Serotype distribution and antibiotic resistance of *Streptococcus agalactiae* isolated from patients in Hue Central Hospital

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Abstract

Group B streptococcus (GBS) infections are still the leading cause of invasive infections in neonates, specically they also seriously cause mortality and morbidity with underlying diseases in adults. Curently, there are ten GBS serotypes (Ia, Ib, and II-IX) and the resistance characteristic of GBS is important to clinical treatment. **Objectives and methods**: 30 clinical isolates of GBS were obtained from patients in Hue Central Hospital, Vietnam, from January 2016 until Jun 2019. Then the isolated GBS was conducted antimicrobial susceptibility test to determine the antibiotic resistance and serotypes by a multiplex PCR method. **Results**: GBS strains were resistant to tetracycline (100%), azithromycin (82.6%), erythromycin (80%) and clindamycin (80%). Resistance rates were lower with levofloxacin (45%), chloramphenicol (52.6%) and ceftriaxone (6.7%) whereas resistance was not observed in ampicillin, vancomycin and penicillin G. The distribution rate of serotype V (66.67%) was higher than type I (33.33%). **Conclusions**: Antibiotic resistance characteristics of GBS in samples are mostly familiar with other studies: β -lactams and vancomycin were the most susceptible antibiotics to GBS, the resistance rate in second line drug like clindamycin and erythromycin were high but there were large differences between studies. This study determined two GBS serotypes of Ia and V among isolated strains.

Keyword: *Streptococcus agalactiae, GBS, antibiotic resistance, serotype.*

1. INTRODUCTION

Group B streptococcus (GBS) is known as a commensal flora in human gastrointestinal and genitourinary tracts. However, this opportunistic pathogen can cause invasive infections [1]. In addition to being a major source of invasive infections in newborns, GBS are also a substantial cause of mortality or morbidity in adults with underlying illnesses [2]. Besides, GBS infections has been reported in neonates, children, and adults, non-pregnant adults, pregnant women, older adults with different disease manifestations such as urinary tract infections, pneumonia, osteomyelitis, endocarditis, skin and soft tissue infections… [3]. GBS has several virulence factors cause these clinical manifestations due to various capabilities of host cells invasion, penetration of the blood-brain barrier, and escape from host immune responses. There are ten GBS serotypes (Ia, Ib, and II-IX) described base on the capsular polysaccharide structure which is one of GBS important virulence factor; 98% of all colonizing GBS isolates have been identified as belonging to bacterial serotypes I–V [4, 5]. In additions, it was reported that antibiotic resistance, neonatal diseases, or specific organ involvement seem to be associated with specific GBS serotypes [5].

Although GBS is a group of bacteria that affect a large number of patients and the epidemiological distribution of the serotype will influence the antibiotic clinical treatment in the hospital, there were no related studies in Thua Thien Hue province. Because of the above reasons, we carried out the research "Serotype distribution and antibiotic resistance of *Streptococcus agalactiae* isolated". This study aimed to determine the antibiotic resistance of GBS strains isolated and to determine the serotype distribution of isolated GBS strains.

2. MATERIAL AND METHODS

Study subjects

An experimental study was recruited among 30 clinical isolates of GBS obtained from patients admitted to Hue Central Hospital, Vietnam, from January 2016 until June 2019.

The identification of GBS isolates was carried out in the Department of Microbiology of Hue Central Hospital using standard tests of CAMP test. The further tests were carried out in Department of Microbiology of Hue Central Hospital and Department of Microbiology of Hue University of Medicine and Pharmacy Hospital.

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Antimicrobial Susceptibility Test

Antimicrobial susceptibilities of GBS isolates were determined by means of the Kirby-Bauer disk-diffusion test on Mueller-Hinton agar. Zone Diameter breakpoints and quality-control protocols were used according to standards established by the Clinical and Laboratory Standards Institute [6]. Isolates with intermediate susceptibility were classified as resistant for analysis.

Serotyping

Every GBS strains was serotyped with a multiplex PCR method.

The GBS strains were extracted DNA by Phenol-Chloroform method, followed by PCR examination. The PCR products were analyzed for the patterns of different serotypes using gel electrophoresis.

In this study, we used primers specific described in Poyart et al for serotype Ia, Ib, II-V which were the most common serotypes of GBS [4, 5, 7] (Table 1). There were three PCR primer sets used for the examination. The first contained primer pairs specific for serotypes: Ia, III; the second contained primer pairs for serotypes: Ib and II; and the third contained a primer pair for GBS- serotypes: IV, V.

Statistical methods

The collected data were stored in MS Excel 2016. SPSS 20.0 software was used for analysis and processing the data.

3. RESULTS

Clinical characteristics of the study subjects

Totally, 30 GBS strains were performed antimicrobial susceptibility tests and PCR for determining specific GBS serotypes. The result indicated that in 30 patients at Hue Central Hospital who isolated group B streptococcus, the distribution rate for male patients was 46.7%, as opposed to 53.3% for female patients. The highest rate of isolates of group B Streptococcus was from vaginal discharge (30%) and urine specimen (30%). The lower rate samples such as blood, pus, and other fluids accounted for 10% of each type (Table 2).

Antimicrobial susceptibility profile of isolated GBS strains

The antimicrobial susceptibility profile of isolated GBS strains was presented in Table 3. Group B streptococcus was resistant to tetracycline (100%), azithromycin (82.6%), erythromycin (80%) and clindamycin (80%). Resistance rates were lower with levofloxacin (45%), chloramphenicol (52.6%) and ceftriaxone (6.7%) whereas resistance was not observed in ampicillin, vancomycin and penicillin G (Table 3).

Serotypes distributions of isolated GBS strains

There were two kinds of GBS serotypes detemined from 30 GBS strains: serotype V and serotype Ia; other serotypes (Ib, II-IV) was not detected. In 9 patients who isolated group B streptococcus, the distribution rate of serotype V (66.67%) was higher than type Ia (33.33%) (Table 4).

The relationship between antibiotic susceptibility rates and serotypes of isolated GBS was described in Table 5. The antibiotic susceptibility testing showed 100% susceptibility to β -lactams group included ampicillin, ceftriaxion, penicillin G and vancomycin, irrespective of serotypes. No relationship was found between antibiotic susceptibility rates and serotypes of isolated GBS strains (Table 5).

Table1. Primer sequence

Primer Name	Sequence 5' → 3'	Reference
Serotype Ia Forward	GGTCAGACTGGATTAATGGTATGC	[7]
Serotype Ia Reverse	GTAGAAATAGCCTATATACGTTGAATGC	
Serotype Ib Forward	TAAACGAGAATGGAATATCACAAACC	
Serotype Ib Reverse	GAATTAACTTCAATCCCTAAACAATATCG	
Serotype II Forward	GCTTCAGTAAGTATTGTAAGACGATAG	
Serotype II Reverse	TTCTCTAGGAAATCAAATAATTCTATAGGG	
Serotype III Forward	TCCGTACTACAACAGACTCATCC	
Serotype III Reverse	AGTAACCGTCCATACATTCTATAAGC	
Serotype IV Forward	GGTGGTAATCCTAAGAGTGAACTGT	
Serotype IV Reverse	CCTCCCCAATTTCGTCCATAATGGT	
Serotype V Forward	GAGGCCAATCAGTTGCACGTAA	
Serotype V Reverse	AACCTTCTCCTTCACACTAATCCT	

Table 2. Background characteristics of the study group

	Features	Frequency	Percentage (%)
Sex	Female	16	53.3
	Male	14	46.7
	Total	30	100.0
Specimens	Vagina discharge	9	30
	Urine	9	30
	Blood	3	10
	Pus	3	10
	Sputum	1	3.3
	Amniotic fluid	1	3.3
	Pleural fluid	1	3.3
	Other fluid	3	10
	Total	30	100

Table 3. The antimicrobial susceptibility profile of isolated GBS strains

Antibiotic types	N	Resistant	
		n	%
Tetracycline	19	19	100.0
Ampicillin	24	0	0.0
Chloramphenicol	19	10	52.6
Erythromycin	30	24	80.0
Clindamycin	30	24	80.0
Ceftriaxone	30	2	6.7
Vancomycin	24	0	0.0
Levofloxacin	20	9	45.0
Azithromycin	23	19	82.6
Penicillin G	30	0	0.0

Table 4. Serotype distribution of isolated GBS strains

Serotypes	Amount	Percentage (%)
Ia	3	10
Ib	0	0
II	0	0
III	0	0
IV	0	0
V	6	20
Other	21	70
Total	30	100

Table 5. Antibiotic susceptibility of GBS serotypes

Features	Serotype			
	Ia		V	
	n	%	n	%
Tetracylcline susceptible	0	0%	0	0%
Ampicillin susceptible	2	100%	5	100%
Chloramphenicol susceptible	0	0.00%	4	100%
Erythromycin susceptible	1	33.30%	2	33.30%
Clindamycin susceptible	1	33.30%	2	66.70%
Ceftriaxone susceptible	3	100%	6	100%
Vancomycin susceptible	2	100%	6	100%
Levofloxacin susceptible	2	100%	2	50%
Azithromycin susceptible	0	0.00%	3	50%
Penicillin G susceptible	3	100%	6	100%

4. DISCUSSION

This study focused on determining the antibiotic resistance rate by the Kirby-Bauer disk-diffusion test on Mueller-Hinton agar and serotype of GBS. There was no difference between the sex ratio of GBS isolated patients, the diverse types of specimens were mainly urine (30%) vaginal fluid (30%) and other body fluids.

Due to the high susceptibility rate, β-lactam antibiotics like penicillin G, ampicillin, and cephalosporin are the first choice for treating GBS [8]. According to our research, GBS strains were not resistant to ampicillin or penicillin G, and there was 6.7% resistant to ceftriaxone. This is consistent with other studies in Taiwan with a sensitivity rate of almost 100% to penicillin, cephalosporin 2nd, 3rd, ampicillin [9]. Similar results also appear in other regions such as France, Arab, China and a study in 2020 in Nghe An, Vietnam [10-13].

Clindamycin is the recommended alternative to penicillin for women who have a high risk of anaphylaxis or a severe, uncommon delayed-onset reaction, but only if the GBS isolate is known to be susceptible to clindamycin. Erythromycin was once added, but due to the rising resistance rate, it was rarely used [8]. Our study found that 80% of patients were resistant to erythromycin and clindamycin, which is a much higher rate than that found in other studies. According to CDC reports in 2020, 58.2% of GBS isolates were resistant to erythromycin and 47.2% of GBS isolates were resistant to clindamycin [14]. The results of another study in Taiwan were 48.9% and 51.4% susceptibility to erythromycin and clindamycin respectively [5]. China has particularly high rates of erythromycin resistance, with rates of 74.1% found in both colonizing and invasive isolates [15]. In the USA, erythromycin and clindamycin resistance rates are 54.8% and 44.8%, respectively [16]. In another study with lower rates of resistance in the Brazilian region, resistance rates to clindamycin (2 - 16.7%) and erythromycin (4 - 14%) [17].

Vancomycin is a last resort antibiotic used to treat GBS infections [8]. The results of our study have a susceptibility rate of 100% to vancomycin, which is similar to the results of the study in Nghe An province [13]. This result was also correspond to the study on pregnant women in Arab and the studies on newborns in France and China [10-12].

For other antibiotics, they are not unnoticed because of the high rate of resistance and are often not used in clinical practice. In our study, tetracycline had a 100% resistance rate corresponding to the results of systematic studies with a resistance rate of over 80%[18]. Levofloxacin resistance rate of 45% is much higher than the rate of 2.3% in the US study [16]. Other antibiotics such as azithromycin and chloramphenicol have high rates of resistance and are not used for clinical treatment.

Our study found only 2 serotypes Ia and V with the rate of 10% and 20%, respectively. According to a large systematic study, the two predominant serotypes in Southeast Asia are I (20%) and V (27%) so it is rather consistent with the results of this study [4]. Compared to a study in Nghe An Provine of Vietnam, the most common serotype in the study was III, accounting for 39.1% in pregnant women, followed by V (31.9%), while serotypes Ia, Ib, II, VI

and VII account for a lower percentage, ranging from 1.4 to 11.6% [13]. Reasons for the different prevalence of GBS serotypes can be explained as the study at different geographical locations, sources of bacterial isolates, population profiles studied and different time periods in these studies.

In other studies, clindamycin, erythromycin, and chloramphenicol resistance in serotype III was comparatively higher [9]. Besides, resistance to macrolide and clindamycin was associated with serotype Ib, III, and V GBS isolates [5, 19]. In our study, due to the limited number of bacteria identified with serotypes that it was difficult to determine the statistical relation between serotype and resistance to antibiotics.

The limitations of our study include the experimental study design without observing clinical manifestation and the limited study population. However, this was the first study in analyzing the serotypes of GBS strains isolated in Hue city. Therefore, the result of this study could contribute to supply data about GBS serotype for further deep studies.

5. CONCLUSIONS

Antibiotic resistance characteristics of GBS in samples were mostly familiar with other studies: β-lactam and vancomycin were the most susceptible antibiotic to GBS, the resistance rate in second line drug like clindamycin and erythromycin were high but there were differences between studies. This study determined two GBS serotypes of Ia and V among isolated strains.

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