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Indian Journal of Microbiology Research

Journal homepage: <https://www.ijmronline.org/>

Original Research Article

Prevalence and the risk factors for the carriage of beta-haemolytic streptococci among women visiting a tertiary care hospital in South India

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ARTICLE INFO

Article history:

Received 29-09-2021

Accepted 06-10-2021

Available online 15-12-2021

Keywords:

Prevalence

Risk factors

Beta-haemolytic streptococci

GAS

GBS

ABSTRACT

Introduction: Streptococci are gram positive cocci arranged in chains and are part of normal flora of humans and animals. The present study is carried out to determine the prevalence and risk factors for the carriage of beta-haemolytic streptococci (BHS) among women visiting Dr. VRK Women's Teaching Hospital & Research Centre, Hyderabad.

Materials and Methods: Vaginal swabs were collected from 250 patients attending outpatient department (OPD) of Dr. VRK Women's Teaching hospital. Swabs were inoculated onto 5% sheep blood agar plates and incubated for 24 h at 37°C in a candle jar. BHS isolates were phenotypically identified by standard microbiological techniques, all the isolates presumptively identified as BHS were tested for Bacitracin susceptibility. Sensitive isolates were presumptively identified as GAS and resistant isolates were identified as non-group A BHS (NGABHS). Presumptively identified GAS & NGABHS isolates were serogrouped by Lancefield grouping using a commercially available latex agglutination test.

Results: BHS were isolated from 12.4% of samples. As many as 12 BHS isolates were identified as GAS and 19 were identified as NGABHS. Ten of nineteen were identified as group B *Streptococci* (GBS), 4 (12.9%) were identified as group C *Streptococci* (GCS) and 5 (16.12%) were identified as group G *Streptococci* (GGS). Among six clinical groups, the prevalence of GAS is highest i.e. 7.5% in female patients visiting Gynaecology OPD with history of white discharge. Prevalence of NGABHS was more among post insertion (18%) IUCD group compared to pre insertion (8%) IUCD group. GBS were isolated from 7% of samples from IUCD group and 4% of samples from prostitutes.

Conclusion: This study reports the prevalence of BHS among women visiting a tertiary care hospital in Hyderabad. This study also identified certain risk factors such as IUCD usage and working as a FSW are associated with the increased prevalence of NGABHS especially GBS.

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1. Introduction

Streptococci are gram positive cocci arranged in chains and are part of normal flora of humans and animals. *Streptococcus pyogenes* is a beta-hemolytic bacterium belonging to Lancefield serogroup A, also known as the group A streptococci (GAS). GAS are known to be a cause of vulvo-vaginitis in women. It is a widely carried

pathogen in the female genital tract and causes rare but serious infectious conditions in women: Toxic Shock-Like Syndrome (TSLs) which is associated with early onset shock and multi-organ system failure, necrotizing fasciitis (NF) which involves local necrosis of subcutaneous soft tissues and skin, and bacteremia which can cause focal infections including meningitis, pneumonia, peritonitis, osteomyelitis, septic arthritis, myositis, postoperative wound infections and puerperal sepsis, the latter being a major cause of maternal mortality. Incidence of these

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invasive infections is very low, around 1–8 per 100,000 population per year, but has a potential case fatality rate ranging from 5% to 20%, and in the case of septic shock up to 30–45%.^{1–4} The risk of invasive GAS infection is very high for postpartum patients, with a case-fatality rate of 3.5%.⁵ There is an increased risk of GAS colonized women developing invasive infections when compared to males (female/male ratio 4:1).⁶ The risk of developing invasive GAS infections will increase in pregnancy being 20-fold higher than in non-pregnant women.⁷

Group B Streptococcus (GBS, *Streptococcus agalactiae*) is frequently found in the normal microbiota of the gastrointestinal and vaginal tracts of women.^{8,9} GBS are a well-recognized cause of maternal sepsis in the puerperium and can cause life-threatening infections in neonates, with maternal colonization being the principal route of transmission. Early-onset GBS disease (EOD) results from ascending infection of the womb or by neonatal acquisition during vaginal passage and manifests on days 0–6 of life with pneumonia or respiratory distress commonly advancing to sepsis. Late-onset disease (LOD) is classified with onset from days 7 to 90, arising from maternal, nosocomial, or community sources, and presents with bacteremia with a high complication rate of meningitis.^{10,11} In non-pregnant adults, the incidence of infection is continuously increasing.^{12,13} Elderly and immunocompromised persons with underlying conditions, such as diabetes mellitus and cancer, are at increased risk of invasive GBS disease.^{14,15} Skin and soft-tissue infection, primary bacteraemia and urinary tract infection are the most frequent clinical manifestations of invasive GBS disease in the elderly.^{16,17}

Group C *Streptococcus* (GCS) and group G *Streptococcus* (GGS) of human origin are now considered to constitute a single subspecies, *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE).¹⁸ SDSE have been identified as part of the normal flora of the human respiratory, gastrointestinal, and female genital tracts. Thus, for many years colonization by these bacteria has been considered harmless. However, the pathogenicity of SDSE has now been better recognized. Recent epidemiological studies have shown that SDSE causes a variety of diseases similar to those caused by *S. pyogenes* such as skin and soft tissue infections range from superficial wound infections to severe invasive infections, life-threatening Streptococcal toxic shock syndrome (STSS) and bacteremia.^{19–21}

The present study is carried out to determine the prevalence and risk factors for the carriage of beta-haemolytic streptococci among women visiting Dr. VRK Women's Teaching Hospital & Research Centre, Hyderabad.

2. Materials and Methods

This cross sectional study was carried out in the Dr VRK Women's Teaching hospital & Research Centre, a tertiary care hospital, at Aziznagar from March 2014 to June 2015. High vaginal swabs were collected from 250 patients attending outpatient department (OPD) of Dr. VRK Women's Teaching hospital. Patients were divided into 6 clinical groups.

1. Patients attending family planning OPD for copper T insertion and post insertion follow-up.
2. Patients visiting Gynaecology OPD with history of white discharge and irregular menstrual periods
3. Pregnant women attending antenatal OPD
4. Patients from postnatal septic wards
5. Women in labour
6. Prostitutes

2.1. Sample collection and processing

Vaginal swabs were collected from 250 female patients belonging to various clinical groups. The demographic profile was taken from each case. Vaginal swabs were put into Amie's transport medium and transported at 4°C in a temperature monitored cooler to the Clinical Microbiology Laboratory, where they were processed within 2 hours. Swabs were inoculated onto 5% sheep blood agar plates and incubated for 24 h at 37°C in a candle jar, which can provide an atmosphere of 5% CO₂. Culture plates negative for β-haemolytic colonies were incubated for additional 24 hours to allow the growth of slow growers. Beta-haemolytic streptococci (BHS) isolates were phenotypically identified by standard microbiological techniques: which include β-haemolytic activity on sheep's blood agar, Gram stain revealing Gram positive cocci, and negative catalase test.

All the isolates presumptively identified as BHS were tested for Bacitracin susceptibility. BHS strains inoculated on sheep blood agar plates. Test was performed using 0.04 units Bacitracin discs (Himedia Laboratories, Mumbai, India) as per standard protocol. Bacitracin disc was placed over the medium with the help of sterile forceps and the plates were incubated at 37°C overnight. Isolates with a zone of inhibition ≥ 15 mm diameter were considered as sensitive. Isolates with no zone of inhibition or < 15 mm diameter were considered as resistant. Sensitive isolates were presumptively identified as GAS and resistant isolates were identified as non-group A BHS (NGABHS). Presumptively identified GAS & NGABHS isolates were serogrouped by Lancefield grouping using a commercially available Streptex™ Rapid kit (Thermo Fisher Scientific, India).

3. Results

In this study, a total of 250 female patients belonging to six different clinical groups attending outpatient department of Dr VRK Women's Teaching hospital were screened for BHS. BHS were isolated from 31 (12.4%) of 250 patients (Table 1). Occurrence of BHS was more in patients with intrauterine contraceptive device (IUCD) i.e. 18% followed by antenatal cases (12%), prostitutes (10%), postnatal sepsis (8%) and patients visiting Gynaecology OPD (7.5%) (Table 1). Majority of the BHS, 18 (58%) of 31 were isolated from 15-25 years age group followed by 26-35 years age group (35.48%). Similar trend was observed for NGABHS (Table 2).

As many as 12 (38.7%) of 31 BHS isolates were susceptible to Bacitracin and identified as GAS by serogrouping. Nineteen (61.3%) of 31 isolates were resistant to Bacitracin and presumptively identified as NGABHS. By Lancefield grouping, 10 (32.2%) were identified as group B *Streptococci* (GBS), 4 (12.9%) were identified as group C *Streptococci* (GCS) and 5 (16.12%) were identified as group G *Streptococci* (GGS) (Table 3). As many as 7 (38.9%) of 18 BHS isolated from IUCD group were identified as GBS (Table 3). In postnatal sepsis 1 (50%) of 2 BHS was identified as GBS (Table 3). Two (40%) of five BHS isolated from prostitutes were identified as GBS (Table 3). GCS were isolated from IUCD users and antenatal cases (Table 3). GGS were isolated from IUCD users and prostitutes (Table 3).

We have also studied the prevalence of GAS, GBS, GCS and GGS in 100 women using IUCD. IUCD users were divided into two groups viz., pre insertion & post insertion. Results of our study showed that prevalence of NGABHS was more among post insertion (18%) IUCD group compared to pre insertion (8%) IUCD group (Table 4). However, there is not much difference in the prevalence of GAS among post insertion and pre insertion IUCD groups (Table 4).

4. Discussion

GAS are known to be a cause of vulvo-vaginitis in women. It also causes rare but serious infectious conditions in women viz., TSLs, NF and bacteremia. GBS is frequently found in the normal microbiota of the gastrointestinal and vaginal tracts of women and is a well-recognized cause of maternal sepsis in the puerperium and can cause life-threatening infections in neonates. Other NGABHS like GCS and GGS were responsible for a variety of diseases such as skin & soft tissue infections, life-threatening STSS and bacteremia. Hence it is important for clinician to identify the serogroup of BHS and to record the changes in their epidemiological patterns.

The presumptive identification of GAS is usually done by testing for susceptibility to bacitracin even though there are

reports about the occurrence of bacitracin resistant strains.²² Many laboratories use this as the sole test for diagnosing GAS infections.²³ In this study, all the 12 BHS isolates susceptible to bacitracin were identified as GAS by latex agglutination kit and all the 19 NGABHS isolates were resistant to bacitracin. Hence, bacitracin susceptibility test, along with the Lancefield antigen A test, can be used for greater specificity in the identification of *S. pyogenes*.

Previous studies reported a very low prevalence of GAS.^{24,25} The prevalence of GAS carriage in the genital tract is lower than GBS carriage. In this study, *S. pyogenes* were isolated from 4.8% of the samples. The higher prevalence of GAS in this study could be due to the inclusion of different clinical groups (6) for sample collection. Among six clinical groups, the prevalence of GAS is highest i.e. 7.5% in female patients visiting Gynaecology OPD with history of white discharge. Previous studies also showed that GAS carriage in genital tract is likely to cause symptoms, the commoner being: vaginal soreness and irritation, and discharge associated with inflammation of the vaginal mucosa.²⁶

Severe peripartum GAS infections, a scourge of the pre-antibiotic era, virtually disappeared during the mid-20th century, but have been reported sporadically since the early 1990s. Though still infrequent, such infections must be recognized and treated aggressively to prevent severe morbidity or mortality. In this study, *S. pyogenes* was isolated from 1 (2.86%) of 35 postnatal sepsis cases. Most of the studies also reported 1-2 unrelated cases of postnatal sepsis caused by GAS.²⁷⁻²⁹

The prevalence of NGABHS among BHS isolated from female patients is 61.29% which is in agreement with the previous work.³⁰ In the present study, prevalence of NGABHS was more among post insertion (18%) IUCD group compared to pre insertion (8%) IUCD group (Table 4). This result is in agreement with the previous report,³¹ who found that incidence of NGABHS was more in post insertion group. This could be due to change in the vaginal flora by the presence of foreign body like copper T.

S. agalactiae (GBS) were isolated from 4% of samples. This result is in agreement with the previous studies, where the prevalence of GBS colonization of the lower genital tract of 'normal' women has been reported as 4-18%.^{32,33} In this study, GBS were isolated from 7% of samples from IUCD group (Table 3). Previous studies also reported slightly higher prevalence of GBS in IUCD users compared to other women.^{31,34} GBS were isolated from 4% of samples from prostitutes. A research study by Cools et al. 2016³⁵ also suggested that working as a female sex worker (FSW) is a risk factor for vaginal GBS colonization.

5. Conclusion

This study reports the prevalence of BHS among women visiting Dr. VRK Women's Teaching Hospital & Research

Table 1: Occurrence of BHS in female patients belonging to six different clinical groups

Clinical group	No of cases screened	No of cases positive for BHS	Positive percentage (%)
IUCD users	100	18	18
Gynaecology OPD	40	03	7.5
Postnatal sepsis	35	02	08
Antenatal cases	25	03	12
Women in labour	10	NIL	-
Prostitutes	50	05	10
Total	250	31	12.4

Table 2: Age wise distribution of BHS & NGABHS

Age group (Years)	No of cases investigated	No of cases positive for BHS	Positive percentage (%)	No of cases positive for NGABHS	Positive percentage (%)
15-25	108	18	16.7	13	12
26-35	96	11	11.46	06	6.2
36-45	36	02	5.5	NIL	-

Table 3: Lancefield grouping of BHS isolated from female patients belonging to various clinical groups

Clinical group	No of cases screened	No of cases positive for BHS	Lancefield group			
			A	B	C	G
IUCD users	100	18	05	07	02	04
Gynaecology OPD	40	03	03	-	-	-
Postnatal sepsis	35	02	01	01	-	-
Antenatal cases	25	03	01	-	02	-
Women in labour	10	NIL	-	-	-	-
Prostitutes	50	05	02	02	-	01
Total	250	31	12	10	04	05

Table 4: Occurrence of GAS and NGABHS in women using IUCD

Group	No of cases	No of cases positive for GAS	Positive percentage (%)	No of cases positive for NGABHS	Positive percentage (%)
Pre insertion	50	03	06	04	08
Post insertion	50	02	04	09	18

Centre, a tertiary care hospital in Hyderabad. This study also identified certain risk factors such as IUCD usage and working as a FSW are associated with the increased prevalence of NGABHS especially GBS.

6. Source of Funding

None.

7. Conflict of Interest

All the authors declare that there is no conflict of interest.


References

1. Tyrrell GJ, Lovgren M, Kress B, Grimsrud K. Invasive group A streptococcal disease in Alberta. *J Clin Microbiol.* 2000;43:1678–83.
2. Rimawi BH, Soper DE, Eschenbach DA. Group A streptococcal infections in obstetrics and gynecology. *Clin Obstet Gynecol.* 2012;55:864–74.
3. Centers for Disease Control and Prevention. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, Group A Streptococcus; 2018. Available from: <https://www.cdc.gov/abcs/reports-findings/survreports/gas18.html>.
4. Nelson GE, Pondo T, Toews KA, Farley MM, Lindegren ML, Lynfield R, et al. Epidemiology of Invasive Group A Streptococcal Infections in the United States. *Clin Infect Dis.* 2005;63:478–86.
5. Chuang I, Beneden CV, Beall B, Schuchat A. Population-based surveillance for postpartum invasive group a streptococcus infections. *Clin Infect Dis.* 1995;35(6):665–70.
6. Anderson BL. Puerperal group A streptococcal infection: Beyond Semmelweis. *Obstet Gynecol.* 2014;123(4):874–82.
7. Deutscher M, Lewis M, Zell ER, Taylor TH, Beneden CV, Schrag S. Incidence and severity of invasive Streptococcus pneumoniae, group A Streptococcus, and group B Streptococcus infections among pregnant and postpartum women. *Clin Infect Dis.* 2011;53:114–23.
8. Verani JR, Mcgee L, Schrag SJ. Division of Bacterial Diseases NCFI, Respiratory Diseases CfDC, Prevention. Prevention of perinatal group B streptococcal disease—revised guidelines from CDC. *MMWR Recomm Rep.* 2010;59(RR-10):1–36.
9. Meyn LA, Krohn MA, Hillier SL. Rectal colonization by group B Streptococcus as a predictor of vaginal colonization. *Am J Obstet*

- Gynecol.* 2009;201(1):76.
10. Maissey HC, Doran KS, Nizet V. Recent advances in understanding the molecular basis of group B Streptococcus virulence. *Expert Rev Mol Med.* 2008;10:e27. doi:10.1017/S1462399408000811.
 11. Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AK, Couzens S. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet.* 2012;379(9815):547–56.
 12. Dermer P, Lee C, Eggert J, Few B. A history of neonatal group B streptococcus with its related morbidity and mortality rates in the United States. *J Pediatr Nurs.* 2004;19(5):357–63.
 13. Farley MM. Group B streptococcal disease in nonpregnant adults. *Clin Infect Dis.* 2001;33(4):556–61.
 14. Skoff TH, Farley MM, Petit S, Craig AS, Schaffner W, Gershman K. Increasing burden of invasive group B streptococcal disease in nonpregnant adults. *Clin Infect Dis.* 1990;49(1):85–92.
 15. Kothari NJ, Morin CA, Glennen A, Jackson D, Harper J, Schrag SJ, et al. Invasive group B streptococcal disease in the elderly. *Emerg Infect Dis.* 2003;15(8):1279–81.
 16. Edwards MS, Baker CJ. Group B streptococcal infections in elderly adults. *Clin Infect Dis.* 2005;41(6):839–47.
 17. Shabayek S, Spellerberg B. Group B streptococcal colonization, molecular characteristics, and epidemiology. *Front Microbiol.* 2018;9:437. doi:10.3389/fmicb.2018.00437.
 18. Vandamme P, Pot B, Falsen E, Kersters K, Devriese LA. Taxonomic study of lancefield streptococcal groups C, G, and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. *Int J Syst Bact.* 1996;46(3):774–81.
 19. Brandt CM, Spellerberg B. Human infections due to *Streptococcus dysgalactiae* Subsp. *equisimilis*. *Clin Infect Dis.* 2009;49(5):766–72.
 20. Barnham M, Kerby J, Chandler RS, Millar MR. Group C streptococci in human infection: A study of 308 isolates with clinical correlations. *Epidemiol Infect.* 1989;102(3):379–90.
 21. Gustafson LW, Blaaker J, Helmig RB. Group A streptococci infection. A systematic clinical review exemplified by cases from an obstetric department. *Eur J Obstet Gynecol Reprod Biol.* 2017;215:33–40.
 22. Olender A, Łetowska I, Karyński M, Kiernicka-Ciekot K, Pels K. Problems with identification of beta-haemolytic streptococcus resistant to bacitracin isolated from patients with pharyngitis. *Med Dosw Mikrobiol.* 2012;64:1–10.
 23. Menon T, Lloyd C, Jacob S. Should bacitracin sensitivity be used in the presumptive identification of group A streptococci? *Indian J Med Microbiol.* 2005;23:275.
 24. DeLouvois J, Hurley R, Stanley VC. Microbial flora of the lower genital tract during pregnancy: relationship to morbidity. *J Clin Pathol.* 1975;28(9):731–5.
 25. Beargie R, Lynd P, Tucker E, Duhring J. Perinatal infection and vaginal flora. *Am J Obstet Gynecol.* 1975;122:31–3.
 26. Lewis RFM. Beta-haemolytic streptococci from the female genital tract: clinical correlates and outcome of treatment. *Epidemiol Infect.* 1989;102:391–400.
 27. Nathan L, Peters MT, Ahmed AM, Leveno KJ. The return of life-threatening puerperal sepsis caused by group A streptococci. *Am J Obstet Gynecol.* 1993;169(3):571–2.
 28. Gergis H, Barik S, Lim K, Porter W. Life-threatening puerperal infection with group A Streptococcus. *J R Soc Med.* 1999;92(8):412–3.
 29. Abouzeid H, Wu P, Mohammed N, Al-Samarrai M. Group A streptococcal puerperal sepsis: the return of a potentially fatal disease. *J Obstet Gynaecol.* 2005;25(8):806–8.
 30. Koshi G. Serologic groups of hemolytic streptococci in human infections. *Indian J Med Res.* 1971;59(3):394–400.
 31. Farrag OA, Gawad AA, Antar S. Group B beta haemolytic streptococcal colonisation in women using intrauterine contraceptive devices. *Contraception.* 1985;31:595–602.
 32. Jensen NE, Anderson BL. The prevalence of Group B streptococci in human urogenital secretions. *Scand J Infect Dis.* 1979;11:199–202.
 33. Finch RG, French GL, Phillips I. Group B streptococci in the female genital tract. *BMJ.* 1976;1:1245–7.
 34. Goldacre MJ, Watt B, Loudon N, Milne L, Loudon J, Vessey MP. Vaginal microbial flora in normal young women. *BMJ.* 1979;1:1450–1453.
 35. Cools P, Jaspers V, Hardy L, Crucitti T, Delany-Moretlwe S, Mwaura M, et al. A Multi-Country Cross-Sectional Study of Vaginal Carriage of Group B Streptococci (GBS) and *Escherichia coli* in Resource-Poor Settings: Prevalences and Risk Factors. *PLoS one.* 2016;11(1):148052.

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Cite this article: Sadanand LNR, Raghunath P. Prevalence and the risk factors for the carriage of beta-haemolytic streptococci among women visiting a tertiary care hospital in South India. *Indian J Microbiol Res* 2021;8(4):308-312.