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Colonization of *Streptococcus agalactiae* among pregnant patients in Trinidad and Tobago

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ABSTRACT

Objective: To assess colonization of *Streptococcus agalactiae* [group B streptococcus (GBS)], and delineate capsular serotype distribution and antibiotic susceptibility profiles among pregnant women in Trinidad and Tobago.

Methods: Vaginal swabs were collected from 248 pregnant women attending antenatal clinics in northern Trinidad, and processed using standard microbiological laboratory tests to confirm GBS. Polymerase chain reaction detected *atr* and *cps* serotype genes. Antimicrobial susceptibility tests were performed using the Kirby–Bauer method, and SPSS Version 25 was used for statistical analysis. Prevalence ratio measured the risk, and $P \leq 0.05$ was considered to indicate significance.

Results: The GBS carriage rate was 29% (72/248, 95% confidence interval 23.3–34.8), and carriage was significantly associated with variables including gestational diabetes ($P=0.042$), age 25–35 years ($P=0.006$), multiparity ($P=0.035$) and marital status ($P=0.006$). The most common serotype was type II [47.2% (34/72)], and serotypes V, VI, VII and VIII were not encountered. GBS showed high resistance to amoxicillin-clavulanic acid (37.5%), erythromycin (30.6%), trimethoprim-sulphamethoxazole (58.3%) and tetracycline (97.2%).

Conclusion: GBS colonization among pregnant women and resistance to commonly used antibiotics are high in Trinidad and Tobago. A population-based study is required to obtain accurate figures in order to improve maternal healthcare services.

Introduction

Streptococcus agalactiae commonly colonizes the lower genital tract and gastrointestinal tract in women, especially pregnant women. If not properly cleared or treated, *S. agalactiae* poses a major risk to neonates, causing neonatal sepsis and infections (Meyn et al., 2009; Verani et al., 2010). Pregnant women are at higher risk of infection if labour or rupture of the membranes occurs before 37 weeks of gestation, if there is a urinary tract infection, if a previous child has tested positive for *S. agalactiae* infection, or if there is intrapartum fever (Melin and Efstratiou, 2013; Burcham et al., 2019).

In the mid 1990s, before the introduction of preventative strategies in the USA, *S. agalactiae* was an escalating problem. The implementation of maternal intrapartum chemoprophylaxis triggered a considerable decrease in early-onset streptococcal disease, and a decline in invasive infection among pregnant women. In 2014, new cases of *S. agalactiae* infection reduced to 0.24 per 1000 (Verani et al., 2010; Melin and Efstratiou, 2013). Worldwide, there are approximately 410,000 cases of severe *S. agalactiae* infection annually, including invasive forms that commonly

manifest as bloodstream infections. At least 147,000 cases result in stillbirth and infant death. The highest burden of *S. agalactiae* infection is seen in Africa, where 54% and 65% of cases result in stillbirth and infant death, respectively (World Health Organization, 2017). The US Centers for Disease Control and Prevention have recommended that all pregnant women should be screened for group B streptococcus (GBS) colonization at 35–37 weeks of gestation using vaginal/rectal specimens in order to decrease the morbidity and mortality of GBS-associated neonatal disease (Verani et al., 2010).

Studies have shown that the rate of *S. agalactiae* infection has decreased significantly since chemoprophylaxis was introduced, from 1.7 cases per 1000 live births in the 1990s to 0.22 cases per 1000 live births in 2017. This represents an 80% reduction in the number of cases (Hanna and Noor, 2021). More than 50% of heavily colonized mothers are more likely to transmit *S. agalactiae* to their offspring (Melin and Efstratiou, 2013). In 1994, 1995 and 2003, studies were conducted in Trinidad on colonization among pregnant women during the third trimester, and early-onset infections in children after transmission of *S. agalactiae* from the mother (Orrett and Olagundoye, 1994;

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Simpson and Heard, 1995; Orrett, 2003). Since these studies, there has been no further research to determine the most prevalent serotypes in Trinidad and Tobago, or any new or additional information on GBS. To the authors' knowledge, no local data are available to indicate whether there is a problem with susceptibility to, and contraction of, *S. agalactiae* infection among pregnant women, and its prevalence in transmission to their offspring(s) during birth. As reported elsewhere this has been well documented, although the route of transmission or acquisition in late-onset infections remains unclear; it has been suggested that transmission/acquisition may occur via vertical transmission from mother to neonate, nosocomial transmission, contaminated breast milk or prematurity (Rajagopal, 2009; Le Doare and Kampmann, 2014; Zimmerman et al., 2017).

There is a dearth of information on GBS in Trinidad and Tobago, and evidence of any recent research into this phenomenon and statistics about its occurrence have not been maintained. No national policies or guidelines exist regarding preventative strategies in the nation's hospitals to combat what has represented an escalating public health issue in other parts of the world. Specialists appear to apply their own preventive measures based on their own experience or that of their health facilities. This article sought to delineate and characterize GBS colonization among a cross-section of antenatal patients, including identification of risk factors, capsular serotypes and antibiotic susceptibility profiles.

Materials and methods

Study design

This observational cross-sectional study was carried out among a cross-section of adult pregnant women attending antenatal care at public health facilities in north central Trinidad. More than 300 pregnant women were recruited to this study over a 15-month period (May 2018–July 2019). Following written consent, participants completed a self-administered standardized questionnaire. A convenience random sampling method was used to recruit study participants.

Inclusion and exclusion criteria

The inclusion criteria were as follows: pregnant women in their third trimester; age ≥ 18 years at the time of the study; and attending health-care facilities for antenatal care or admitted to the antenatal ward of the public hospital. Participants without symptoms of urinary tract infection or any symptoms unrelated to pregnancy. Exclusion criteria were: age < 18 years; any symptoms suggestive of infections in the respiratory, gastrointestinal, musculoskeletal (wound infections) body systems etc.; and undergoing antibiotic treatment at the time of specimen collection.

Specimen collection

Participants' biodata were collected using a pre-tested standardized questionnaire, which was administered via in-person interview (face to face). Once consent was given, vaginal swabs were taken by medical staff following techniques reported in the literature (Metcalf et al., 2017). A swab stick was used and then placed in Ames transport medium. Samples were taken immediately to the microbiology laboratory at the Department of Paraclinical Sciences of the University of the West Indies at St. Augustine for analysis.

Laboratory analysis

Each sample was inoculated on an enriched media culture plate; Sheep's blood agar is a media used for the incubation at 35°C overnight in 5% CO₂ before analysis for the growth of colonies. Where there were slow-growing colonies, the plates were left for another 24 h before further examination. When growth was established, further tests – Gram

stain, catalase and Strep kits – were performed. Following these initial presumptive tests to identify the colonies as *Streptococcus* spp., further tests were performed, including antibiotic susceptibility tests with the Kirby–Bauer disk diffusion method and CAMP tests. Antibiotic susceptibility testing of *S. agalactiae* isolates was undertaken, and results were interpreted in accordance with the guidelines of the Clinical and Laboratory Institute Standards (Clinical and Laboratory Institute Standards, 2021).

Bacterial enrichment storage

The GBS isolates from the samples were stored in 2 mL of brain heart infusion enrichment media supplemented with glycerol, and placed in the refrigerator for storage at -70°C until further analysis.

DNA extraction

DNA extraction was performed using the alkaline lysis method and the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Briefly, the alkaline lysis method involved taking three or four pure colonies from an overnight-cultured isolate, and suspending them in a sterile microtube containing 60 μ L of lysis buffer (0.05 N NaOH, 0.25% sodium dodecyl sulfate); samples were vortexed and heated at 95°C for 15 min. Next, 540 μ L of TE buffer (50 mM Tris HCl, 1 mM EDTA, pH 8) was added to the microtube to dilute the cell lysate, and the microtube was centrifuged at 15,000 rpm for 5 min to sediment the cell debris. The supernatant was then transferred to a new sterile microtube and used for polymerase chain reaction (PCR); it was then frozen at -20 °C for further use.

Molecular analysis of *Streptococcus agalactiae* isolates

Two separate PCR programmes (single and multiplex) were used to confirm the GBS isolates and identify the different capsular serotypes. Molecular confirmation for the GBS isolates was achieved by detecting the *atr* gene following steps reported previously (Arabestani et al., 2017), with some modifications. The PCR reaction volumes per reaction were as follows (Promega HotStart PCR kit): PCR buffer, 10 μ L; MgCl₂, 5 μ L; forward primer, 1.0 μ L; reverse primer, 1.0 μ L; DNA template, 2.0 μ L; Taq polymerase, 0.25 μ L; and nuclease free water, ≤ 50 μ L. The primer sequences for the *atr* gene were as follows: forward, CAA CGA TTC TCT CAG CTT TGT TAA; and reverse, TAA GAA ATC TCT TGT GCG GAT TTC.

Multiplex PCR reactions were employed to detect different capsular genes for the GBS serotypes. As listed in Table 1, the primer sequences selected for amplification for the capsular genes were as reported previously (Hickman et al., 1999), with some modifications. Amplification for different serotype genes – cps1aH, cps1bJ, cps1bK, cps2K, cps1a/2/3I, cps1a/2/3J, cps4N, cps5O, cps6I, cps7M, cps8J and dltS – from the GenBank Database libraries was performed to identify the different serotypes of *S. agalactiae* (Hickman et al., 1999). The PCR conditions for this multiplex PCR programme were as follows: initial denaturation at 94 °C for 3 min, followed by 30 cycles of the following conditions: 94 °C for 30 s, 58 °C for 1 min and 72 °C for 1 min. This was followed by a final extension at 72 °C for 5 min. The PCR reaction volumes were as follows: buffer, 10 μ L; and MgCl₂ 5 μ L. Primers Ia, Ib, II, IV and V were run together, and primers III VI, VII and VIII were run together. Each primer was added at 0.5 μ L per reaction; dNTPs, 1.5 μ L; DNA template, 2.0 μ L; Taq polymerase, 1.0 μ L per reaction; and nuclease free water, ≤ 50 μ L.

Statistical analysis

Data were analysed using SPSS Version 21 (IBM Corp., Armonk, NY, USA). Both descriptive and inferential data analysis were used. Descriptive methods included frequency and percentage distribution tables, graphs and charts. Inferential methods were Chi-squared test and 95%

Table 1
Primer pairs for *Streptococcus agalactiae* serotyping (Hickman et al., 1999).

Primer name	Sequence	Gene target (s)	Amplicon size (s), bp
Ia-F	GGTCAGACTGGATTAATGGTATGC	cps1aH	521 and 1,826
Ia-R	GTAGAAATAGCCTATATACGTTGAATGC	cps1aH	
Ib-F	TAAACGAGAATGGAATATCACAAACC	cps1bJ	770
Ib-R	GAATTAACITCAATCCCTAAACAATATCG	cps1bK	
II-F	GCTTCAGTAAGTATTGTAAGACGATAG	cps2K	397
II-R	TTCTCTAGGAAATCAAATAATTCTATAGGG	cps2K	
III-F	TCCGTAACAACAGACTCATCC	cps1a/2/3I	1,826
III-R	AGTAACCGTCCATACATTCTATAAGC	cps1a/2/3J	
IV-F	GGTGGTAATCCTAAGAGTGAAGTGT	cps4N	578
IV-R	CCTCCCAATTTTCGTCCATAATGGT	cps4N	
V-F	GAGGCCAATCAGTTCACGCTAA	cps5O	701
V-R	AACCTTCTCCTTCACACTAATCCT	cps5O	
VI-F	GGACTTGAGATGGCAGAAGGTGAA	cps6I	487
VI-R	CTGTCCGACTATCCTGATGAATCTC	cps6I	
VII-F	CCTGGAGAGAACAAATGCCAGAT	cps7M	371
VII-R	GCTGGTCGTGATTTCTACACA	cps7M	
VIII-F	AGGTCAACCACTATATAGCGA	cps8J	282

confidence intervals (CI). All hypotheses were tested at the 5% level of significance. $P < 0.05$ was considered to indicate significance.

Results

Over 300 participants were targeted and enrolled in the study. However, only 250 of them gave consent, completed the questionnaire, and allowed clinical samples (vaginal swabs) to be taken and analysed. Two of the 250 patients were subsequently excluded as they had received antibiotics recently. As such, 248 patients and samples were included in this study.

Streptococcus agalactiae carriage rate

Of the 248 women recruited into the study, 94.4% (234/248) tested positive for micro-organisms and 5.6% (14/248) tested negative. The analysis of the organisms indicated that *Staphylococcus aureus* were the most common [43.1% (107/248)], followed by *S. agalactiae* [29% (72/248), 95% CI 23.3–34.8] and *Candida* spp. [14.1% (35/248)]; *Proteus* spp., a prominent Gram-negative organism and a member of the Enterobacteriaceae family accounted for 4.8% (12/248) of the isolates. Other microbial agents including *Bacillus* spp. and other *Streptococcus* spp. were also found [3.2% (8/248)].

Characteristics of study participants

As depicted in Table 2, sociodemographic and obstetric characteristics of *S. agalactiae* carriage revealed that several variables were significantly associated with GBS colonization among the study population: multiparity ($P=0.035$); age 24–35 years ($P=0.005$); gestational diabetes ($P=0.042$); and marital status ($P=0.006$).

S. agalactiae serotype genes – *atr* for 1a and 1b, and *cps* for 1a, 1b, II, III and IV – were detected (Figures 1 and 2). The most prevalent serotype encountered among the colonized participants was serotype II [47.2% (34/72)], followed by serotype 1b [23.6% (17/72)]. Serotypes 1a, III and IV accounted for 16.6% (12/72), 9.72% (7/72) and 2.8% (2/72) of isolates, respectively. Serotypes V, VI, VII and VIII were not identified or encountered.

Antimicrobial susceptibility pattern

The results of the susceptibility patterns of *S. agalactiae* isolates in this study are shown in Table 3. The GBS isolates recovered from this cohort of patients were highly resistant (>30%) to several antibiotics

Table 2

Characteristics of study participants with *Streptococcus agalactiae* carriage among a cross-section of pregnant women attending antenatal clinics in Trinidad and Tobago (%).

Characteristics	Frequency	P-value
Ethnicity		0.301
Afro-Trinidadian	32 (44.4)	
Indo-Trinidadian	23 (32)	
Mixed	15 (20.8)	
Other	2 (2.8)	
Pregnancy history		0.035
Primigravida	36 (50)	
Multigravida	36 (50)	
Marital status		0.006
Single	29 (40.2)	
Married	22 (30.6)	
Divorced	0	
Separated	1 (1.4)	
Common law	20 (27.8)	
Age group		0.005
18–23	12 (16.7)	
24–29	33 (45.8)	
30–35	22 (30.5)	
36–41	3 (4.2)	
42–47	2 (2.8)	
Gestational DM		0.042
A	54 (75)	
B	18 (25)	

DM, diabetes mellitus; A, diabetics colonized with *S. agalactiae*; B, non-diabetics colonized with *S. agalactiae*.

$P \leq 0.05$ was taken to indicate significance.

including ampicillin (65.3%), amoxicillin-clavulanic acid (37.5%), erythromycin (30.6%), nitrofurantoin (45.8%), tetracycline (97.2%) and trimethoprim-sulphamethoxazole (58.3%). However, the isolates were highly susceptible to cephalosporins, carbapenems, fluoroquinolones and glycopeptides.

Discussion

The prevalence of *S. agalactiae* in this study was 29%, and this figure can be considered high compared with the worldwide distribution analysis rate (Russell et al., 2017). When compared with other regions of the world, this figure could be considered high or low, as occurrence rates have varied in other reports; for example, 10–40% in the USA, 6.5–36% in Europe, 7.1–16% in Asia, 9.2–25.3% in the Middle East, and 11.9–31.6% in Africa (Johri et al., 2006; Kumar et al., 2006; Mavnyengwa et al., 2010; Madrid et al., 2017). A major observation

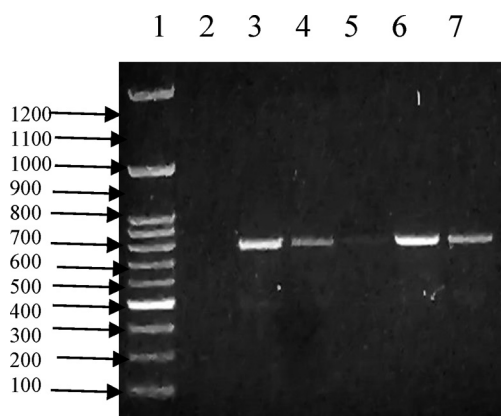


Figure 1. Results of gel electrophoresis from confirmatory polymerase chain reaction assay targeting the 780-bp *atr* gene of the *Streptococcus agalactiae* isolates from Trinidad and Tobago. Lane 1, DNA marker; lane 2, *Enterococcus faecalis* ATCC 29212 (negative control); lane 3, *Streptococcus agalactiae* ATCC 12386 (positive control), lanes 4–7, *Streptococcus agalactiae* isolates with 780 bp.

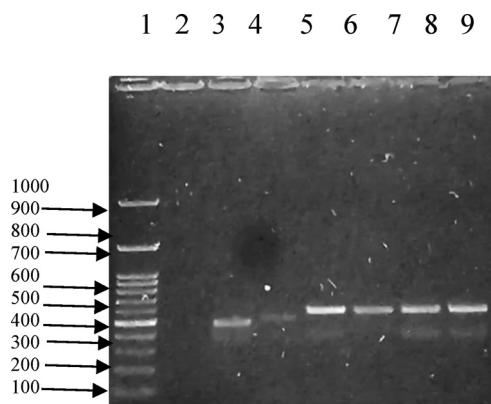


Figure 2. Polymerase chain reaction products of *Streptococcus agalactiae* serotypes recovered from vaginal swabs of antenatal patients from Trinidad and Tobago. Lane 1, DNA marker; Lanes 2–9 *Streptococcus agalactiae* isolates showing predominantly serotype II.

Table 3
Antibiotic susceptibility pattern of *Streptococcus agalactiae* isolates from women attending antenatal clinics in Trinidad and Tobago.

Antibiotics	Susceptible (%)	Resistant (%)
Ampicillin	25 (34.7)	47 (65.3)
AMC	45 (62.5)	27 (37.5)
Azithromycin	69 (95.8)	3 (4.2)
Ceftriaxone	68 (94.4)	4 (5.6)
Cefuroxime	68 (94.4)	4 (5.6)
Ciprofloxacin	66 (91.7)	6 (8.3)
Clindamycin	65 (90.3)	7 (9.7)
Ertapenem	65 (90.3)	7 (9.7)
Erythromycin	50 (69.4)	22 (30.6)
Imipenem	67 (93.1)	5 (6.9)
Levofloxacin	66 (91.7)	6 (8.3)
Norfloxacin	65 (90.3)	7 (9.7)
Meropenem	72 (100)	0
Nitrofurantoin	39 (54.2)	33 (45.8)
SXT	30 (41.7)	42 (58.3)
Tetracycline	2 (2.8)	70 (97.2)
TZP	70 (97.2)	2 (2.8)
Vancomycin	72 (100)	0

AMC, amoxicillin/clavulanic acid; SXT, trimethoprim-sulphamethaxole; TZP, piperacillin-tazobactam.

for the high GBS carriage rate (29%) observed in this study was the significant number of patients who were diabetic (75%, $P=0.04$). This high colonization rate is in line with previous reports that colonization of the genitourinary tract by *S. agalactiae* is very common among pregnant women and those with diabetes (Akkaneesermsaeng et al., 2019; Furfaro et al., 2019). According to Ramos et al. (1997), diabetes is an independent risk factor for colonization during pregnancy, and that seems to have been the case in the present study population, and was also reported by Stapleton et al. (2005). There is no evidence for routine testing for *S. agalactiae* when pregnant patients attend antenatal clinics in Trinidad and Tobago, so colonization may be missed if patients do not have, or do not show, any clinical symptoms. As such, antibiotic prophylaxis would not be administered during labour.

As reported in the literature, there are sociodemographic risk factors associated with GBS colonization in pregnant women, such as ethnicity, maternal age, parity, marital status and educational level (Ramos et al., 1997; Stapleton et al., 2005; Ippolito et al., 2010). In the present study, multiparous women had a higher colonization rate for *S. agalactiae* ($P=0.035$) and were probably higher risk for carriage. Women aged 24–29 years were seen mostly significantly colonized ($P=0.005$). Marital status was shown to be significantly associated with high carriage of GBS in the present study ($P=0.006$).

In this study, detection of the *atr* gene was used to confirm GBS recovered from the vaginal swabs (Arabestani et al., 2017), and the most prevalent capsular serotypes observed were serotypes II, 1a, 1b, III and IV. This is in agreement with the results from a global systemic and meta-analysis study by Madrid et al. (2017), which noted that these serotypes were mainly associated with early-onset infection in neonates. Although serotype 1a was not the most common serotype observed in this study, it has been reported to be the leading cause of maternal colonization in a meta-analysis, and the most common serotype causing and contributing to early-onset disease in neonates (Verani et al., 2010). *S. agalactiae* colonization is community specific, as reported by others (Gibbs et al., 2004; Stapleton et al., 2005); it is therefore not surprising that serotype II was the most prevalent serotype encountered in the present study. As in Europe, the USA and Canada, where serotypes VI–IX are rare, these serotypes, plus serotype V, were not encountered in the present study. However in South-east Asia, East Asia, South Asia and West Africa, these serotypes were more common (Madrid et al., 2017; Furfaro et al., 2019; Bianchi-Jassir et al., 2020).

In this analysis, the prevalence of serotype III was low. This is in contrast to a previous systemic review, which found that this serotype was the most common (Arabestani et al., 2017; Bianchi-Jassir et al., 2020). However, in the systemic review, a good amount of maternal colonization data was available for almost all regions, although this was not the focus of the present study. In support of the findings of this study, the systemic review by Bianchi-Jassir et al. reported that serotype III was less common in Latin America and the Caribbean, South-east Asia, South Asia and West Africa; unfortunately, the reason for this was not given (Bianchi-Jassir et al., 2020). Therefore, the rates and incidences of infection and disease need to be closely examined and evaluated for each community or country in order to institute the most appropriate preventative measure or strategy available.

The high (>30%) resistance rates of GBS isolates to ampicillin and amoxicillin-clavulanic acid observed in this study were a huge contrast to previous findings (Mengist et al., 2016; Rigvava et al., 2019; Jisuvei et al., 2020), and was in agreement with previous findings for vancomycin (all susceptible). The susceptibility profiles observed for these GBS isolates seem to follow the same pattern as that reported for Gram-positive bacteria in Trinidad and Tobago (Akpaka et al., 2017; Hawkins et al., 2017). The only explanation is antibiotic use, which seems to be a perennial problem in the country (Akpaka et al., 2007). However, these GBS isolates were found to be highly susceptible to several cephalosporins, carbapenems, macrolides and glycopeptides, which is very encouraging. A major limitation of this study was the small number of participants.

Conclusion

The frequency of GBS colonization among a cross-section of pregnant women and their resistance to commonly used antibiotics were high (>30%) in Trinidad and Tobago. The prevalent capsular serotypes (1a, 1b, II, III and IV) were similar to those reported in countries in Latin America and the Caribbean. A population-based study is required to obtain accurate data regarding GBS carriage, virulence of these organisms, and prevalent sequence types in this region and throughout the country in order to improve maternal healthcare services.

Author contributions

Patrick Eberechi Akpaka: study design, data analysis, writing manuscript, supervision of study. Khamiya Henry: study design, data collection and analysis, writing of manuscript. Reinand Thompson: data collection and analysis, writing of manuscript. Chandrashekhar Unakal: study design, data analysis, writing manuscript, supervision of study

Ethical approval

Ethical approval was obtained from the Campus Ethics Committee of the University of the West Indies, St. Augustine, Trinidad and Tobago. Permission was also obtained from the relevant health authorities.

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Declaration of Competing Interest

None declared.

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