Determination of Capsular Serotypes, Antibiotic Susceptibility Pattern, and Molecular Mechanism of Erythromycin Resistance among Clinical Isolates of Group B Streptococcus in Isfahan, Iran

Abstract

Background: Documented streptococcal resistance to erythromycin has recently been raised. The aim of this study is to identify the molecular mechanism of erythromycin resistance among Group B Streptococcus (GBS) strains and to correlate with the clinical origin of strains.

Materials and Methods: A total number of 134 colonizing (n = 36), invasive (n = 36), noninvasive (n = 46), and asymptomatic (n = 16) GBS isolates were characterized by the detection of dltS gene, capsular serotyping, antibiotic susceptibility profiles using disc diffusion method, and screening of the ermB, ermTR, and mefA resistance genes. Results: The distribution of capsular serotypes was as follows: serotype III (24.6%), Ia (21.6%), V (17.9%), Ib (14.9%), II (8.9%), IV (8.9%), VI (1.5%), and VII (1.5%). From 134 GBS isolates, 51 (38%) isolates were resistant to erythromycin. The constitutive macrolide lincosamide streptogramin B (MLSB) was the most common resistance phenotype (62.7%), followed by inducible MLSB (27.4%) and M phenotype (9.8%). Erythromycin resistance rate was higher among asymptomatic GBS strains (13/16, 81.2%). Serotype III was the most prevalent type among resistant isolates (41.1%). The ermB gene highly distributed among resistant strains (64.7%), followed by ermTR (21.5%) and mefA (9.8%). The ermB gene was related to constitutive MLSB phenotype (84.3%, P < 0.05) and serotypes III (61.9%), Ib (87.5%), and V (83.3%). All M phenotype strains harbored mefA gene and were in association with serotype Ia (90%). Conclusion: The current study suggests that ribosomal modification with erm genes is the main mechanism of erythromycin resistance. Because of relatively high prevalence of erythromycin resistance, double disc test highly recommended for GBS disease treatment and intrapartum prophylaxis among penicillin intolerant patients in our region.

Keywords: Antibiotic resistance, erythromycin, microbial, Streptococcus agalactiae

Introduction

Streptococcus agalactiae or group B Streptococcus (GBS) based on Lancefield classification is a leading cause of serious neonatal and adults infections.[1] GBS is often a part of normal flora of gastrointestinal and genitourinary tracts of healthy women.[2] Transmission during labor from colonized mothers to their babies can cause life-threatening infections and development of early onset disease (EOD) and late onset disease (LOD). Since colonized mothers can act as reservoir of GBS, Centers for Disease Control and Prevention, and the American Academy of Pediatrics recommended intrapartum antibiotic prophylaxis (IAP) to prevent perinatal GBS disease.[3] According to these recommendations, antenatal screening of pregnant women at 35–37 weeks of pregnancy with swab culture of rectovaginal secretions is done.[4] Pregnant mothers who have a positive urine or rectovaginal swab culture should receive IAP during delivery.[5] Penicillin is drug of choice for the treatment of GBS infections and IAP. However, reduced susceptibility to penicillin has been reported.[6] Erythromycin and clindamycin are used as alternative therapeutic agents for β-lactam allergic patients. Resistance to the erythromycin and clindamycin is not uncommon and increasingly has been observed worldwide.[7] Erythromycin resistance is mediated by two mechanisms that were first elucidated in Group A Streptococcus and Streptococcus pneumoniae. These mechanisms are ribosomal methylation of 23S rRNA by erm (erythromycin ribosome methylation) genes.
gene and efflux pump. Methylase enzymes encoded by over the forty variants with ermA, ermB, and ermTR identified in Streptococcus. They block binding of macrolide (including erythromycin), lincosamides (including clindamycin), and streptogramin B (MLSB) to the 50S ribosomal subunit, leading to cross-resistance. Based on the constitutive or inducible expression of erm gene, two MLSB phenotypes have been described. Inducible MLSB phenotype occurs when resistance to erythromycin induces resistance to clindamycin. This phenotype can detect with double-disc diffusion test (D-zone test) that make a D-shape zone of inhibition around the clindamycin disc. With a single disc diffusion test, these strains appear susceptible to the clindamycin. In the clinical laboratories, D-zone test should be considered as a routine test for the detection of the inducible MLSB (iMLSB) phenotype to prevent treatment failure with clindamycin and emergence of constitutive MLSB phenotype. Another resistance mechanism is mediated by an efflux pump encoded by the mefA/E gene. This pump confers the resistance to macrolides but not lincosamides, a characterization called M phenotype.

Capsular typing is a traditional method for the classification of GBS. According to the capsular polysaccharide (CPS), GBS classified to the ten serotypes (Ia, Ib, and II-IX). The CPS is one of the most important virulence factors and a target helping vaccine development.

Despite the clinical impact of GBS infections and increasing resistance rates to some antimicrobial agents, there are a limited number of studies reporting antimicrobial susceptibility profiles among GBS strains circulating in Iran. We undertook this study to document antimicrobial susceptibility testing, capsular genotyping and the presence of three macrolide resistance genes among GBS strains isolated from colonizing, invasive, noninvasive, and asymptomatic infections.

Materials and Methods

Bacterial isolates

In this cross-sectional study from July 2016 to September 2018, a total number of 134 nonduplicate GBS were collected from Alzahra and Shahid Beheshti hospitals in Isfahan, Iran. These isolates included colonizing GBS (n = 36) collected from swab culture of rectovaginal secretions of pregnant women at 35–37 weeks of gestations, invasive GBS collected from sterile body fluids of nonpregnant women such as blood and joint infections (n = 36), noninvasive GBS obtained from urinary tract infections, vaginal discharges, abscess, and tracheal tube secretions (n = 46, male = 9, female = 37), as well as asymptomatic women with positive urine culture with colony count <10³ CFU/mL (n = 16). All isolates identified with morphology of colony, beta hemolysis on the sheep blood agar, Gram stain, catalase reaction, CAMP test, and polymerase chain reaction (PCR) amplification and detection of 952 bp dltS gene specific for GBS species after DNA extraction.

Antibiotic susceptibility profile

The disc susceptibility method based on the criteria of the Clinical and Laboratory Standards Institute (CLSI) 2018 edition was utilized to determine the antimicrobial susceptibility patterns to the following nine antimicrobial discs (Mast, Merseyside, UK) and concentrations: penicillin (10 units), vancomycin (30 µg), ceftriaxone (30 µg), tetracycline (30 µg), clindamycin (2 µg) and erythromycin (15 µg), cefepime (30 µg), cefotaxime (30 µg), and levofloxacin (5 µg). For clindamycin, the detection of inducible resistance was performed by double disc diffusion testing (DD-test) in order to categorize the isolates as iMLSB, constitutive MLSB (cMLSB) (constitutive MLSB), M and L phenotypes.

DNA extraction

The genomic DNAs of GBS isolates were extracted using a simple boiling method. Briefly, a loopful of bacterial biomass was suspended in 300 µl of TSE buffer (50 mM Tris hydrochloride [pH 7.5], 1% SDS, 25 mM EDTA), and the suspension was heated at 95°C for 20 min and centrifuged at 10,000 g for 10 min. The supernatant was taken as DNA lysate and was kept at −20°C for the molecular assay.

Capsular genotyping

Capsular genotyping was performed using nine pairs of primers and multiplex PCR assay for the detection of Ia, Ib, and II-VIII serotypes as previously described. A primer pairs (dltS-F and dltS-R) targeting the GBS-specific dltS gene were also included as an internal positive control.

Detection of macrolide resistance genes

All erythromycin-resistant GBS surveyed for the presence of the ermTR, ermB, and mefA genes using PCR method with previously published primers.

Statistical analysis

The SPSS Statistics V. 20.0 (IBM SPSS Statistics for Windows, Armonk, NY, United States: IBM Corp) were used for statistical analysis. Association between capsular serotype with the distribution of erythromycin-resistance genes was assessed by applying Fisher’s exact test. Differences were considered statistically significant at P < 0.05.

Results

Of 134 GBS isolates studied in this study, 51 isolates were resistant to erythromycin. A number of 15/51 colonizing isolates (29.4%), 15/51 (29.4%) invasive isolates, 8/51 (15.6%) noninvasive GBS, and 13/51 (25.4%) asymptomatic isolates were erythromycin resistant.
According to the source of GBS isolates, 41.6% of colonizing GBS (15/36), 41.6% of invasive GBS (15/36), 17.3% of noninvasive GBS (8/46), and 81.2% of asymptomatic GBS isolates (13/16) were resistant. Therefore, we found high presence of erythromycin resistance among asymptomatic adults.

Capsular genotyping of 134 GBS isolates showed that all isolates were typeable and except for serotype VIII and IX, all types were detected. The distribution of capsular serotypes was as follow: serotype III (33, 24.6%), Ia (29, 21.6%), V (24, 17.9%), Ib (20, 14.9%), II (12, 8.9%), IV (12, 8.9%), VI (2, 1.5%), and VII (2, 1.5%). For colonizing GBS, serotype Ia was frequently detected (11, 30.5%), followed by III (8, 22.2%) and Ib and V (each 6, 16.6%). For invasive isolates, serotype III (11, 30.5%) followed by Ib and V (each 5, 13.8%), IV and Ia (each 4, 11.1%). For noninvasive GBS strains, serotypes III and V (each 10, 21.7%) were the most common capsular types. Asymptomatic GBS isolates had serotype Ia as most common type (31.2%). Distribution of serotypes across source was demonstrated in Figure 1.

All isolates were susceptible to penicillin, cefotaxime, and vancomycin. Based on CLSI criteria, two isolates were nonsusceptible to cefepime and ceftiraxone. Resistance to the levofloxacin was detected in 15 isolates (11.1%). The overall frequency of tetracycline resistance was 91%. Out of the 134 isolates studied, 51 (38%) isolates were resistant to erythromycin and 42 (31.3%) of them to clindamycin [Table 1]. Results of double-disc diffusion test revealed that the cMLSB phenotype was the most frequent (62.7%), followed by iMLSB (27.4%) and M phenotype (9.8%) [Table 2]. In respect to the prevalence of serotypes among erythromycin-resistant GBS, we found that 41.1% of resistant isolates were belonged to serotype III (P < 0.05). Among the cMLSB-GBS strains, CPS-III and V (18/32, 56.2%) and iMLSB-GBS strains CPS-III and Ib (10/14, 71.4%) were the most frequent serotypes. In contrast, M phenotype strains were predominately associated with serotype Ia isolates (4/5, 90%). Details are summarized in Table 3. The ermB gene and to a lesser extent ermTR gene were widely distributed (64.7% and 21.5%, respectively). However, only five isolates were positive for mefA gene (9.8%). Resistance genes ermB and ermTR were combined in one invasive strain with CPS-V and cMLSB phenotype. In this study, modification of 23S rRNA of 50S subunit of ribosome due to methylases encoded by ermB and ermTR genes was the most common reason of resistance to erythromycin. The cMLSB phenotype was in association with ermB gene (84.3%) (P < 0.05). Besides, two genes ermB and ermTR were found in approximately equal amounts in iMLSB phenotype strains. The M phenotype was detected only in isolates harboring the mefA gene (100%) [Table 2].

According to source of GBS isolates, cMLSB phenotype/ermB gene was most common phenotype and genotype among colonizing, invasive, noninvasive, and asymptomatic strains [Figure 2].

Discussion

The increasing trend in the rates of resistance to erythromycin and clindamycin among GBS isolates has raised concerns about the use of these antibiotics as alternative agents for the prophylaxis or treatment of GBS infections in beta-lactam allergic patients. In current study, a rate of 38% resistance to erythromycin was detected. In

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**Table 1: Antibiotic susceptibility profile of 134 Group B Streptococcus isolates**

| Antibiotic | Susceptible, n (%) | Intermediate, n (%) | Resistant, n (%) |
|------------|--------------------|---------------------|-----------------|
| Penicillin | 134 (100)          | -                   | -               |
| Cefepime   | 132 (98.5)         | 2 (1.5)             | -               |
| Ceftriaxone| 132 (98.5)         | 2 (1.5)             | -               |
| Cefotaxime | 134 (100)          | -                   | -               |
| Vancomycin | 134 (100)          | -                   | 122 (91.1)      |
| Tetracycline| 12 (8.9)          | 106 (79.1)          | 13 (9.7)        |
| Levofloxacin| 61 (45.5)         | 31 (23.2)           | 42 (31.3)       |
| Erythromycin | 53 (39.6)         | 30 (22.4)           | 51 (38)         |

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**Figure 1: Distribution of serotypes according to the source of 134 Group B Streptococcus isolates**

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**Figure 2: Distribution of erythromycin resistance phenotypes/genotypes according to source of 51 resistant isolates**
addition, we found most of the erythromycin-resistant isolates were obtained from the rectovaginal samples of pregnant women and sterile body fluids of women with invasive infections (30/51, 58.8%). This issue leads to an increase in using vancomycin and cefazolin for the prophylaxis and treatment, which could enhance the emergence of vancomycin-resistant strains in the future.\cite{5} A meta-analysis by Khademi and Sahebkar found that the overall prevalence of resistance to erythromycin among pregnant women was 21%.\cite{5} However, Jalalifar et al. found a higher presence of erythromycin resistance among adults with urinary tract infections.\cite{14} Results of a study showed lower rate of 25% resistance to the erythromycin among colonizing and invasive GBS in adults.\cite{15} Besides, the erythromycin resistance was particularly high in the USA (54%), France (41.7%), and China (74.1%), but in Spain, only 20.7% of GBS strains were resistant.\cite{7, 16, 17} Comparison of distribution of resistant isolates in each group showed this rate was higher among asymptomatic GBS (13/16, 81.2%). Borchardt et al. reported a high prevalence of resistance to erythromycin among asymptomatic adults in comparison to invasive ones.\cite{18} Other study from Poland showed the proportion of erythromycin resistance among invasive versus carriage and noninvasive isolates was similar.\cite{19}

Capsular serotyping of GBS is crucial in determining the pathogenicity of the isolates and vaccine development.\cite{20} Placental transfer of anti-CPS-specific GBS antibodies from the mother to the fetus reduces the risk of invasive GBS disease with the evidence of protection against both EOD and LOD. Five serotypes Ia, Ib, II, III, and V are the predominant types of GBS that cause infections in neonates and adults.\cite{1} Literature of review showed that a hexavalent polysaccharide–protein conjugate vaccine (Ia, Ib, II, III, IV, and V) has the potential to prevent up to 93% of worldwide maternal colonizing isolates, 95% of maternal invasive GBS disease, 99% of GBS-associated stillbirth, and 99% of infant invasive GBS disease. Although evidence is still limited, a vaccine targeting maternal colonization could provide additional protection against neonatal disease.\cite{5, 20, 21} Our results showed serotypes Ia, III, and V accounted for 64.1% of GBS isolates. With regard to the source of isolates, the most prevalent serotype in colonizing and asymptomatic GBS isolates was Ia and in invasive and noninvasive GBS were serotypes III and V. Maternal colonization by serotype Ia is seen globally, but prevalence and geographical distribution of serotypes is different.\cite{5} Regarding maternal colonization, previous studies in our country showed despite our results, CPS III was the most common serotype.\cite{22, 23} Serotype Ia was the most prevalent type in the United States, United kingdom, and South Korea contributing to maternal colonization and EOD.\cite{5} CPS III is well known for its association with infections in neonates and adults, and it has been reported throughout the world.\cite{5} Several investigations revealed the importance of serotype V for its association with infections in nonpregnant adults.\cite{5} In this study, most of serotype V strains were belonged to patients with noninvasive infections. Another studies in the United Kingdom, South Africa, and France found serotype V as dominant CPS among maternal disease and colonization.\cite{22, 24} In this study, a high prevalence of serotype III among erythromycin-resistant

| Table 2: Distribution of resistance genes across erythromycin resistance phenotypes |
|------------------------------------------|-----------------|-----------------|-----------------|
| Erythromycin resistance genes | Erythromycin resistance phenotype | Total, n (%) |
|------------------------------------------|-----------------|-----------------|
| ermB | cMLSB, n (%) | iMLSB, n (%) | M phenotype, n (%) |
|------------------------------------------|-----------------|-----------------|
| ermTR | 27 | 6 | - | 33 (64.7) |
| mefA | 4 | 7 | - | 11 (21.5) |
| Negative PCR | 5 | 1 | - | 5 (9.8) |
| Total | 32 (62.7) | 14 (27.4) | 5 (9.8) | 51 (100) |

MLS: Macrolides, lincosamides, and streptogramin B, cMLSB: Constitutive MLSB, iMLSB: Inducible resistance to MLSB, PCR: Polymerase chain reaction

| Table 3: Distribution of resistance phenotypes and genotypes across serotypes among 51 erythromycin-resistant Streptococcus Group B |
|------------------------------------------|-----------------|-----------------|-----------------|
| Serotypes | Group B Streptococcus | Total, n (%) |
|------------------------------------------|-----------------|-----------------|
| Ia, n (%) | 8 (15.6) | 8 (15.6) | 21 (41.1) | 3 (5.8) | 6 (11.7) | 2 (3.9) | 1 (1.9) | 51 (100) |
| Ib, n (%) | 7 (21.8) | 13 (39.3) | 2 (5.7) | 5 (14.2) | 1 (2.8) | 33 (64.7) |
| III, n (%) | 7 (21.8) | 1 (2.8) | 13 (41.3) | 2 (5.7) | 5 (14.2) | 1 (2.8) | 33 (64.7) |
| IV, n (%) | 1 (8.3) | 1 (8.3) | 5 (41.6) | 2 (16.6) | 1 (2.8) | 33 (64.7) |
| V, n (%) | - | 1 (8.3) | 2 (16.6) | 1 (2.8) | 33 (64.7) |
| VI, n (%) | - | - | - | - | - | - | - |
| VII, n (%) | - | - | - | - | - | - | - |
| Total | 32 (62.7) | 14 (27.4) | 5 (9.8) | 51 (100) |

Two serotypes III had negative results in PCR amplification of all three genes. MLS: Macrolides, lincosamides, and streptogramin B, cMLSB: Constitutive MLSB, iMLSB: Inducible resistance to MLSB, PCR: Polymerase chain reaction
GBS isolates was observed. Previous studies found an association between erythromycin resistance with serotypes II, III, or V.\cite{8,14}

Results of double-disc diffusion test revealed that cMLSB phenotype was the most common among erythromycin resistant strains followed by iMLSB and M phenotype. Many studies have confirmed the high prevalence of the cMLSB phenotype over the other two phenotypes.\cite{15,16,26}

Based on our finding, 64.7% of the erythromycin-resistant strains carried the resistance gene ermB and cMLSB phenotype was strongly related to this gene. Other investigations in our country, France, and China confirmed our results and ermB was the most frequent gene related to erythromycin resistance.\cite{16,24,27} However, all M phenotype strains in this study were associated with the mefA gene. The efflux pump encoded by mefA gene unable to pump out clindamycin and other lincosamides even in the presence of erythromycin. Hence, strains harboring mefA gene cannot develop MLSB or L phenotype. This issue confirmed with other previous studies.\cite{28}

Previous studies have suggested a possible association between serotype V or III strains and the presence of the ermB gene and between serotype Ia and the mefA gene.\cite{9,24,29} We also found such associations: the mefA gene was found in 50% of the resistant serotype Ia strains, and the ermB gene in 61.9% of the resistant serotype III strains, 87.5% of the resistant serotype Ib strains, and 83.3% of the resistant serotype V strains.

Erythromycin resistance in two of our strains was not associated with either the mefA or the ermB and ermTR genes. Such resistance in beta-hemolytic streptococci may be related to mutations in ribosomal proteins, as previously reported for S. pneumoniae.\cite{30}

**Conclusion**

All GBS isolates included in this study were susceptible to penicillin, cefotaxime, and vancomycin. These results confirm that the use of penicillin as a drug of choice for prophylaxis and treatment of GBS infections in Iran is appropriate. A high rate of erythromycin resistance (38%) was found among the GBS isolates studied. These results agree with data from the other parts of our country, which showed a resistance rate of 21%\cite{13} but differ from the 52% erythromycin resistance rate reported by others.\cite{14} This discrepancy may be related to the different isolate collection periods and source of GBS. Erythromycin and clindamycin should no longer be relied upon as an alternative agent for prophylaxis and treatment of GBS infections in Iran without susceptibility testing. Most of resistant isolates were serotype III/cMLSB/\textit{ermB}\textsuperscript{+}. Dominant distribution of cMLSB phenotype showed ribosomal modification with \textit{ermB} gene is the main mechanism of erythromycin resistance. Since iMLSB-GBS isolates appear susceptible to clindamycin but may be inappropriate for the treatment of infection, the use of the double disc diffusion test is strongly recommended.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Shabayek S, Spellerberg B. Group B streptococcal colonization, molecular characteristics, and epidemiology. Front Microbiol 2018;9:437.

2. Madrid L, Seale AC, Kohli-Lynch M, Edmond KM, Lawn JE, Heath PT, \textit{et al}. Infant group B streptococcal disease incidence and serotypes worldwide: Systematic review and meta-analyses. Clin Infect Dis 2017;65:S160-72.

3. Steer PJ, Russell AB, Kochhar S, Cox P, Plumb J, Gopal Rao G. Group B streptococcal disease in the mother and newborn – A review. Eur J Obstet Gynecol Reprod Biol 2020;252:526-33.

4. Pietrocola G, Arciola CR, Rindi S, Montanaro L, Speziale P. \textit{Streptococcus agalactiae} non-pilus, cell wall-anchored proteins: involvement in colonization and pathogenesis and potential as vaccine candidates. Front Immunol 2018;9:602.

5. Furfaro LL, Chang BJ, Payne MS. Perinatal streptococcus agalactiae epidemiology and surveillance targets. Clin Microbiol Rev 2018;31:e00049-e00018.

6. Seki T, Kimura K, Reid ME, Miyazaki A, Banno H, Jin W, \textit{et al}. High isolation rate of MDR group B streptococci with reduced penicillin susceptibility in Japan. J Antimicrob Chemother 2015;70:2725-8.

7. Hayes K, O’Halloran F, Cotter L. A review of antibiotic resistance in Group B \textit{Streptococcus}: The story so far. Crit Rev Microbiol 2020;46:1-17.

8. Oppegaard O, Skrede S, Mylvaganam H, Kittang BR. Emerging threat of antimicrobial resistance in β-hemolytic streptococci. Front Microbiol 2020;11:797.

9. Poyart C, Tazi A, Réglier-Poupet H, Billoët A, Tavares N, Raymond J, \textit{et al}. Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci. J Clin Microbiol 2007;45:1985-8.

10. Mobascherzadeh S, Shojaei H, Azadi D, Havaei SA, Rostami S. Molecular characterization and genotyping of methicillin-resistant \textit{Staphylococcus aureus} in nasal carriage of healthy Iranian children. J Med Microbiol 2019;68:374-8.

11. Ogbolu DO, Alli OA, Ohuremi AS, Onifade CO. Erythromycin resistance determinants in a clinical gram positive cocci isolated from Nigerian patients. J Clin Diagn Res 2018;12:5-10.

12. Seale AC, Bianchi-Jassir F, Russell NJ, Kohli-Lynch M, Tann CJ, Hall J, \textit{et al}. Estimates of the burden of group B streptococcal disease worldwide for pregnant women, stillbirths, and children. Clin Infect Dis 2017;65:S200-19.

13. Khademi F, Sahebkar A. Group B \textit{Streptococcus} drug resistance in pregnant women in Iran: A meta-analysis. Taiwan J Obstet Gynecol 2020;59:635-42.

14. Jalalifar S, Havaei SA, Motallebirad T, Moghim S, Fazeli H,
Esfahani BN. Determination of surface proteins profile, capsular genotyping, and antibiotic susceptibility patterns of Group B Streptococcus isolated from urinary tract infection of Iranian patients. BMC Res Notes 2019;12:1-6.

15. Khodaei F, Najafi M, Hasani A, Kalantar E, Sharifi E, Amini A, et al. Pilus-encoding islets in \textit{S. agalactiae} and its association with antibacterial resistance and serotype distribution. Microb Pathogen 2018;116:189-94.

16. Bergal A, Loucif L, Benouareth DE, Bentorki AA, Abat C, Rolain JM. Molecular epidemiology and distribution of serotypes, genotypes, and antibiotic resistance genes of \textit{Streptococcus agalactiae} clinical isolates from Guelma, Algeria and Marseille, France. Eur J Clin Microbiol Infect Dis 2015;34:2339-48.

17. López Y, Parra E, Cepas V, Sanfeliú I, Juncosa T, Andreu A, et al. Serotype, virulence profile, antimicrobial resistance and macrolide-resistance determinants in \textit{Streptococcus agalactiae} isolates in pregnant women and neonates in Catalonia, Spain. Enferm Infec Microbiol Clin 2018;36:472-7.

18. Borchardt SM, DeBusscher JH, Tallman PA, Manning SD, Marrs CF, Kurzynski TA, \textit{et al.} Frequency of antimicrobial resistance among invasive and colonizing Group B streptococcal isolates. BMC Infect Dis 2006;6:1-7.

19. Sadowy E, Matynia B, Hryniewicz W. Population structure, virulence factors and resistance determinants of invasive, non-invasive and colonizing \textit{Streptococcus agalactiae} in Poland. J Antimicrob Chemother 2010;65:1907-14.

20. Bianchi-Jassir F, Paul P, To KN, Carreras-Abad C, Seale AC, Jauneikaitė E, \textit{et al.} Systematic review of Group B Streptococcus capsular types, sequence types and surface proteins as potential vaccine candidates. Vaccine 2020;38:6682-94.

21. Song JY, Lim JH, Lim S, Yong Z, Seo HS. Progress toward a group B streptococcal vaccine. Hum Vaccin Immunother 2018;14:2669-81.

22. Mansouri S, Ghasami E, Shahabi Najad N. Vaginal colonization of group B streptococci during late pregnancy in southeast of Iran: Incidence, serotype distribution and susceptibility to antibiotics. J Med Sci 2008;8:574-8.

23. Beigverdi R, Jabalameli F, Mirsaleheian A, Hantoushzadeh S, Boroumandi S, Taherikalani M, \textit{et al.} Virulence factors, antimicrobial susceptibility and molecular characterization of \textit{Streptococcus agalactiae} isolated from pregnant women. Acta Microbiol Immunol Hung 2014;61:425-34.

24. Nabavinia M, Khalili MB, Eslami G, Vakili M, Azartoos N, Mojihiyan M. Distribution of Pilus island and antibiotic resistance genes in \textit{Streptococcus agalactiae} obtained from vagina of pregnant women in Yazd, Iran. Iran J Microbiol 2020;12:411-6.

25. 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;4:130-5.

26. Lu B, Chen X, Wang J, Wang D, Zeng J, Li Y, \textit{et al.} Molecular characteristics and antimicrobial resistance in invasive and noninvasive Group B \textit{Streptococcus} between 2008 and 2015 in China. Diagn Microbiol Infect Dis 2016;86:351-7.

27. Wu B, Su J, Li L, Wu W, Wu J, Lu Y, \textit{et al.} Phenotypic and genetic differences among group B \textit{Streptococcus} recovered from neonates and pregnant women in Shenzhen, China: 8-year study. BMC Microbiol 2019;19:185.

28. Cattoir V, Leclercq R. Resistance to Macrolides, Lincosamides, and Streptogramins. In: Mayers D., Sobel J., Ouellette M., Kaye K., Marchaim D. (eds) Antimicrobial Drug Resistance. Springer, Cham. 2017;269-80.

29. Gizachew M, Tiruneh M, Moges F, Adefris M, Tigabu Z, Tessema B. \textit{Streptococcus agalactiae} from Ethiopian pregnant women; prevalence, associated factors and antimicrobial resistance: Alarming for prophylaxis. Ann Clin Microbiol Antimicrob 2019;18:3.

30. Akoğlan Kitanan FN, Mustak IB, Hascelik G, Saricam S, Gurler N, Diker KS. Erythromycin-resistant \textit{Streptococcus pneumoniae}: Phenotypes, genotypes, transposons and pneumococcal vaccine coverage rates. J Med Microbiol 2019;68:874-81.