR were negative by culture (Table 2).

In conducting the test both vaginal and rectal samples of the same individuals were used and it was observed that 37 vaginal samples were positive by RT PCR for GBS while 22 were negative when tested using the rectal samples.

Also when 27 rectal samples were positive for GBS by RT PCR, 12 vaginal samples were negative in the same individuals by RTPCR.

RT PCR revealed positive for both specimens (vaginal and rectal) in only 15 individuals (Table 3).

In addition when both specimens were used, RT PCR was able to detect 49/100 GBS positive samples whereas when only vaginal swabs and rectal swabs were utilised separately the results were (37/100) and (27/100), respectively.

Table 1. Comparison between vaginal and rectal swabs by culture and realtime PCR

time i en			
	Culture	RT PCR	
Vaginal swabs	18	37	
Rectal swabs	0	27	
Vaginal and/or rectal swabs	18	49	

Table 2. Comparison between culture and real-time PCR.

	Cu	lture	
	Positive	Negative	Total
PCR			
Positive	17	32	49
Negative	1	50	51
Total	18	82	100

Table 3. Comparison between vaginal and rectal swabs by real-time PCR.

	Vagina	al swabs	
	Positive	Negative	Total
Rectal swabs			
Positive	15	12	27
Negative	22	51	73
Total	37	63	100

Socio-demographic features

The socio-demographic and risk factors of the 100 pregnant women screened for vaginal and rectal GBS colonisation in this study are summarised in Table 4. Mean age of participants was 29 years within the range between 17 and 44 years. GBS was predominantly found in the age groups ranging from 21 to 35 years. In the study, 80% of the population had either General Certificate of Education Ordinary Level (GCE O/L) or GCE Advanced Level (GCE A/L). Only 12% of the mothers had an education level below GCE O/L.

Majority of the study population was not employed (74%) and GBS colonisation was found to have a significant relationship with nonemployees (p = .043). The predominant age of marriage was 21–25 years. Out of 14 obese pregnant females, 7 (50%) had GBS colonisation and of 28 overweight females, 13 (46.4%) were colonised by GBS. Furthermore, out of the 15 underweight pregnant females, 7 (46.6%) were colonised by GBS. However, out of 43 normal weight females, 23 (53.5%) were colonised.

Participants' obstetric risk factors

It was identified that out of the 38 primigravida pregnant women, 18 (47.3%) were colonised with GBS. Furthermore, it was observed that out of the participants having 1 child, 2 children, 3 children and 4 children, 16 (48.4%), 9 (56.2%) and 5 (50%) and 2 (66.6%) were colonised, respectively.

In considering the obstetric history, it has been observed that among 20 mothers who complained of abortion/miscarriage, 45% (9/20) had GBS colonisation while amongst 4 mothers who had a previous history of perinatal death, 50% were seen to be GBS colonised although it was not statistically significant. Further 2 (66.6%) out of 3 mothers who had premature babies were colonised and both mothers (2) with a history of ectopic pregnancies were also colonised by GBS.

Table 4. Socio-demographic characteristics and GBS status.

Variable	Number 100	GBS positivity rate (50)	p value
Age (years)			
15–20	8 (8%)	4 (50%)	
21–25	21 (21%)	10 (47.6%)	.37
26–30	27 (27%)	17 (63%)	
31–35	24 (24%)	12 (50%)	
36-40	17 (17%)	7 (41.2%)	
41–45	3 (3%)	0 (0%)	
Religion			
Buddhist	69 (69%)	33 (47.8%)	.341
Catholic	15 (15%)	9 (60%)	
Hindu	5 (5%)	1 (20%)	
Islam	11 (11%)	7 (63.6%)	
Occupation			
Employed	26 (26%)	9 (34.6%)	.043
No employed	74 (74%)	41 (56.2%)	
Age of marriage			
15–20	28 (28%)	15 (53.5%)	.653
21–25	35 (35%)	18 (51.4%)	
26–30	28 (28%0	14 (50%)	
31–35	3 (3%)	2 (66.6%)	
36–40	5 (5%)	1 (20%)	
41–45	1 (1%)	0 (0%)	
Education			
<gce level<="" ordinary="" td=""><td>12 (12%)</td><td>6 (50%)</td><td></td></gce>	12 (12%)	6 (50%)	
GCE ordinary level	38 (38%)	17 (44.7%)	.476
GCE advanced level	42 (42%)	23 (54.8%)	
Degree/Diploma	8 (8%)	4 (50%)	
BMI			
Under weight – $<$ 18.5	15 (15%)	7 (46.6%)	
Normal – 18.6–24.9	43 (43%)	23 (53.5%)	.936
Over weight – 25–29.9	28 (28%)	13 (46.4 %)	
Obesity – >30	14 (14%)	7 (50%)	

Considering the obstetric history of the current pregnancy, 50 mothers were treated with an antifungal for the complaint of abnormal vaginal discharge and 62% (31/50) of them were colonisers for GBS. GBS colonisation was found to have a significant relationship with the history of vaginal discharge (p = .016).

Thirteen mothers had hypertension in the current pregnancy, whilst 27 had gestational diabetics, 11 reported urinary tract infection and 3 were with endocrine disorders and their GBS colonisation rates were 6/13 (46.1%), 11/27 (40.7%), 6/11 (54.5%), 1/3 (33.3%), respectively.

When frequency of sex was considered, 45 mothers who claimed the practice for more than 2 times/week had the highest GBS colonisation rate (55.5%) (Table 5).

Antimicrobial susceptibility testing

All GBS isolates were sensitive to penicillin, ampicillin, cefotaxime and vancomycin. Of the 18 isolates, 5 were non-susceptible for erythromycin and 4 for clindamycin by both disk diffusion and MIC detection. Thirteen (68.4%) isolates were sensitive to erythromycin while 4 (21%) isolates were intermediate sensitive and 1 (5.2%) isolate was resistant (Table 6). All isolates with intermediate sensitivity were found to have MIC₅₀ 0.5 μ g/ml and \geq 1.0 μ g/ml. Three (15.8%) isolates demonstrated intermediate sensitivity to clindamycin (MIC₅₀-0.5 μg/ml) and 1 (5.2%) was resistant. Four isolates showed constitutional resistance to clindamycin while inducible

Table 5. Participants' obstetric risk factors.

Variable	Number 100	GBS positive (50)	p value
Parity			
Primigravida	38 (38%)	18 (47.3%)	
1 Child	33 (33%)	16 (48.4%)	
2 Children	16 (16%)	9 (56.2%)	.429*
3 Children	10 (10%)	5 (50%)	
4 Children	3 (3%)	2 (66.6%)	
Previous pregnancies history			
Abortion/miscarriage	20 (20%)	9 (45%)	.719*
Perinatal death	4 (4%)	2 (50%)	_
Premature baby	3 (3%)	2 (66.6%)	_
Ectopic pregnancy	2 (2%)	1 (50%)	_
Current pregnancy			
Treated with antifungal	50 (50%)	31 (62%)	.016*
Frequency (>2) having sex/week	45 (45%)	25 (55.5%)	.236*
Clinical history			
Hypertension	13	6 (46.1%)	.826*
Gestational diabetics	27 (27%)	11 (40.7%)	.260*
Urinary tract infection	11 (11%)	6 (54.5%)	.749*
Endocrine disorder	3 (3%)	1 (33.3%)	_

^{*}p value was calculated by Chi-square test, -: p value cannot be calculated.

Table 6. Antibiotic susceptibilities of 18 GBS isolates from pregnant women.

Antibiotic	No. and % of isolates Sensitive	Intermediate	Resistant
		carace	
Penicillin 10 units	18 (100%)	_	_
Erythromycin 15 μg	13 (68.4%)	4 (21%)	1 (5.2%)
Clindamycin 2 µg	14 (73.7%)	3 (15.8%)	1 (5.2%)
Ampicillin 10 μg	18 (100%)	_	_
Cefotaxime µg	18 (100%)	_	_
Vancomycin 30 μg	18 (100%)	-	_

clindamycin resistance was not detected. One isolate was resistant only to erythromycin.

Discussion

In this study, the overall GBS colonisation rate which was detected using both culture and PCR was 50%. Studies done previously in Sri Lanka gave a prevalence rate of 30% in 2015 (Dissanayake et al. 2015) whilst in 2006, a prevalence of 26.7% was determined (Fernandopulle et al. 2006). When compared to previous studies a higher proportion of GBS was observed in this study due to the inclusion of a new tool, the RT PCR. As documented previously (Carrillo-Avila et al. 2018) the greater sensitivity of the RT PCR is emphasised by this study too.

In contrast to our findings of 50% prevalence, recent studies have reported a lower prevalence of rectovaginal GBS colonisation in the region. A low prevalence was seen in India (2% to 16%) (Saha et al. 2017) whilst Chan et al. (2013) detected a GBS colonisation rate of 7.7% in Bangladesh (Chan et al. 2013), and a rate of 8.5%in Pakistan (Chaudhry et al. 2010).

Further studies from Europe, North and South America and Africa interestingly have reported a higher rate than that seen in the South East Asia with a colonisation rate of 22% in Belgium (El Aila et al. 2010), 21% in Netherlands, 19.7-24% in North and South America and 21-22% in Sub Saharan Africa (Kwatra et al. 2016).

The variations of rate of colonisation between countries could be attributed to many factors such as sample size, type of sampling sites, use of non-selective media for culture, use of different culture methods, use of various gene targeting PCR techniques, socio-economic status of study subjects, and the socio-demographic factors (Carrillo-Avila et al. 2018).

Further, the low prevalence seen in South East Asia and the high rates seen in this study and in Europe, America and Africa could be due to the different colonising serotypes. Of the 10 GBS serotypes, some serotypes (especially serotype111) are associated with greater virulence and thereby a higher propensity to invasive disease. Serotype 111 was found less frequently in South East Asian region (Russell et al. 2017). Knowledge of serotype distribution in Sri Lanka is sparse and is an urgent necessity. Since the colonising rate is higher than that of the region, a study on the serotypes would enable to assess the risk of invasive disease in this population of pregnant women from Sri Lanka. Serotyping of the isolates though essential was not carried out in this study and was envisaged in the future and is a limitation currently.

As highlighted colonisation rate of GBS with culture method (18%) was low in comparison to RT PCR (49%). RT PCR assay showed considerable increase in the identification of GBS. The advantage of using PCR over culture in this setting is due to the ability of the PCR to detect a higher positive rate due to its ability to detect nonviable bacteria and or low bacterial loads in the clinical specimens. In addition, the qPCR assay used in this study was capable of detecting 2.5 copies/µl of GBS. The limit of detection (LOD) was superior to the studies of Michele Berger et al., in 2018 which detected 10 copies/µl of GBS and El Aila et al who described a LOD of 20 copies/μl.

Further, the drawbacks of culture methods as an identification process are that growth of the rectal vaginal microbiota such as enterococci, staphylococci or other streptococci species can inhibit the GBS even when using a selective medium such as Todd-Hewitt broth. In the current study, both lower vaginal swabs and rectal swabs were collected and processed separately. Using double-sampling for each detection method separately may have influenced the difference between the rate of colonisation of culture and PCR, because the load of bacteria might have been different in the two sampling swabs.

A possible explanation for one sample, for which culture was positive and PCR negative may be the presence of inhibitors for PCR.

The culture method may not be very effective in isolation of GBS and it is a time-consuming method, requiring at least 48 h for identification of GBS. In contrast, the RT PCR technique provides rapid and accurate results.

Further, in order to administer intrapartum antibiotic prophylaxis, culture method as a screening tool to detect GBS colonisation in pregnant women in Sri Lanka is costeffective. However, introducing the RT PCR with its higher detecting ability of GBS colonisation for those who are negative for cultures would enable to reduce the risk of infection in the newborn.

The drug of choice for GBS infection is penicillin, however, there is a considerable proportion of penicillin-allergic people. Thus, in the treatment of those penicillin-allergic people, erythromycin and clindamycin are used. Erythromycin and clindamycin resistance have been cited in previous reports as up to 37% and 17%, respectively (Gibbs et al. 2004). Dissanayake et al. (2015) previously detected 26.7% isolates resistant to erythromycin and 6.7% isolates resistant to clindamycin showing that while erythromycin-resistant rates for Sri Lanka remain unchanged at 26.2% in the current study, clindamycin resistant rates have increased to 21% by both disk diffusion and MIC methods. Interestingly, four isolates of GBS could not be treated with either clindamycin or erythromycin due to the presence of constitutive MLS B resistance. Neighbouring countries like India with its low prevalence of GBS have also reported resistance to erythromycin (Sharmila et al. 2011). In other reports, variable erythromycin resistance ranging from 0% to 22% has been seen globally (Kulkarni et al. 2001; Orrett 2003; Dzowela et al. 2005; Nwachukwu et al. 2007). Castellano-Filho et al. (2010) in Brazil found that antibiotic resistance to clindamycin and erythromycin were 50% and 22.7%, respectively. In addition in a recent study in Iran (2018) resistance to penicillin (16.66%) and vancomycin (16.66%) also was detected (Daramroodi and Keshavarzi 2018).

Considering the sampling site when using RTPCR enabled detection from both sites (vaginal and rectal) whilst using culture techniques it has been identified that only vaginal swabs were positive for GBS. Previous studies have shown that in order to determine greatest carriage of GBS, use of both rectal and vaginal swabs is important. Further rectovaginal swabs have yielded a maximum growth rate (El Aila et al. 2010).

In this study, a significant association is seen between GBS colonisation and vaginal discharge. All of these pregnant women had admitted obtaining antifungal treatment for vaginal discharge. Although isolation of Candida species was not within the purview of this study, carriage of Candida has been considered to be a risk factor for GBS colonisation (Pidwill et al. 2018). Although mechanisms are poorly understood, synergistic interplay between Candida albicans and GBS has been suggested to promote colonisation of both organisms. Further, GBS strains serotype 111 and 1a are also known to physically interact with Candida albicans enabling GBS to exhibit tropism for Candida filaments (Pidwill et al. 2018).

Although previously reported, GBS colonisation was not statistically significant with the bad obstetric history namely abortion or miscarriage, perinatal death, premature babies, ectopic pregnancies in this study (Tam et al. 2012). A high proportion of GBS was observed in those with bad obstetric history in the present study. Colonisation may be transient, intermittent or even chronic(Ahmadzia and Heine 2014) hence every pregnancy needs to be evaluated as per risk from GBS colonisation as further supported by a study done by Darabi et al. (2017) in Iran.

In conclusion, this study showed high prevalence of GBS colonisation among pregnant women in the Western Province of Sri Lanka. It is recommended to establish national policies for screening of pregnant women of more than 35 weeks of gestation and treatment guidelines. Penicillin can

be the antibiotic of choice for prophylaxis against GBS. Further, it is recommended to include both rectal and vaginal swabs in screening for GBS. Use of PCR has a place in those culture-negative mothers.

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Disclosure statement

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