ORIGINAL ARTICLE

Identification of Serotypes of non-typeable Group B Streptococci by Polymerase Chain Reaction

MADIHA MUMTAZ[1], SIDRA SALEEM[2], ABDUL SATTAR[3], *ABDUL WAHEED[4], SHAH JAHAN[5], MARIA GILL[6]*
[1]Senior Demonstrator Pathology, Sialkot Medical College Sialkot
[2,5]Associate professor of Pathology, University of Health Sciences Lahore,
[3]Associate Professor of Pathology, Khawaja Muhammad Safdar Medical college Sialkot,
[4]Lecturer, Government College University Faisalabad
[6]Assistant Professor of Physiology, CMH Institute of Medical Sciences Multan
Correspondence to: Dr. Madiha Mumtaz Email: mrzwan33@gmail.com Cell: 0321-3656214

ABSTRACT

Aim: To identify the most prevalent serotype of non-typeable Group B Streptococci among pregnant women at 35 weeks of gestation.
Study Design: Descriptive study
Place and duration of study: Department of Microbiology and Resource Laboratory of University of Health Sciences, Lahore from 1st January 2019 to 30th June 2019.
Methods: A total of 200 pregnant women at 35 weeks of gestation were selected.Bacterial identification Lancefield's grouping and conventional serotyping of group B Streptococci was done. Some non type able strains of group B Streptococci were obtained after conventional serotyping. Molecular identification of those nontypeable strains of group B Streptococci were done by polymerase chain reaction. Conventional polymerase chain reaction conditions were optimized in a trial and error approach.
Results: All the non typeable strains of group B Streptococci belonged to serotype III while no other group B Streptococci strains were found.
Conclusion: Serotype III is the most prevalent serotype in our population. In future studies on larger scale should be carried out throughout the Pakistan to find out the most prevalent and invasive serotype of group B Streptococci.
Key words: Group B Streptococci, Polymerase chain reaction, Serotype III

INTRODUCTION

Neonatal bacterial infections are one of major health problem all over the world. Throughout the world these infections are a prominent cause of illness and death in newborns.[1] Commonly the causative infectious agents inhabit the genital tract of mother. Baby is more prone to get these infections while passing through the birth canal.[2] Betahemolytic streptococci are divided into groups by Lancefield grouping.[3]Group B streptococci (GBS) are the most common cause of sepsis in neonates and obstetrical patients.[4]

Incidence and fatality rates of GBS infections is different among countries of same continent and within the same country. The mean incidence rate of GBS infection is 0.53 per 1000 live birth. According to WHO Africa remains the region with highest rates of GBS infection and Southeast Asia remains the lowest and the fatality is reported 10 % globally. In the past 25 years GBS has emerged as leading cause of illness and death among newborns in US and other developed countries.[5]

All group B streptococci own specific type of polysaccharide capsular antigen and is categorized into ten serotypes (Ia, Ib, II through IX) Some serotypes are non type-able (NT) that is they do not show any recognized cps type.[6] All the nine capsular types are associated with human diseases.[7]

The epidemiology of GBS serotypes not only differ in different geographical areas but also vary with time. From 1980 to 2011 the predominating serotype was III and remaining types account for more than 85% of the cases.Serotype III is an invasive serotype causing neonatal infection and it is also commonly present in asymptomatic mother therefore techniques other than conventional serotyping would be useful for the identification of this virulent strain emphasizing the need of antibiotic prophylaxis[8].

Because of the high discriminatory powers and reproducibility molecular serotyping methods are attractive theoretically. To detect and genotype GBS isolates PCR based methods have been used, however, there is need for further development to make them feasible for usage of serotype recognition. Therefore we planned this study to identify the non-type-able serotypes of GBS by PCR.

MATERIALS AND METHODS

This descriptive study conducted in the Microbiology Department and Resource Laboratory of University of Health Sciences, Lahore from 1stJanuary 2019 to 30thJune 2019. Two hundred pregnant women at 35 weeks of gestation were selected. Non type-able Group B streptococci were used which were obtained after conventional serotyping of the 19 clinical isolates of GBS.GroupB streptococci which could not be typed by conventional serotyping were selected, while type able were excluded. TIA Namp Bacteria DNA Kit was used for DNA extraction. Manufacturer instructions were followed to complete the process. **A**mplification of DNA was done by specific primers genes are given below in table1.

Received on 25-08-2019
Accepted on 03-02-2020

Table 1: Primers for different genotypes

Type Ia	
F	5GGCCTGCTGGGATTAATGAATATAGTTCCAGGTTT G3
R	5GTATAACTTCTATCAATGGATGAGTCTGTTGTAGTA CGG3
F	F5GATAATAGTGGAGAAATTTGTGATAATTTTCTCAA AAAGAC3
R	5CCTGATTCATTGCAGAAGTCTTTACGATGCGATAG G3
Type III	
F	5'GAATACTATTGGTCTGTATGTTGGTTTTATTAGCAT CGC3'
R	5'GTATAACTTCTATCAATGGATGAGTCTGTTGTAGTA CGC 3'
Type IV	
F	5CCCAAGTATAGTTATGAATATTAGTTGGATGGTTTT TGG 3'
R	5'GGGTCAATTGTATCGTCGCTGTCAACAAAACCAAT CAAATC 3'
Type V	
F	5'CCCAGTGTGGTAATGAATATTAGTTGGCTAGTTTT TGG3'
R	5'CCCCCCATAAGTATAAATAATATCCAATCTTGCATA GTCAG3
Type VI	
F	5'CCTTATTGGGCAAGGTATAAGAGTTCCCTCCAGTG TG3"
R	5'GAAGCAAAGATTCTACACAGTTCTCAATCACTAAC TCCG3

RESULTS

In our study two hundred pregnant women attending antenatal clinic of a tertiary care hospital of Lahore were enrolled over a period of six months. All the participants were screened for vaginal and rectal GBS colonization. Frequency of GBS colonization was 9.5%. These 19 clinical isolates of GBS were processed further.10% of the non-typeable serotypes were used for molecular study.Lancefield'sgroupingwas performed by using Streptococcal grouping Kit(Pro-Lab USA). Conventional serotyping (CS) was performed by using Streptococcal Serotyping Kit(Pro-Lab USA). Conventional serotyping of 19 clinical isolates of GBS was performed and results obtained were shown in the table. Percentages of different serotypes wereIII (36.8%) then serotypeV(21%) Ia(15%) II (10%) and VII (5%). Non typeable serotypes were 10%.

Fig. 1: Serotyping distribution of GBS isolates (n=19)

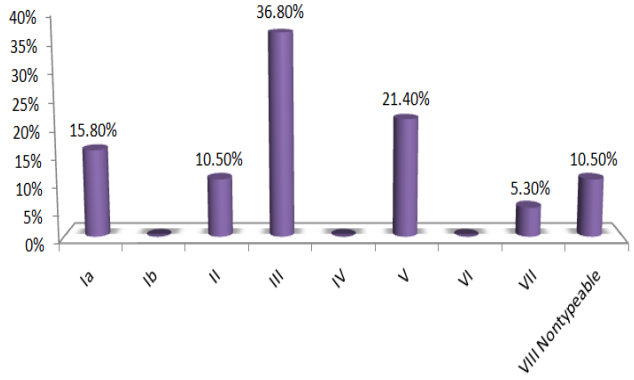
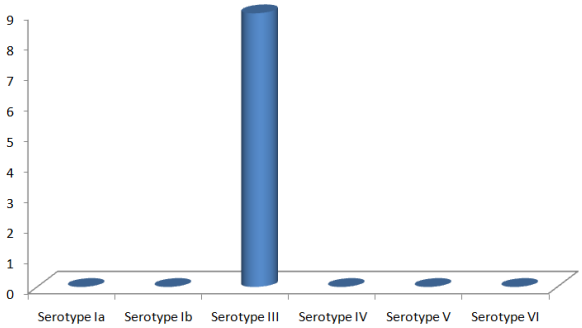


Fig 2: Showing the PCR results

Fig. 3: Molecular serotype distribution of non typeable samples



Molecular serotyping of non typeable serotypes was then performed. Non type able group B streptococci were further processed for DNA extraction by using standard protocol of kit manual. PCR was carried on these samples by using specific primers of Serotypes Ia, Ib, II, III, IV, V and VI. After PCR the product was run on 2% agarose gel as shown in figures. serotype III was present in all organisms. No other serotype was detected.

DISCUSSION

Group B Streptococci are capable of causing aggressive disease in neonates, pregnant ladies, and immune suppressed individuals. Fundamental feature of GBS in its ability to cause a disease is making of an antigenically changeable polysaccharide. This variable polysaccharide capsule is used for strain typing[9].

In our study serotype III was present in all organisms. No other serotype was detected. A study was conducted by Soareset al[10] in Brazil and they reported that serotype III was most common in clinical samples of Group B streptococci isolated from pregnant women. The findings of this study are similar to our study results.[10]Ippolito et al from Washington, USA reported similar results.[11] A study was conducted by Motlova et al in Czech Republic from 2001 to 2002 and they showed that serotype III was most communal serotype amongst all clinical isolates of GBS.[12] A study was led by Von Both et al[13] in Germany and they reported 27% isolates were carrying serotype III.Charlene

et al reported serotype V as predominant strain followed by serotype III in Western Cape region of South Africa. So in that region serotype V may be more common in ours serotype III.[14]

Reason for the non type ability is not clearly known. Three possible reasons for non typeable strains of GBS as described by Sarah et al[15] are(i) isolate is non-encapsulated variant (ii) isolates produces an uncharacterized polysaccharide for which antibodies are not yet available (iii) isolates has an insertion or mutation in genes essential for capsule expression.

CONCLUSION

Serotype III is the most prevalent serotype in our population.In future studies on larger scale should be carried out throughout the Pakistan to find out the most prevalent and invasive serotype of GBS.

REFERENCES

1. Le Roux DM, Zar HJ. Community-acquired pneumonia in children - a changing spectrum of disease. PediatrRadiol 2017; 47: 1392-8.
2. Jamal S, Srivastava R. A retrospective analytical study of the epidemiology and causes of preterm birth. Int J ReprodContraceptObstet Gynecol. 2017; 6(12): 5453-57
3. Raabe VN, Shane AL. Group B *Streptococcus* (*Streptococcus agalactiae*).MicrobiolSpectr 2019; 7(2): 1-21.
4. Kalin A, Acosta C, Kurinczuk JJ, Brocklehurst P, Knight M. Severe sepsis in women with group B Streptococcus in pregnancy: an exploratory UK national case-control study.*BMJ Open* 2015; 5(10):e007976.
5. Madrid L, Seale AC, Kohli-Lynch M, Edmond KM, Lawn JE, Heath PT et al. Infant group B Streptococcal disease incidence and serotypes worldwide: Systematic review and meta-analyses. *Clin Infect Dis* 2017; 65(suppl 2): S160–72.
6. Mengist A. Group B Streptococcus: Vaccine Development Art. EC Microbiology 2018; 14(3): 92-107.
7. Gao XY, Zhi XY, Li HW, Klenk HP, Li WJ. Comparative genomics of the bacterial genus *Streptococcus i*lluminates evolutionary implications of species groups. PLoS ONE 2014; 9(6): e101229.
8. Wang P, Ma Z, Tong J, Zhao R, Shi W, Yu S, et al. Serotype distribution, antimicrobial resistance, and molecular characterization of invasive group B Streptococcus isolates recovered from Chinese neonates. Int J Infect Dis 2015; 37: 115-8.
9. Song JY, Lim JH, Lim S, Yong Z, Seo HS. Progress toward a group B streptococcal vaccine, Human Vaccines Immunotherap 2018; 14:11, 2669-2681
10. Soares GCT, Alviano DS, Santos GDS, Alviano CS, Mattos-Guaraldi AL, Nagao PE. Prevalence of Group B Streptococcus serotypes III and V in pregnant women of Rio de Janeiro, Brazil. BrazJMicrobiol2013; 44: 869-872.
11. Ippolito DL, James WA, Tinnemore D, Huang RR, Dehart MJ, Williams J et al.. Group B streptococcus serotype prevalence in reproductive-age women at a tertiary care military medical center relative to global serotype distribution. BMC Infect Dis2010; **10:** 1.
12. Motlova J, Strakova L, Urbaskova P, Sak P, Sever T. Vaginal & rectal carriage of Streptococcus agalactiae in the Czech Republic: incidence, serotypes distribution & susceptibility to antibiotics. Indian JMedRes2004;119: 84
13. Von-Both U, John A, Fluegge K, Siedler A, Berner R. Molecular epidemiology of invasive neonatal Streptococcus agalactiae isolates in Germany. Pediatr Infect Dis J 2008; 27: 903-6.
14. Africa CWJ, Kaambo E. Group B streptococcus serotypes in pregnant women from the Western Cape Region of South Africa. Front. Public Health 2018; 6:356.
15. Teatero S, Ferrieri P, Martin I, Demczuk W, McGeer A. Serotype distribution, population structure, and antimicrobial resistance of group B S*treptococcus* strains recovered from colonized pregnant women NahuelFittipaldi for the Toronto invasive bacterial diseases network. J ClinMicrobiol 2017;55(2):412-22.