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Original Article

Serotype Determination of *Streptococcus Agalactiae* Detected from Vagina and Urine of Pregnant Women in Yazd, Iran-2015

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Background and Aims: Group B streptococcus (GBS), is a bacterium that colonize in the vagina and/or rectum of pregnant, as well as non-pregnant women. The frequency of GBS varies in different geographical areas. Capsular serotyping of the bacterium could result in efficient vaccine designation. Serotyping data of GBS in Iranian pregnant women is limited. The aim of this study was to investigate the GBS molecular capsular serotyping of pregnant women in Yazd, Iran.

Materials and Methods: In this cross-sectional study, a total of 346 vaginal and urine samples collected from pregnant women were cultured on blood agar and following incubation, the suspected colonies were identified according to standard protocol. Capsular serotyping was carried out by multiplex-polymerase chain reaction assay.

Results: Three hundred forty six samples were collected from pregnant women out of which 57 (16.47%) and 33 (9.5%) samples were identified as GBS of vagina, and urine, respectively. Serotype III was predominant in both vaginal and urine samples by frequencies of 54.4% and 51.5 %, respectively. Other serotypes in vaginal GBS were as II (26.3%), Ia (12.3%), Ib (3.5%), and V (3.5%); while in urine GBS were as Ia (21.2%), II (18.2%), Ib (6.1%), and V (3%).

Conclusions: This study revealed that capsular serotype III of GBS is the dominant serotype among pregnant women in Yazd, Iran. Moreover vaginal and urine GBS serotypes were significantly correlated. These data could be helpful for future possible formulation of a GBS conjugate vaccine.

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Introduction

Streptococcus agalactiae, known as Streptococcus Group B (GBS), is a facultative gram-positive coccus, isolated for the first time from mastitis, and since 1970s it is known as a serious cause of some human diseases [1] in the regions such as rectum, vagina, and urinary tracts of the pregnant as well as non-pregnant healthy people. It cannot cause disease under normal conditions but can be fatal in the elderly and those who bear weak immune system [2]. The bacterium causes pneumonia, bacteremia, sepsis, fetal obstetric infections, osteomyelitis, endocarditis, and meningitis [3]. The importance of this bacterium has been determined since 1970s due to its lethal infections mostly affecting newborns [4, 5]. In infants, GBS diseases are classified into two groups: early-onset diseases (EODs) and late-onset diseases (LODs). EODs appear in the first week of birth but LODs emerge between the second and third months. In pregnant women, infections resulting from this bacterium increases the risk of EODs in the newborns [6].

Different investigation have revealed that 11-30% of pregnant women carry the GBS species in their vagina and about 50-70% of their infant take up the bacterium during labor or birth [7]. Epidemiological studies in the city of Yazd have demonstrated that about 15-20% of pregnant women are carriers of GBS in their vagina [8, 9]. Recent reports have shown that approximately 4% of the infants born from contaminated mother are at risk of infection as sepsis, meningitis and pneumonia [9]. There are several factors involved in the pathogenicity of the GBS. Capsular polysaccharide is one of the most important virulence factors which activates host C3 complement on the surface and inhibits opsonization phagocytosis [10]. Based on antigenic properties of GBS capsule, the bacterium has been categorized into ten serotypes: Ia, Ib and II- IX [10]. Epidemiological surveys around the world have indicated that distribution of GBS serotypes are geographically different. The majority of investigations regarding GBS serotypes have indicated that serotype III is the most predominant. In a study conducted by Sadeh et al. in Yazd it was revealed that 50% of the GBS detected from 650 vaginal swab samples were serotype III, followed by serotypes II, Ia, V and Ib [9].

The aim of this study was as follows:

1- Identifying serotypes of GBS detected from both vagina and urine of the pregnant women

2- Comparing vaginal serotypes and urine GBS serotypes to explore the similarity between the type of GBS in vagina and urinary tract system

3- Representing the sensitivity of urine sample for GBS detection

Materials and Methods

Sample obtaining and culture

The present cross-sectional study was conducted from June 2015 to December 2016. All ethical issues were considered and approved by the Ethics Committee of Shahid Sadoughi university of medical sciences, Yazd, Iran. A total of 346 vaginal and urine samples were collected from the same pregnant

women referring to Mojibiyan Hospital, Seyyedoshohada Clinic, and Baghayipour Clinic (Yazd, Iran). The samples were transferred to microbiology laboratory of Shahid Sadoughi university of medical sciences immediately. Consequently, demographic information including age and number of pregnancies were asked and recorded in an answer sheet. It is important to note that those cases who had used antibiotic for at least 10 days prior to sample collection were removed from the study.

Bacteria identification

All samples were inoculated on sheep blood agar medium (Liofilchem, Italy) and incubated at 35°C. About 18-24 hours after incubation, the suspected β-hemolysis colonies were selected for gram staining. Gram positive coccus was further tested for final GBS identification using catalase, CAMP (Christie-Atkins-Munch-Petersen) and hyporate hydrolysis tests. Then the isolated GBS were stored at -20°C for further molecular examination.

Genomic DNA extraction

Salting-out method was used for genomic DNA extraction [11]. Then the quality and quantity of extracted DNA was measured using 0.7% agarose gel electrophoresis and nanodraop respectively. The product was kept in -20 °C for further examining.

Molecular detection of GBS

Molecular detection for GBS isolates were performed based on Poyart et al. work [12] using the specific primer pairs of dlts-F and dlts-R (Table 1, Pishgam Biotech Company). Three microliter of template DNA was used as a template in a final volume of 20 µl of polymerase chain reaction (PCR) mixture containing the following: 4.5 µl of water, 10 µl of 2X PCR Master Mix (Amplicon, Denmark), and 2.5 µl of primer with final concentration of 10 pmol. Amplification was processed in an automated PCR machine (Eppendorf, Germany) as follows: One cycle initial denaturation for 300 seconds at 94°C followed by 30 cycles at 94°C for 60 seconds, 55°C for 60 seconds, and 72°C for 60 seconds. The final extension was performed with 72°C for 300 seconds. Simultaneously, all reference GBS species were directed to PCR as control. All serotypes of GBS as reference were kindly dedicated by Prof. Kong from Center of Infectious Diseases, New South Wales in Australia.

Molecular serotyping using multiplex-PCR

Detected GBS isolates were serotyped using multiplex-PCR with specific primers (Table 1) [12]. Molecular serotyping was performed using two sets of multiplex PCR reactions (Table 1, Pishgam Biotech Company): amplification of both multiplex reactions with the final volume of 20 µl for PCR reaction (2 µl of water, 10 µl of 2X PCR Master Mix) (Amplicon, Denmark), 5 µl of working primers with final concentration of 10 pmol, and template DNA (3 µl). Amplification was carried out with thermocycler (Eppendorf, Germany). The thermal profile for serotype Ia-V was as follows: 94°C for 300 seconds as initial denaturation for one cycle, 94°C for 60 seconds, 49.9°C for 60 seconds, and 72°C for 60 seconds, for 35 cycles, with a final extension at 72°C for 300 seconds. On the other hand, the thermal profile for serotype V-VIII was

as follow: 94°C for 300 seconds as initial denaturation for one cycle, 94°C for 60 seconds, 60°C for 60 seconds, and 72°C for 60 seconds, for 35 cycles, with a final extension at 72°C for 300 seconds. One percent agarose gel electrophoresis was performed on PCR products and sequenced for further confirmation.

Table 1. Capsular polysaccharide type-specific primers and amplicon size of PCR products

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	GAATTAACTTCAATCCCTAAACAATATCG	cps1bK	
II-F	GCTTCAGTAAGTATTGTAAGACGATAG	cps2K	397
II-R	TTCTCTAGGAAATCAAATAATTCTATAGGG	cps2K	
III-F	TCCGTACTACAACAGACTCATCC	cps1a/2/3I	1,826
III-R	AGTAACCGTCCATACATTCTATAAGC	cps1a/2/3J	
IV-F	GGTGGTAATCCTAAGAGTGAACTGT	cps4N	578
IV-R	AGTAACCGTCCATACATTCTATAAGC	cps4N	
V-F	GAGGCCAATCAGTTGCACGTAA	cps5O	701
V-R	AACCTTCTCCTTCACACTAATCCT	cps5O	
VI-F	GGACTTGAGATGGCAGAAGGTGAA	cps6I	487
VI-R	CTGTCGGACTATCCTGATGAATCTC	cps6I	
VII-F	CCTGGAGAGAACAATGTCCAGAT	cps7M	371
VII-R	GCTGGTCGTGATTTCTACACA	cps7M	
VIII-F	AGGTCAACCACTATATAGCGA	cps8J	282
VIII-R	TCTTCAAATTCCGCTGACTT	cps8J	
dlts-F	AGGAATACCAGGCGATGAACCGAT	dltS	952
dlts-R	TGCTCTAATTCTCCCCTTATGGC	dltS	

Statistical analysis

Chi-square test was performed by SPSS software (Ver. 16) for statistical analysis. $P<0.05$ was considered as significant.

Results

Frequency of GBS among pregnant women

Out of 346 samples collected separately from both vagina and urine, 57 samples (16.47%) of vagina and 33 samples (9.5%) of urine were positive for GBS.

GBS serotyping

As Figures 1 and 2 indicate, following multiplex-PCR, serotypes Ia, Ib, II, III and V were detected from both vagina and urine samples. Note that, serotypes IV, VI, VII and VIII were not detected in this study. Results obtained from multiplex-PCR among vaginal isolates revealed that 54.4% of GBS were serotype III; and prevalence of serotypes Ia, Ib, II, and V were as 12.3%, 3.5%, 26.3%, and 3.5%, respectively (Fig. 3). In urine isolates, the frequency of serotype III was 51.5%, and for Ia, Ib, II, and V serotypes, it was 21.2%, 6.1%, 18.2%, and 3% respectively. The remaining serotypes (IV, VI, VII, and VIII) were not found in the tested samples.

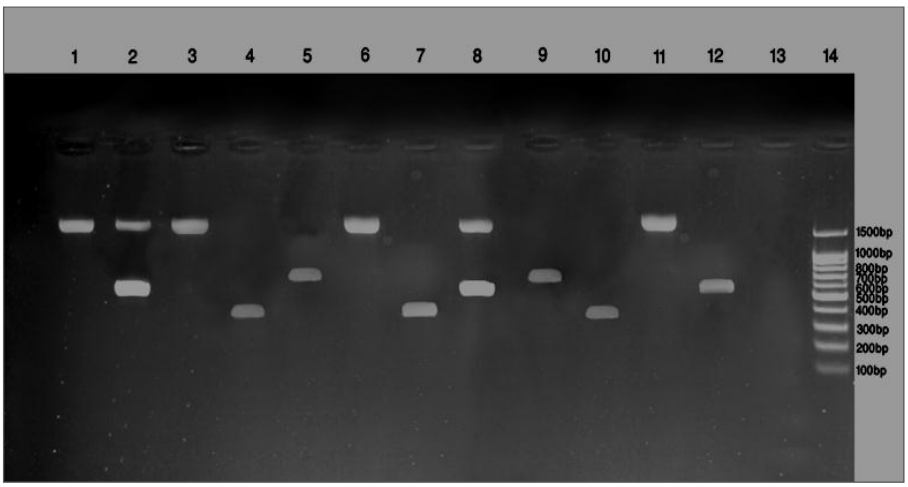

Fig. 1. Gel electrophoresis of the multiplex PCR reaction 1: serotype III (lane 1, 3 and 6), serotype Ia (lane 2), serotype II (lane 4 and 7), serotype Ib (lane 5), positive control serotype (Ia, Ib, II-IV) (lane 8-12), lane 13 is the negative control and lane 14 is molecular size standard (100-bp. Ladder; Fermentas).

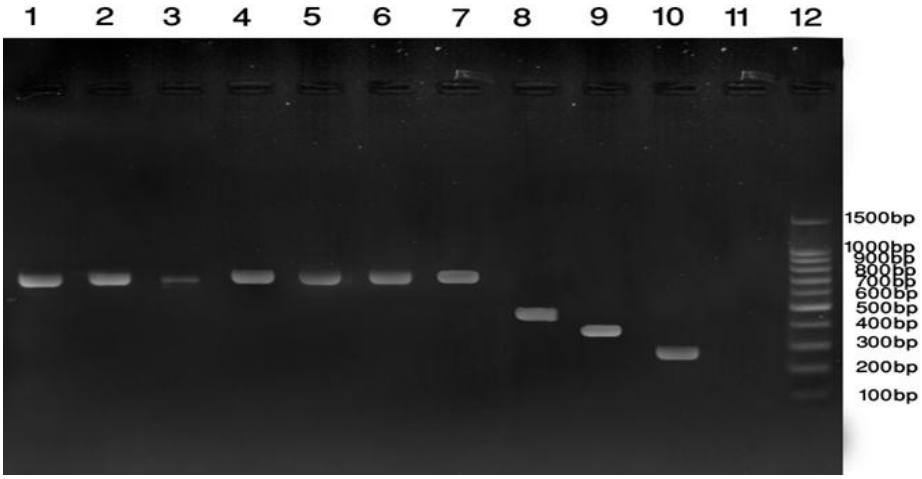

Fig. 2. Gel electrophoresis of the multiplex PCR reaction 2: serotype V (lane 1- 6), positive control serotype (V-VIII) (lane 7-10), lane 11 is the negative control and lane 14 is molecular size standard (100-bp. Ladder; Fermentas).

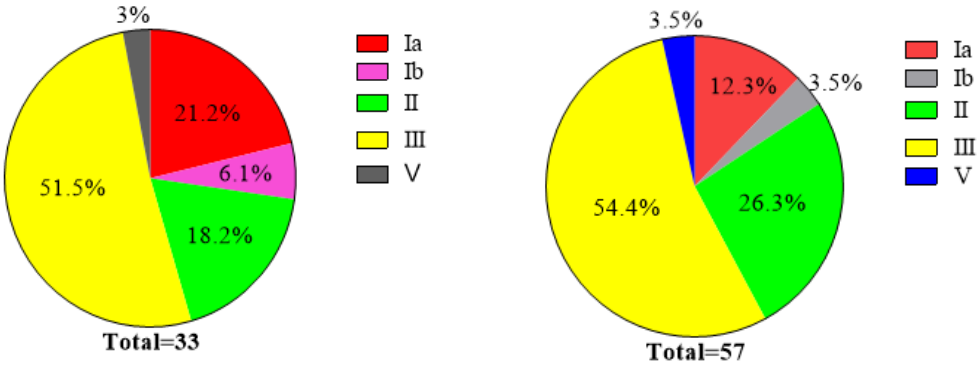

Fig. 3. Frequency of GBS serotypes in vaginal (right) and urine (left) isolates.

Correlation between the GBS serotypes with age, number, and month of pregnancy

By grouping the cases into <30 and ≥30 in terms of age; first, second and more than second in terms of the number(s) of pregnancy; as well as the first 4.5 months and the second 4.5 months in terms of the months of pregnancy, our result showed no significant correlation between the age of pregnancy (p=0.41 for urine isolates, and p=0.13 for vaginal isolates), number of pregnancy (p=0.51 for urine isolates, and p=0.95 for vaginal isolates), and months of pregnancy (p=0.86 for urine isolates, and p=0.37 for vaginal isolates) with serotypes.

Correlation between GBS serotypes detected from vaginal and urine

The association between different vaginal and urine serotypes was investigated here. Out of 346 pregnant women, 26 were GBS positive for both vaginal and urine samples, and in all cases, the urine isolates serotypes were approximately the same as vaginal serotypes. Seven serotypes Ia were found in urine samples whereas only 5 were detected in the same women's vaginal samples. However, the 2 remaining serotypes were identified as II (Fig. 4.). Chi-square test showed that urine and vaginal isolates serotypes are significantly correlated (p=0.000).

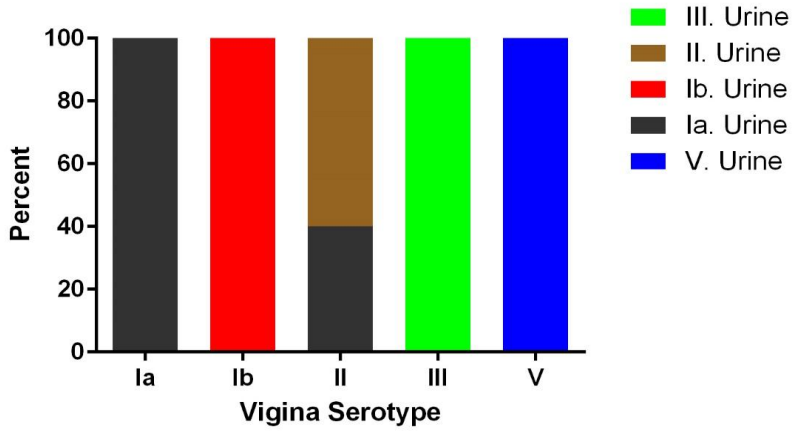

Fig. 4. Correlation between GBS serotypes detected from vaginal and urine

Discussion

In the present study, 346 vaginal and urine samples were collected from pregnant women in Yazd (Iran). Following inoculation, their serotypes were identified. Most of GBS carrying women were under 30 years old (data have not shown). The frequency of GBS among vaginal samples was 16.47%, while for urine sample was 9.53%. The result is in line with that of Absalan et al. (2013). These investigations demonstrated the prevalence of GBS in the 15 to 40 year-old pregnant women who had referred to Healthcare Center of Yazd to be 19.6% [8]. The prevalence of GBS among pregnant women differs in various countries. Moreover, due to cultural differences, even the age of GBS infected

pregnant women varies in different regions. In a study conducted by Rausch et al. (2009) in Sweden, the prevalence of GBS was found to be 21% [13] while in the report by Lu et al. taken place in China, the prevalence amounted to 7.1% [14]. Similar research conducted by Busetti et al. (2007) in Italy revealed the prevalence of GBS up to 17.9% [15]. In addition to geographical location and cultural differences, the use of antibiotics may also induce such differences.

In the present study, 51.5% of the urine GBS isolates were found to be serotype III, followed by Ia (21.2%), II (18.2%), Ib (6.1%) and V (3%). Serotype IV, VI, VII, and VIII were not found. In addition, results of vaginal isolates revealed that serotype III is the most prevalent one followed by serotype II, Ia, Ib, and V by frequency of 26.3%, 12.3%, 3.5%, and 3.5%, respectively. In vaginal isolates like urine isolates, serotypes IV, VI, VII, and VIII were not found at all. However, Rahnama et al. [16] showed that the most dominant serotype of GBS in Tehran population is serotype III (by frequency of 50%) followed by serotype V (16%), serotype Ia (14%) and serotype II (14%). The prevalence of serotype III in our study was the same as that of this study, but serotype V was more prevalent in Rahnama's study (~ 3.5% vs. 16%). Serotypes Ia and II were more prevalent in our study than the others (2-3 times). The differences may be dependent upon the source of samples as urine, vagina and semen. Janati et al. [17] showed that the prevalence of different serotypes of GBS in recto-vaginal samples of Ardabil is as follow: Serotype V (19.6%), III (12.5%), VI (10.7%), Ib (8.9%), VII (5.3%), and VIII (3.5%). The results of our study is not consistent with this study. Based on Janati's research [17], the prevalence of serotype III is relatively low (10.7%). Serotype V was the most prevalent serotype in Ardabil population (19.6%) while in our study, serotype V had a very low prevalence (~ 3%). Serotype IV was not found in our study as well as that of Absalan's et al. [8] but it was found to be prevalent in that of Janati's et al (12.5%) [17]. In addition, serotype VI and VIII were also found in Ardabil population which was not consonant with that of ours as well as Absalan's. Overal, our results are consistent with Absalan's et al. [8], both carried out in Yazd, but being in contrast with the study of Janati et al. [17]. Besides geographical location, these differences may be due to the type of study. In this study PCR technique was used whereas Janati's et al. [17] work was performed using serological method which is less sensitive compared to molecular techniques [9, 12, 16]

In addition, studies of Florindo et al. [18] carried out in Portugal, showed that serotype III, Ia, and V are the most prevalent serotypes, followed by serotypes II, IV, and Ib. Serotype VI and VIII were not found in this study. Comparing these results with those of ours, it was revealed that prevalence of serotype III and Ia is relatively the same in these two countries but serotype V is more prevalent in Portugal.

Interestingly, in a recent study by Sadeh et al. [9] which was also carried out in Yazd,

the prevalence of GBS in pregnant and non-pregnant women amounted to 15.6%. In addition, serotype III was the most prevalent serotype (52%) followed by serotype II, Ia, V and Ib by frequency of 20, 16.7, 6.7, and 6.7%, respectively. This study is strongly consistent with that of ours.

In the last part of our study, the correlation between different urine and vaginal isolates was assessed. Out of 346 pregnant women, 26 women were positive for both urine and vaginal samples. Our analysis revealed that there is a strong correlation between vagina and urine serotypes (p=0.000). According to the present data, all urine serotypes were correlated with vaginal serotype, except vaginal serotype II which was identified Ia in 40% of urine isolates.

Conclusions

This study highlighted that the GBS isolates form both urine and vagina of pregnant women are the same serotypes. However, this study together with few other investigations [8, 9] in our microbiology department have provided valuable information regarding both prevalence and serotype epidemiology of GBS in pregnant and non-pregnant women in the city of Yazd. These data may be efficient enough for GBS conjugate vaccine against serotype III, II, Ia, V and Ib.

Conflicts of Interest

We declare that there is no conflict of interest.

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References

[1]. Baker CJ, Barrett FF. Transmission of group B streptococci among parturient women and their neonates. J Pediatr. 1973; 83(6): 919-25.

[2]. Farley MM, Strasbaugh LJ. Group B streptococcal disease in nonpregnant adults. Clin Infec Dis. 2001; 33(4): 556-61.

[3]. High KP, Morven SE, Carol JB. Group B streptococcal infections in elderly adults. Clin Infec Dis. 2005; 41(6): 839-47.

[4]. Baker CJ, Barrett FF, Gordon RC, Yow MD. Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. J Pediatr. 1973; 82(4): 724-29.

[5]. Franciosi RA, Knostman JD, Zimmerman RA. Group B streptococcal neonatal and infant infections. J. Pediatr. 1973; 82 (4): 707-18.

[6]. Edwards MS, Baker CJ. Group B streptococcal infections, Infectious diseases of the fetus and the newborn infant, 5th ed. Philadelphia PA: WB Saunders Co; 2001: 1091-1156.

[7]. Cunningham FG, Williams JW. Williams Obstetrics. 22nd ed. New York: McGraw-Hill: Medical Pub Division; 2005: 39-90.

[8]. Absalan M, Eslami F, Zandi H, Mosaddegh A, Vakili M, Khalili MB. Prevalence of Recto-Vaginal Colonization of Group B Streptococcus in Pregnant Women. JIMS. 2013; 30(220): 2367-375.

[9]. Sadeh, M, Firouzi R, Derakhshandeh A, Khalili MB, Kong F, Kudinha T. Molecular characterization of streptococcus agalactiae isolates from pregnant and non-pregnant women at Yazd University Hospital, Iran. Jundishapur J Microbial. 2016; 9(2): e30412.

[10]. Slotved HC, Kong F, Lambertsen L, Sauer S, Gilbert GL. Serotype IX, a Proposed New Streptococcus agalactiae Serotype. J Clin Microbiol. 2007; 45(9): 2929-936.

[11]. Jens L, Samarut J, Hölttä E. A nontoxic and versatile protein salting-out method for isolation of DNA. Biotechniques 1994; 17(2): 316-18.

[12]. Poyart C, Tazi A, Réglier-Poupet H, Billoët A, Tavares N, Raymond J, et al. Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci. J clin microbiol. 2007; 45 (6): 1985-988.

[13]. Rausch AV, Gross A, Droz S, Bodmer T, Surbek DV. Group B Streptococcus colonization in pregnancy: prevalence and prevention strategies of neonatal sepsis. J perinatal Med. 2009; 37(2): 124-29.

[14]. Lu B1, Li D, Cui Y, Sui W, Huang L, Lu X. Epidemiology of Group B streptococcus isolated from pregnant women in Beijing, China. Clin Microbiol Infec. 2014; 20(6): O370-O373.

[15]. Busetti, M, Pierlanfranco DA, and Cesare C. Group B streptococcus prevalence in pregnant women from North-Eastern Italy: advantages of a screening strategy based on direct plating plus broth enrichment. J Clini Pathol. 2007; 60(10): 1140-143.

[16]. Rahnama S, Forouhesh Tehrani H, Amirmozafari N, Azadmanesh K, Biglari SH. Distribution of capsular serotypes in group B streptococci clinical. Razi J Med Sci. 2010; 17(78): 25-33.

[17]. Jannati E, Roshani M, Arzanlou M, Habibzadeh S, Rahimi G, Shapuri R. Capsular serotype and antibiotic resistance of group B streptococci isolated from pregnant women in Ardabil, Iran. Iran J Microbiol. 2012; 4(3): 130-35.

[18]. Florindo C, Damiao V, Silvestre I, Farinha C, Rodrigues F, Nogueira F, et al. Epidemiological surveillance of colonising group B Streptococcus epidemiology in the Lisbon and Tagus Valley regions, Portugal (2005 to 2012): emergence of a new epidemic type IV/clonal complex 17 clone. Eurosurveillance. 2014; 19(23): 20825.