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First Study of *scpB* Gene of *Streptococcus agalactiae* in Misiones, Argentina

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Streptococcus agalactiae* (GBS) is the leading cause of neonatal infections. GBS presents different surface proteins of antigenic characteristics. These proteins are encoded by several genes associated with virulence and host interaction acting on bacteria involved in invasiveness. The study of these surface protein antigens is important for the understanding of the pathogenesis and epidemiology of the infection. Several of these antigens have been proposed as components of multivalent conjugate vaccines. This study focuses on the presence of *scpB* gene encoding the C5a peptidase in GBS. Recent studies indicate that C5a peptidase vaccine formulations cause a long-term immune response and prevent GBS infection.
Methodology: A total of 200 *Streptococcus agalactiae* isolates were collected from vaginal or rectal swabs of pregnant women with 35 – 37 weeks gestational age. Conventional biochemical tests and commercial kits for latex particle agglutination (Phadebact Strep B Test-ETC-Bactus International AB Sweden) were used to identify those isolates. GBS strains were preserved in 20% skim milk at -80°C and were recovered to search the gene *scpB*. The *scpB* gene was investigated by conventional PCR. PCR products were visualized on 2% agarose gel gel stained with GelRed® (Biotium, Inc.; US) and visualized of bands in UV trans illuminator (MUV Model 21-312-220), using

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MP (D0017 marker DNA fragments made up of 10 double-stranded DNA molecular size of 100 to 1000 pb-INBIO, Argentina).
Results: The presence of the *scpB* gene was detected in 100% of *Streptococcus agalactiae* strains studied.
Conclusion: Our findings highlight the importance of including C5a peptidase in the design of a regional vaccine. The high specificity of the *scpB* gene in GBS, and the lack of homologous sequences in other bacterial genera and species, makes it a molecular marker that can be used in the diagnosis of colonization or infection by this microorganism.

Keywords: Streptococcus agalactiae; ScpB gene; C5a peptidase.

1. INTRODUCTION

Streptococcus agalactiae, Lancefield group B beta-hemolytic streptococci (GBS), is an important human pathogen which causes a wide range of human diseases. GBS was first described as a human pathogen associated with puerperal fever [1]. Currently, this microorganism is the most common cause of life-threatening neonatal infections, producing pneumonia, sepsis, and meningitis in new-borns and infants [2-4]. The colonization of the genital tract of pregnant women at the end of their gestational age is significantly associated with these infections. Infants acquire GBS in the uterus by the ascending route through intact membrane rupture or during the birth process [5-7].

While GBS is regarded as the agent responsible for the most severe diseases that human beings in their first hours of life can suffer, it has lately been recognized as an agent of disease in non-pregnant adult with underlying chronic diseases and the elderly [8,9]. GBS is responsible for a rising number of severe invasive infections including endocarditis, septic arthritis, meningitis, bacteraemia, skin and soft-tissue infections [10-13].

GBS has different virulence factors for developing the disease. One of these virulence factors is the capsule of polysaccharide structure which also allows their differentiation into 10 serotypes (Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX) [14,15]. The GBS serotype IX, was recently described for Argentina by our working group, with published data [16].

In combination with the capsular polysaccharide, GBS has surface proteins that act as virulence factors involved in the different steps of the road damage to produce disease. These include, α and β protein, which belong to C protein complex; Lmb, HylB, beta-haemolysin, Rib protein and C5a peptidase [17,18]. They are encoded by several genes and mediate the adhesion, colonization and bacterial invasion; they are also antigenic and therefore activate host immune response. These proteins may be used in the development of maternal vaccines against GBS [19-21].

The study of these surface protein antigens is important for the understanding of the pathogenesis and epidemiology of infection. Several of these antigens have been proposed as components of multivalent conjugate vaccines. In this study we investigated the presence of *scpB* gene encoding the C5a peptidase. Recent studies indicate that C5a peptidase vaccine formulations cause a long-term immune response and prevent GBS infection [22]. Particularly, the streptococcal C5a peptidase, encoded by *scpB* gene, has been widely studied, as a highly specific adhesion and protease / invasion in GBS [23].

The C5a peptidase of GBS is a serine protease surface-associated that inactivates human C5a (a neutrophilic chemotactic protein during complement activation) [24]. The C5a peptidase binds to fibronectin and helps to invade cells, it plays a fundamental role in the virulence of this organism and its structure was recently discovered [17] [23-25]. This streptococcal protein is also important in the pathogenesis of GBS by inhibiting neutrophil recruitment and helps reduce the host inflammatory response [17,26].

Recent studies showed that all strains isolated from human GBS exhibit the C5a peptidase gene, *scpB* gene, and only some of the isolates from cattle [27,28]. This specificity associated to the host and bacterial species determine the use of *scpB* gene as a molecular marker to determine the mother carrying GBS or GBS disease [29].

The nucleotide sequence of the C5a peptidase reveals no significant homology to other bacterial

proteins and neither was the gene found in other bacterial species [30].

The frequency of these proteins and the genes encoded usually vary according to the origin of the strains (colonization or invasive), study periods, geographic areas, social status and ethnicity of the studied population [31].

The aim of this study was to investigate the presence of *scpB* gene encoding peptidase c5a in colonizing strains of GBS, to contribute to the knowledge of virulence of GBS, and to provide input for the development of a regional vaccine.

The importance of this study lies in the absence of similar data from the province of Misiones and from other provinces in Argentina. Misiones is a particular province in Argentina from the epidemiological point of view, because it shares 90% of its borders with other countries in the region.

2. MATERIALS AND METHODS

2.1 GBS Isolates

A total of 200 GBS isolates were collected from vaginal or rectal swabs of pregnant women with 35-37 weeks of gestational age.

Swabs were inoculated into tubes of selective Todd-Hewitt broth (Britania, Argentina) supplemented with antibiotics (colistin 10 µg / ml - nalidixic acid 10 µg/ml), and were incubated at 35°C for 18 – 24 h. The broths were sub cultured onto sheep blood agar plates and were incubated at 35°C in 5% CO2 for 18 – 24 h. Isolates were identified as GBS based on cultural characteristics, Gram stain and the following tests: catalase, esculin hydrolysis, hippurate hydrolysis, PYR (pyrrolidonyl aminopeptidase), and Christie, Atkins, and Munch-Peterson (CAMP) reaction.

Serologic confirmation of group B *streptococcus* was performed with a commercial kit for latex agglutination (Phadebact Strep B Test International ETC, Bactus AB, Switzerland), according to the protocol provided by the manufacturer.

2.2 Storage of Strains

GBS strains were preserved in 20% skim milk at -80°C and were recovered to search the gene *scpB*.

2.3 DNA Extraction for Polymerase Chain Reaction (PCR)

The prokaryotic DNA extraction was performed with a liquid culture of 24 h according to the extraction protocol of Sambrook [32]. Evaluating the quality of the DNA was performed by electrophoresis in a 1% agarose gel stained with GelRed® (Biotium, Inc.; US) and visualized of bands in UV trans illuminator (MUV Model 21-312-220).

2.4 Detection of *SCPB* Gene

The search the *scpB* gene was performed by conventional PCR. The PCR mixture consisted of buffer (10 mM Tris-HCl [pH 8.3], 50 Mm KCl), 2 mM $MgCl_2$, 0.2 mM each of the four deoxynucleoside triphosphates (K1404, InBio Highway, Argentina), 10 ppm primer, 1µl de DNA, and 0.5 U of Taq DNA polymerase (E1201, Inbio Highway, Argentina) in a total volume of 20 µl. The primers used are shown in Table 1; their sequences were matched with GenBank and were synthesized by Invitrogen (Korea). Each sample was subjected to the first cycle of denaturation (2 min at 94°C). Each of the 30 subsequent cycles consisted of denaturing at 94°C for 30 s, annealing at 58°C for 1 min, and extension at 72°C for 1 min. The last cycle included an extension at 72°C for 2 min. A negative control test was included in each test, which consisted of the same reaction mixture using water instead of DNA.

In addition, a positive control (GBS BB47), containing the same reaction mixture with template of DNA, was also included. PCR products were visualized on 2% agarose gel using the same equipment as the one for assessing quality of genomic DNA, using MP (D0017 marker DNA fragments made up of 10 double-stranded DNA molecular size of 100 to 1000 pb-INBIO, Argentina).

2.5 Automated DNA Sequencing

Automated sequencing of PCR products is performed by Automatic Sequencing Service Macrogen, Korea.

3. RESULTS

3.1 Detection of *scpB* Gene

The *scpB* gene encoding the C5a peptidase was detected in 200 GBS strains included in this

work. Amplified products separated by electrophoresis in a 2% agarose gel and bands visualized as white bands on a black background by UV trans illuminator are show in Fig. 1. Each figure shows the PCR products obtained by GBS serotype.

3.2 Nucleotide Sequence Analysis

Using primers 5´-AGCCATATGCTGCGATCTCT-3´ and 5´-GGGTTGAACCAAGTGTGCTT-3´ a fragment of 198 bp was obtained. This fragment was sequenced and subjected to bioinformatic analysis. The sequence was studied with the NCBI database using BLASTn. The analyzes are based on the similarity with the aligned sequences in the BLAST search.

The search results yielded a 100% identity with the gene *scpB* (GenBank U56908.1).

4. DISCUSSION

During the past four decades, GBS remains the agent of the most serious diseases that newborns in their first hours of life can suffer, such as sepsis, bacteremia and meningitis [5,7]. In the last years, invasive disease in patients with underlying chronic diseases or elderly [8,9] have been described and even new serotypes of GBS have been included [33].

Current scientific developments are focused on the development of a maternal vaccine that can prevent this severe neonatal disease and in new strategies for the new challenges caused by GBS. Research studies focusing on providing data for the advancement of science in this regard are relevant.

Table 1. Sequence of primers used in PCR to amplify the *scpB* gene in isolates of *Streptococcus agalactiae*

Gene	Forward 5´- 3´	Reverse 5´ - 3´	Amplicon (bp)	TM (°C)	GenBank
cspB	AGCCATATGCTGCGATCTCT	GGGTTGAACCAAGTGTGCTT	198	58	U56908.1

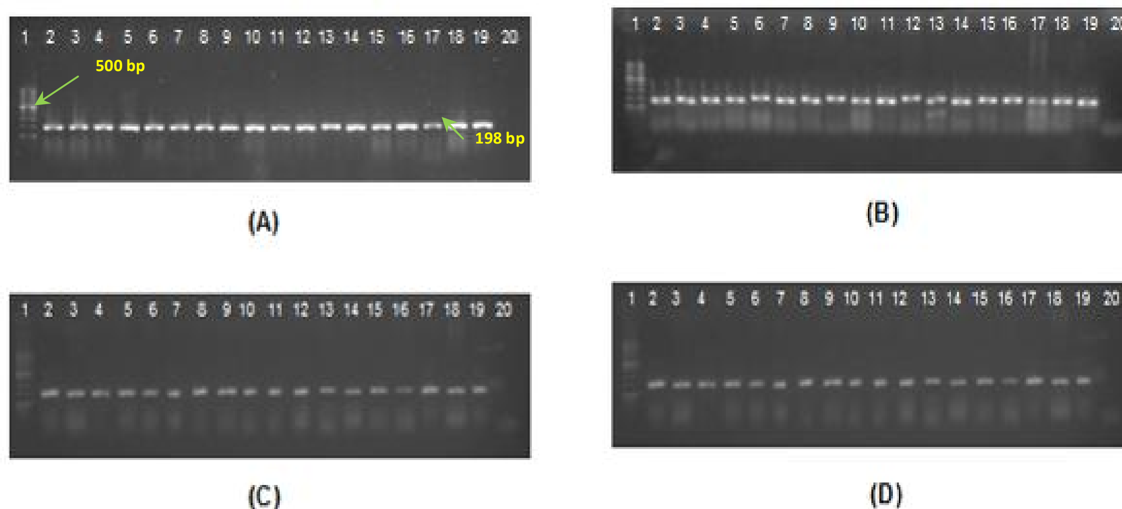

Fig. 1. Electrophoresis in agarose gel 2% of PCR products for *scpB* gene of *Streptococcus agalactiae*

First lane, molecular weight marker, Marker DNA (D0017) 100 a 1000 pb–INBIO, Argentina; lane 2-18, strains Streptococcus agalactiae (GBS): (A) 23, 48, 96, 99, 74, 155, 165, 211, 213, 297, 390, 349, 489, 500, 835, 883, 1057; (B) 1072, 1105, 1111, 1123, 1143, 1155, 1184, 1186, 1187, 1211, 1214, 1217, 1256, 1265, 1286, 1316, 1317; (C) 223, 262, 540, 561, 901, 934, 1041, 1052, 1385, 459, 1485, A10, Z5, 57, 113, 130, 198; (D) 109, 137, 170, 375, 485, E6149, 36VR, 29, 31, 32, 93, 222, 1020, 1090, 1249, 1337, 836; lane 19, positive control, last lane, negative control

C5a peptidase encoded by the *scpB* gene plays an important role in virulence of GBS; it is a serine protease localized on the bacterial surface that inactivates human C5a, a neutrophils chemo attractant during complement activation [17,26]. In addition, C5a peptidase allows adhesion to fibronectin by interaction with another surface protein, the Lmb protein encoded by *lmb* gene [26].

Both genes are located within a putative composite transposon [26]. These features convert to a streptococcal C5a peptidase in a highly specific proteases and adhesin/invasion [34].

In this work, *scpB* gene encoding C5a peptidase was detected in 100% of GBS strains studied. Our results agree with some authors who have detected equal percentages of carrying the gene *scpB* [35] and differ from others where *scpB* gene was presented at various frequencies 88.3% [36], 94.7% [37], 96.1% [38] and 97.6% [39], all equally high.

Previous studies have shown that *scpB* gene can be used as a molecular marker in GBS strains [30]. This gene encoding the C5a peptidase is present in all isolates of streptococci group A, B and G recovered from humans [28][40]. Specifically, *scpB* gene has a deletion in the 3' portion of the gene; the *scpA* and *scpG* genes have not this deletion in group A and G streptococcal strains, respectively [29,40].

This deletion in the 3 ' portion of *scpB* gene GBS, allowed the development of diagnostic methods based on conventional PCR with quick results and higher specificity and sensitivity to the cultures [29,41]. The importance in the search and characterization of this gene is well demonstrated.

GBS presents other surface proteins encoded by different genes including Rib, Lmb, HylB, Bac and Bca (both members of the protein complex C), which are typically detected at much lower frequencies than 100% [36-38], so they would not be good candidates to be included in the development of vaccines against GBS.

The well-studied streptococcal C5a peptidase, a highly conserved surface protein that is expressed by all serotypes of *Streptococcus* tested to date, proves to be an excellent candidate for both maternal vaccines that induce mucosal and systemic immunity [22,42]. The C5a peptidase free or encapsulated induces mucosal and systemic immunity in immunized mice while developed vaccines induce protection against GBS disease in puppies born to vaccinated mothers [42].

Our findings support the idea of including surface protein C5a peptidase of GBS in the design of regional vaccines and contribute to the knowledge of the virulence factors that determine the severity of the disease caused by GBS.

Our aim was to provide findings of the distribution of virulence factors that determine the way to damage GBS and that present geographic and ethnic variability; provide regional data and generate future development actions of maternal GBS vaccine or rapid diagnostic techniques. These findings may enable a speedy introduction of intrapartum antibiotic prophylaxis, a current preventive measure by the detection of breast carrying, which resulted in a significant reduction in neonatal disease.

Our results represent the first study of carrying the *scpB* gene of GBS to Misiones, a province of Argentina which has 90% of its neighbouring borders with other countries in the region, Paraguay and Brazil, the latter currently involved in the production of vaccines.

5. CONCLUSION

Our results for 100% detection of *scpB* gene in 200 GBS strains studied confirm the usefulness of the protein C5a peptidase in the development of regional maternal immunization to prevent neonatal disease.

The high specificity of the *scpB* gene in GBS, and the lack of homologous sequences in other bacterial genera and species, makes it a molecular marker that can be used in the diagnosis of colonization or infection by this microorganism.

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the doctoral thesis by the first author of this paper.

COMPETING INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Fry RM. Fatal infections by haemolytic *Streptococcus* Group B. The Lancet. 1938;1:199-201.
   Available:http://dx.doi.org/10.1016/S0140-6736(00)93202-1.
2. Bekker V, Bijlsma MW, van de Beek D, Kuijpers TW, van der Ende A. Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study. Lancet Infect Dis. 2014;14:1083-9.
   DOI: 10.1016/S1473-3099(14)70919-3.
3. Giménez M, Sanfeliu I, Sierra M, Dopico E, Juncosa T, Andreu A, et al. Group B streptococcal early-onset neonatal sepsis in the area of Barcelona (2004-2010). Analysis of missed opportunities for prevention. Enferm Infecc Microbiol Clin. 2014;pii:S0213-005X(14)00360-7.
   DOI: 10.1016/j.eimc.2014;10.015.
4. Manning, SD. Emergence of a hypervirulent neonatal pathogen. Lancet Infect Dis. 2014;14(11):1028-30.
   DOI: 10.1016/S1473-3099(14)70938-7.
5. Schuchat, A. Epidemiology of group B streptococcal disease in the United States: shifting paradigms. Clin Microbiol Rev. 1998;11(3):497-513.
6. Nobbs AH, Lamont RJ, Jenkinson HF. *Streptococcus* adherence and colonization. Microbiol Mol Biol Rev. 2009;73(3):407-450.
   DOI: 10.1128/MMBR.00014-09.
7. Poyart C, Réglier-Poupet H, Tazi A, Billoët A, Dmytruk N, Bidet P, et al. Invasive group B streptococcal infections in infants, France. Emerg Infect Dis. 2008;14(10): 1647-1649.
   DOI: 10.3201/eid1410.080185.
8. Kumar V, Desai A, Alva A, Board TN. Group B streptococci as an unusual cause of septic arthritis of the hip: association with childbirth. BMJ Case. 2013;pii: bcr2012008059.
   DOI: 10.1136/bcr-2012-008059.
9. Skoff TH, Farley MM, Petit S, Craig AS, Schaffner W, Gershman K, et al. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990-2007. Clin Infect Dis. 2009;49(1):85-92.
   DOI: 10.1086/599369.
10. Vittorino R, Hui-Yuen J, Ratner AJ, Starr A, McCann T. Case report: Group B *Streptococcus* meningitis in an adolescent. F1000Res. 2014;3:167.
    DOI: 10.12688/f1000research.4651.1.
11. Oyanguren B, Esteban L, Guillán M, de Felipe A, Alonso Cánovas A, Navas E, et al. Central nervous system involvement in adult patients with invasive infection caused by *Streptococcus agalactiae*. Neurologia;2014. pii:S0213-853(13)00288-0.
    DOI: 10.1016/j.nrl.2013;12.002.
12. Sendi P, Johansson L, Norrby-Teglund A. Invasive group B Streptococcal disease in non-pregnant adults: A review with emphasis on skin and soft-tissue infections. Infection.2008;36(2):100-11.
    DOI: 10.1007/s15010-007-7251-0.
13. Eskandarian N, Neela V, Ismail Z, Puzi SM, Hamat RA, Desa MN, Nordin SA. Group B streptococcal bacteremia in a major teaching hospital in Malaysia: A case series of eighteen patients. Int J Infect Dis. 2013;17(9):e777-80.
    DOI: 10.1016/j.ijid.2013.01.011.
14. Liébana-Martos Mdel C, Cabrera-Alavargonzalez J, Rodríguez-Granger J, Miranda-Casas C, Sampedro-Martínez A, Gutiérrez-Fernández J, et al. Serotypes and antibiotic resistance patterns in beta-hemolytic *Streptococcus agalactiae* isolates in colonized mothers and newborns with invasive disease. Enferm Infecc Microbiol Clin. 2014;33(2):84-8.
    DOI: 10.1016/j.eimc.2014.02.023.
15. Wang P, Tong JJ, Ma XH, Song FL, Fan L, Guo CM, et al. Serotypes, antibiotic susceptibilities, and multi-locus sequence type profiles of *Streptococcus agalactiae* isolates circulating in Beijing, China. PLoS One. 2015;10(3):e0120035.
    DOI: 10.1371/journal.pone.0120035.
16. Laczeski M, Pegels E, Oviedo P, Quiroga M, Vergara M. Molecular profiles and antimicrobial susceptibility of first isolates of *Streptococcus agalactiae* serotype IX in Argentina. Adv in Microbiol. 2014;4(1):474-483.
    Available:http://dx.doi.org/10.4236/aim.2014.48052

17. Lindahl G, Stålhammar-Carlemalm M, Areschoug T. Surface proteins of *Streptococcus agalactiae* and related proteins in other bacterial pathogens. Clin Microbiol Rev. 2005;18(1):102-27.
18. Herbert MA, Beveridge CJ, Saunders NJ. Bacterial virulence factors in neonatal sepsis: group B *Streptococcus*. Curr Opin Infect Dis. 2004;17(3):225-9.
19. Heath PT, Feldman RG. Vaccination against group B *Streptococcus*. Expert Rev Vaccines. 2005;4(2):207-18.
20. Chang YC, Olson J, Beasley FC, Tung C, Zhang J, Crocker PR. Group B *Streptococcus* engages an inhibitory Siglec through sialic acid mimicry to blunt innate immune and inflammatory responses *in vivo*. PLoS Pathog. 2014;10(1):e1003846.
DOI: 10.1371/journal.ppat.1003846.
21. Chen VL, Avci FY, Kasper DL. A maternal vaccine against group B *Streptococcus*: past, present, and future. Vaccine. 2013;31:D13-D19.
DOI: 10.1016/j.vaccine.2012.12.080.
22. Santillan DA, Rai KK, Santillan MK, Krishnamachari Y, Salem AK, Hunter SK. Efficacy of polymeric encapsulated C5a peptidase-based group B *Streptococcus* vaccines in a murine model. Am J Obstet Gynecol. 2011;205(3):249.e1-8.
DOI: 10.1016/j.ajog.2011.06.024.
23. Brown CK, Gu ZY, Matsuka YV, Purushothaman SS, Winter LA, Cleary PP, et al. Structure of the streptococcal cell wall C5a peptidase. Proc Natl Acad Sci U S A. 2005;102(51):18391-6.
24. Bohnsack JF, Takahashi S, Hammitt L, Miller DV, Aly AA, Adderson EE. Genetic polymorphisms of group B *Streptococcus* *scpB* alter functional activity of a cell-associated peptidase that inactivates C5a. Infect Immun. 2000;68(9):5018-25.
25. Hull JR, Tamura GS, Castner DG. Interactions of the streptococcal C5a peptidase with human fibronectin. Acta Biomater. 2008;4(3):504-13.
DOI: 10.1016/j.actbio.2008.01.009.
26. Gleich-Theurer U, Aymanns S, Haas G, Mauerer S, Vogt J, Spellerberg B. Human serum induces streptococcal c5a peptidase expression. Infect Immun. 2009;77(9):3817-25.
DOI: 10.1128/IAI.00826-08.
27. Dmitriev A, Tkáciková L, Suvorov A, Kantíková M, Mikula I, Totolyan A. Comparative genetic study of group B streptococcal strains of human and bovine origin. Folia Microbiol (Praha). 1999;44(4): 449-53.
28. Franken C, Haase G, Brandt C, Weber-Heynemann J, Martin S, Lämmler C. Horizontal gene transfer and host specificity of beta-haemolytic streptococci: the role of a putative composite transposon containing *scpB* and *lmb*. Mol Microbiol. 2001;41(4):925-35.
29. Dmitriev A, Suvorov A, Shen AD, Yang YH. Clinical diagnosis of group B streptococci by *scpB* gene based PCR. Indian J Med Res. 2004;119(Suppl):233-6.
30. Tkacikova E, Mikula I, Dmitriev A. Molecular epidemiology of group B streptococcal infections. Folia Microbiol (Praha). 2004;49(4):387-97.
31. Manning SD, Ki M, Marrs CF, Kugeler KJ, Borchardt SM, Baker CJ, et al. The frequency of genes encoding three putative group B streptococcal virulence factors among invasive and colonizing isolates. BMC Infect Dis. 2006;6:116. DOI:10.1186/1471-2334-6-116.
32. Sambrook J, Rusell DW. Molecular cloning: A laboratory manual. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York, Third Edition. 2001;1-3.
33. Slotved HC, Kong F, Lambertsen L, Sauer S, Gilbert GL. Serotype IX, a proposed new *Streptococcus agalactiae* serotype. J Clin Microbiol. 2007;45(9):2929-2936.
34. Beckmann C, Waggoner JD, Harris TO, Tamura GS, Rubens CE. Identification of novel adhesins from Group B streptococci by use of phage display reveals that C5a peptidase mediates fibronectin binding. Infect Immun. 2002;70(6):2869-76.
35. Dutra VG, Alves VM, Olendzki AN, Dias CA, de Bastos AF, Santos GO, et al. *Streptococcus agalactiae* in Brazil: serotype distribution, virulence determinants and antimicrobial susceptibility. BMC Infect Dis. 2014;14(1):323-330.
DOI: 10.1186/1471-2334-14-323.
36. Udo EE, Boswihi SS, Al-Sweih N. Genotypes and virulence genes in group B streptococcus isolated in the maternity hospital, Kuwait. Med Princ Pract. 2013;22(5):453-7.
DOI: 10.1159/000349932.
37. Hannoun A, Shehab M, Khairallah MT, Sabra A, Abi-Rached R, Bazi T, et al. Correlation between group B Streptococcal

genotypes, their antimicrobial resistance profiles, and virulence genes among pregnant women in Lebanon. 2009; 2009:796512.
DOI: 10.1155/2009/796512.

38. Eskandarian N, Ismail Z, Neela V, van Belkum A, Desa MN, Amin Nordin S. Antimicrobial susceptibility profiles, serotype distribution and virulence determinants among invasive, non-invasive and colonizing *Streptococcus agalactiae* (group B streptococcus) from Malaysian patients. Eur J Clin Microbiol Infect Dis. 2015;34(3):579-84.
DOI: 10.1007/s10096-014-2265-x.
39. Beigverdi R, Jabalameli F, Mirsalehian A, Hantoushzadeh S, Boroumandi S, Taherikalani M. et al. Virulence factors, antimicrobial susceptibility and molecular characterization of *Streptococcus agalactiae* isolated from pregnant women. Acta Microbiol Immunol Hung. 2014;6(4): 25-34.
DOI: 10.1556/AMicr.61.2014.4.4.
40. Chmouryguina I, Suvorov A, Ferrieri P, Cleary PP. Conservation of the C5a peptidase genes in group A and B streptococci. Infect Immun. 1996;64(7): 2387-90.
41. Elbaradie SM, Mahmoud M, Farid M.Maternal and neonatal screening for Group B streptococci by *scpB* gene based PCR: a preliminary study. Indian J Med Microbiol. 2009;27(1):17-21.
42. Santillan DA, Andracki ME, Hunter SK. Protective immunization in mice against group B streptococci using encapsulated C5a peptidase. Am J Obstet Gynecol. 2008;198:114.e1-6.

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