



The expression of virulence genes in Group B Streptococcus isolated from symptomatic pregnant women with term and preterm delivery

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OUR TEAM

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INTRODUCTION

Group B Streptococcus (GBS)



- ❖ Group B Streptococcus (GBS) are β -hemolytic, gram-positive bacteria that colonize the lower gastrointestinal tract and urogenital tract of nearly 18% of pregnant women worldwide (Kwatra et al., 2016)
- ❖ GBS vaginal colonization during pregnancy is identified as one of the risk factors for preterm delivery (Bianchi-Jassir et al., 2017)

ASCENDING INTRAUTERINE INFECTION

Heavy GBS colonization of vagina



GBS get access to the uterus via cervix



Invasion of chorioamniotic membrane, amniotic cavity and fetus



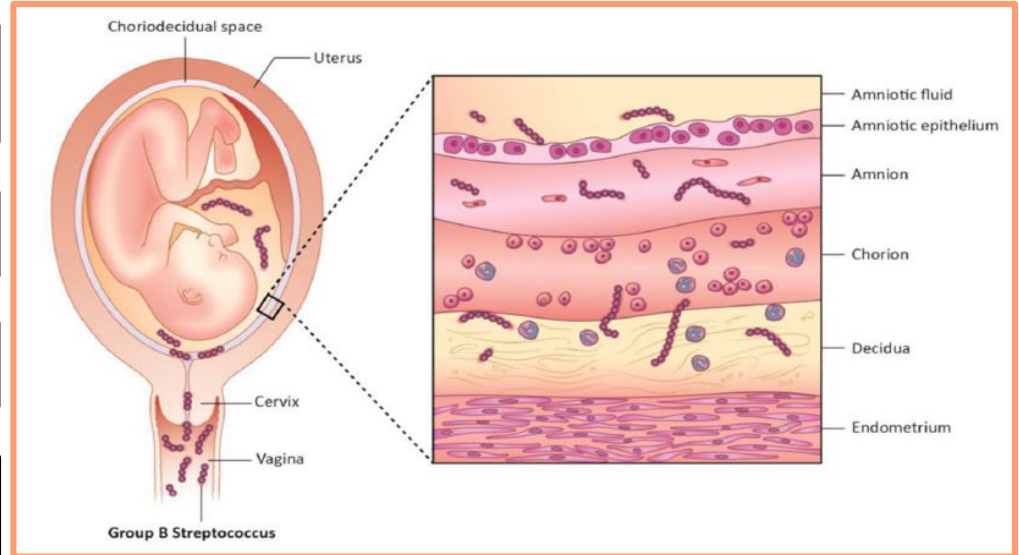
Induction of inflammatory reaction



Early activation of mechanisms of labour



Preterm delivery



PRETERM DELIVERY



- ❖ Preterm delivery is defined as the delivery that occurs before 37 weeks of gestation and the rate of preterm birth ranges between from 8.1% to 11.2% in Malaysia (Jeganathan et al., 2015)
- ❖ Preterm birth affects 1 in 10 of babies born globally, resulting in an estimated 15 million babies being born prematurely each year (WHO 2012; Blencowe et al., 2013; Vogel et al., 2018)
- ❖ About 30–70% of colonized mothers deliver GBS colonized newborns and 1–2% of these develop early-onset infections (Anthony et al., 1979; Barcaite et al., 2008; Melin, 2011; Melin et al., 2013)

SUMMARY OF VIRULENCE FACTORS

Virulence factors	Functions
Hemolytic pigment encoded by <i>CyIE</i>	GBS vaginal colonization, ascending intrauterine infection and preterm delivery in mice and non human primate GSB resistance to killing by macrophages and neutrophils (Whidbey et al., 2013), (Randis et al., 2014), (Boldenow et al., 2016; Liu et al., 2004)
Hyaluronidase encoded by <i>HylB</i>	GBS vaginal colonization, ascending intrauterine infection and preterm delivery in mice (Kolar et al., 2015, Vornhagen et al., 2016). (Milligan et al., 1978; Pritchard et al., 1993; Musseer et al., 1989)
Serine-rich repeat (Srr) proteins encoded by <i>Srr</i>	GBS vaginal colonization in mice (Seo et al., 2013), (Sheen et al., 2011; Wang et al., 2014)
Bacterial surface adhesin of GBS encoded by <i>BsaB</i>	GBS vaginal colonization (in vitro studies) (Buscetta et al., 2014; Jiang et al., 2014)

AIM & HYPOTHESIS

AIM

To investigate the association between mRNA expression of virulence genes in GBS isolates obtained from symptomatic pregnant women and preterm delivery

HYPOTHESIS

The mRNA expression of virulence genes in GBS isolates obtained from symptomatic pregnant women with preterm delivery is elevated when compared to symptomatic pregnant women with term delivery



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METHODOLOGY



SAMPLE COLLECTION IN HTAA



Study Design

A prospective laboratory-based follow up study on symptomatic pregnant women attending Department of Obstetrics and Gynaecology of Hospital Tengku Ampuan Afzan (HTAA) in Kuantan

SAMPLE COLLECTION IN HTAA

Ethical Approval

Kulliyyah of Medicine Postgraduate Committee (KPGC)

Kulliyyah of Medicine Research Committee (KRC)

IIUM Research Ethics Committee (IREC)

National Medical Research Register (NMRR)

Medical Research & Ethics Committee (MREC)

SAMPLE COLLECTION IN HTAA

Sample Size Calculation

The Single Proportion method formula was chosen to calculate the sample size

The most optimum sample size is 34

Taking into account 20% of margin -:

$$34.1 \times 20\% = 6.82$$

$$\text{Thus, } 34.1 + 6.82 = 40.9 = 41$$

SAMPLE COLLECTION IN HTAA

Inclusion Criteria

Symptomatic pregnant women with < 37 weeks gestational age presented preterm labour, preterm premature rupture of membrane (pPROM), vaginal bleeding, vaginal discharge

Exclusion Criteria

Symptomatic pregnant women with <37 weeks gestational age, who received antibiotics in the two weeks before the high vaginal swab samples were collected

SAMPLE COLLECTION IN HTAA



Demographic details

Maternal age, race, body mass index (BMI), education level, occupation, income, smoking habit, alcohol consumption, parity and gestational age

Obstetrical details

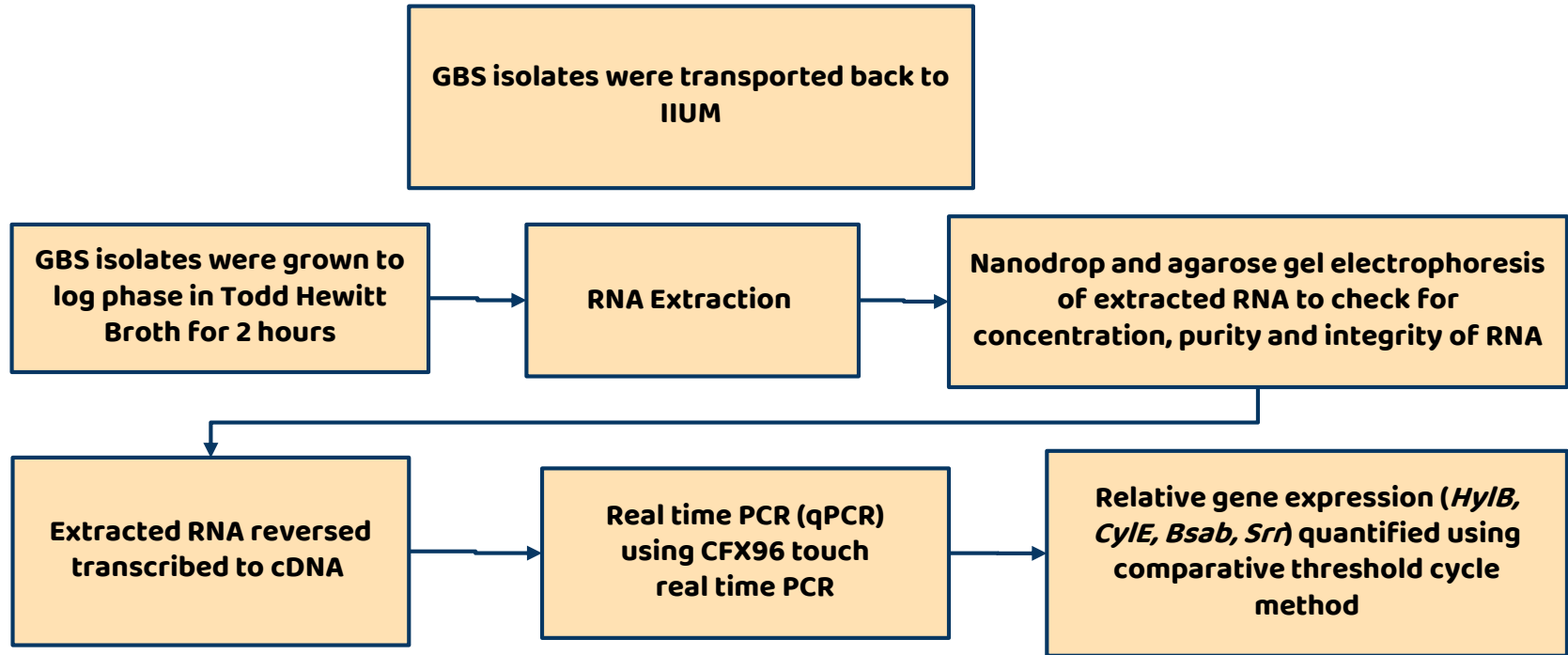
Previous preterm delivery, multiple pregnancy, habitual abortion, cervical incompetence, gestational diabetes, pregnancy-induced hypertension, intra-hepatic cholestasis of pregnancy, placenta previa, and placental abruption and delivery outcomes

SAMPLE COLLECTION IN HTAA

Processing of GBS isolates

- ❖ The high vaginal swab samples were streaked onto blood agar and incubated aerobically for 24 hours
- ❖ GBS colonies that exhibited beta hemolysis (small zone of hemolysis around each colony) were differentiated from other beta-hemolytic organisms
- ❖ Once GBS colonies were isolated, gram stain was performed and Christie, Atkinson, Munch, Peterson (CAMP) test was used for identification of GBS

LABORATORY METHODS





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**RESULT &
DISCUSSION**

Association between Maternal Characteristics and Preterm Delivery

Table I. Demographics and clinical characteristics of GBS-positive symptomatic pregnant women

Demographics & clinical characteristics	GBS-positive pregnant women		P
	term delivery (n=18)	preterm delivery (n=22)	
Maternal age (years)			0.983
≤19 (n=2)	1	1	
20-24 (n=6)	2	4	
25-29 (n=14)	6	8	
30-34 (n=12)	6	6	
≥35 (n=6)	3	3	
Race			1.000
Malay (n=37)	17	20	
Chinese (n=3)	1	2	
Indian (n=0)	0	0	
BMI			0.538
<18.5 (underweight) (n=2)	0	2	
18.5-24.9 (normal) (n=12)	6	6	
25-29.9 (overweight) (n=14)	5	9	
≥30 (obese) (n=12)	7	5	
Education levels			1.000
No formal education (n=1)	0	1	
Primary (n=0)	0	0	
Secondary (n=14)	6	8	
Tertiary (n=25)	12	13	
Occupation			1.000
Housewife (n=20)	9	11	
Employed (n=20)	9	11	
Income (RM)			1.000
B40 (<RM4,360) (n=34)	15	19	
M40 (>RM4,360-RM9,619) & T20 (>RM9,619) (n=6)	3	3	

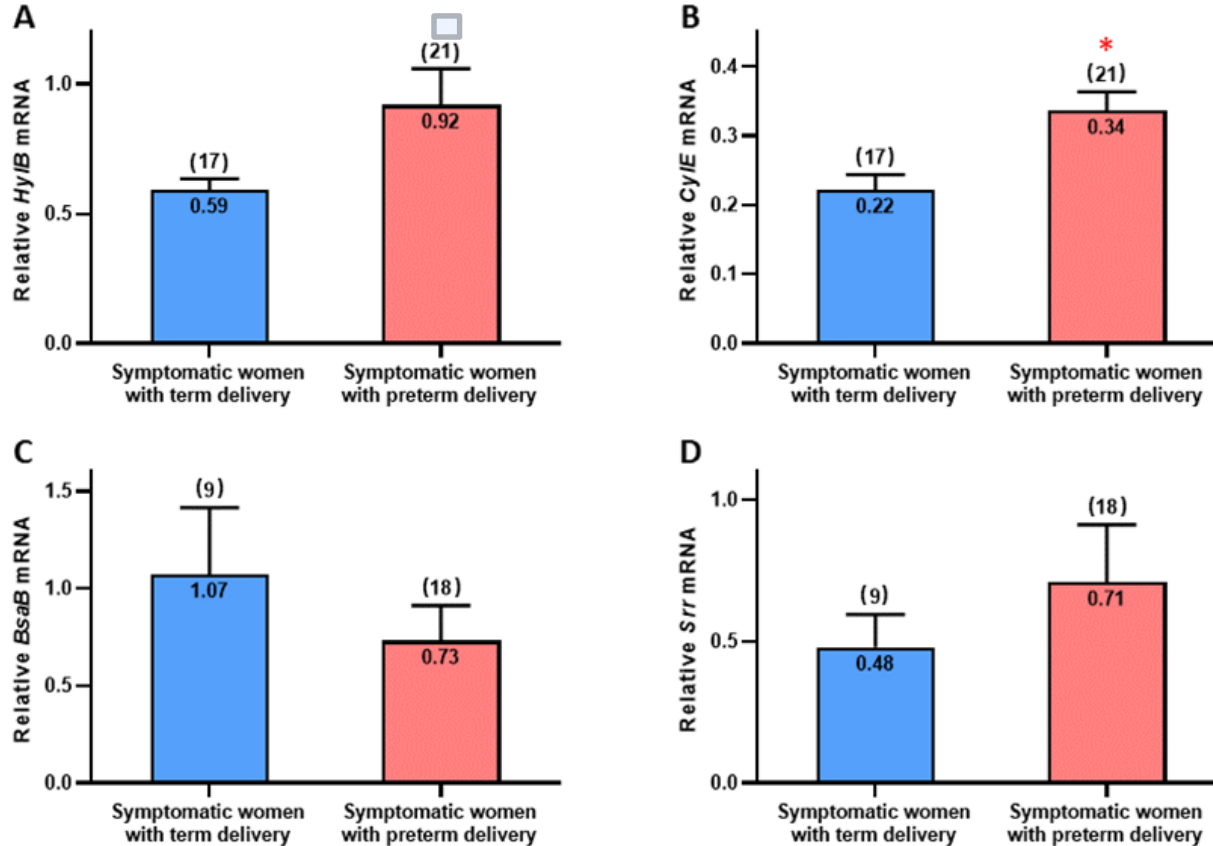
Smoking habit			0.624
Smoker (n=1)	1	0	
Passive smoker (n=18)	7	11	
Non-smoker (n=21)	10	11	
Parity			0.253
0 (nulliparous) (n=11)	3	8	
1 (primiparous) (n=10)	6	4	
2-4 (multiparous) (n=17)	9	8	
≥5 (grand-multiparous) (n=2)	0	2	
Gestational age (weeks)			0.282
22-24 (n=1)	0	1	
25-27 (n=3)	3	0	
28-30 (n=3)	1	2	
31-33 (n=12)	4	8	
34-36 (n=21)	10	11	
Previous preterm delivery			0.105
Yes (n=7)	1	6	
No (n=33)	17	16	
Previous multiple pregnancy			1.000
Yes (n=2)	1	1	
No (n=38)	17	21	
Gestational diabetes			1.000
Yes (n=11)	5	6	
No (n=29)	13	16	
Placenta previa			1.000
Yes (n=1)	0	1	
No (n=39)	18	21	

Note: Chi squared test was applied and Fischer's exact test was applied in cell <5.

Level of significance was set at 0.05.

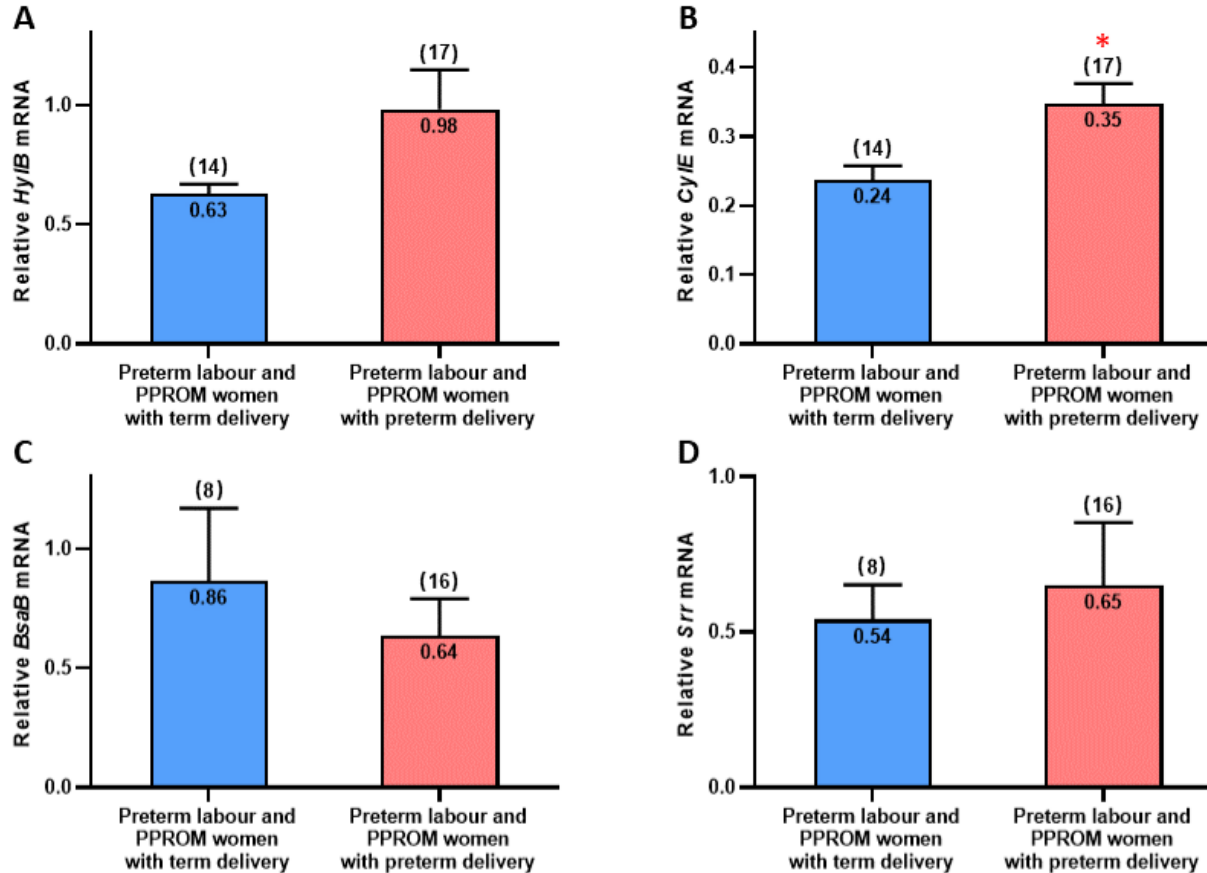
Abbreviations: BMI, Body Mass Index; RM, Ringgit Malaysia

Association between Expression of GBS Virulence Genes with Preterm Delivery



- indicates statistical significance between symptomatic pregnant women with term and preterm delivery

Association between Expression of GBS Virulence Genes with Preterm Delivery



- indicates statistical significance between preterm labour and PPROM women with term and preterm delivery



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CONCLUSION



CONCLUSION



- Following vaginal colonization, both *CyIE* and *HyIB* genes of GBS possibly contribute to ascending intrauterine infection and inflammation that triggers preterm delivery in these symptomatic pregnant women
- Hemolytic pigment and hyaluronidase may be targeted for the exploratory and pre-clinical stages of vaccine development as an alternative to the intrapartum antibiotic prophylaxis

STUDY LIMITATIONS

- ❖ GBS screening is not routinely done among the pregnant women in public hospitals in Malaysia, thus only symptomatic pregnant women, whom high vaginal swabs were taken as part of a diagnosis were included in the study

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