ORIGINAL PAPER

The prevalence of Beta Hemolytic Streptococcus in a Children's Tertiary Care Hospital in Timisoara

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Conflicts of interests

Nothing to declare

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Abstract

Background. Beta hemolytic streptococcus (BHS) represents a worldwide health problem because of the complications that can occur. Thus, it is important to identify the presence and type of BHS in time to start treatment if needed, avoiding the complications.

Aim. Our aim was to identify the prevalence of Beta Hemolytic Streptococcus in a Children's Tertiary Care Hospital in Timisoara.

Methods. A cross-sectional study was conducted from April-June 2018 in Emergency

Hospital for Children Timisoara. The study lot consists from 1100 children, ages between 0-18 years, which were not given, in the preceding two weeks, any antibiotics. We compared the study with two studies from different cities in Nepal, with the same subject as ours. Group A and Group C Streptococci were identified by beta hemolytic colonies, bacitracin sensitivity, catalase negativity test and latex agglutination test (Oxoid Streptococcal Grouping kit). ASO (Antistreptolysin O) test was also performed from serum samples, for patients with positive cultures. We also tested the antibiotic sensitivity to: Cefepime, Clindamycin, Erythromycin, Tetracycline.

Results. The prevalence of BHS (Beta Hemolytic Streptococcus), especially GABHS (Group A

Beta Hemolytic Streptococcus) was 4%, in children from a tertiary care hospital in Timisoara, Romania; which is comparable to the findings of similar studies. In the first study, the prevalence of GABHS was 7.2%, and in the second study was 9%. The patients with positive cultures were also tested for ASO, 28 patients (52%) had high levels, while 26 (48%) had normal levels. On all positive patients an antibiogram was made and we observed that most of the isolates were sensitive to the antibiotics used. A few isolates in Group A were resistant.

Conclusions. Considering the limited data found on our subject, further epidemiological studies on streptococcal disease complex are needed.

Introduction

Beta hemolytic streptococcus represents a worldwide health problem. Thus, it is important to identify the presence and type of BHS (Beta Hemolytic Streptococcus) in time to start treatment if needed, avoiding the complications as aforementioned. The objectives of the treatment is to eradicate the organism, improve the clinical symptoms and signs and prevent the transmission of the disease.

Group A and group C streptococci, known as colonizers of human skin and mucous can produce several infections like scarlet fever, pharyngitis, acute otitis media, erysipelas, cellulitis, impetigo, necrotizing fasciitis, pneumonia, septic arthritis, bacteremia and toxic shock syndrome, and others. Group A streptococcal infections have been associated with serious morbidity and mortality, however in recent times a decline in the incidence and severity of this infections has been recorded.

Group A beta hemolytic streptococcus (GABHS, *Streptococcus pyogenes*) causes a wide variety of infections in infants, children, and adults. Group A streptococcal infections have been associated with serious morbidity and mortality, until the middle of the 20th century, when an important decline in the incidence and severity of this infections happened [1].

Bacteria are responsible for approximately 5-10% of pharyngitis cases. Group A beta hemolytic streptococci represents the most common bacterial etiology [2].

Streptococci belong to a heterogeneous group of positives, spherical or ovoid, pairs or chains, immobile, optionally anaerobic, lacking cytochromes and catalases, with complex nutritional needs [3].

Group A beta hemolytic streptococci (GABHS) consist of *Streptococcus pyogenes* which produce infections with various localizations and clinical manifestations. The pathogenesis of GAS (Group A Streptococcus) is mediated by a variety of factors, such as Streptolysin „O”, which damag-

es cell membranes and accounts for the hemolysis produced on agar blood plates [4].

About 5% of all cases of streptococcus infection in human are caused by beta hemolytic group C streptococci [2].

Materials and methods

A cross-sectional study was conducted from April-June 2018 in „Louis Turcanu” Emergency Hospital for Children Timisoara. The patients lot consists from 1100 children.

The including criteria were: patients from the ambulatory of „Louis Turcanu” Emergency Hospital for Children, age between 0-18 years and patients who were not administrated, in the preceding two weeks, any antibiotics.

Throat swabs were collected and processed for BHS (Beta Hemolytic Streptococci) following standard microbiological procedures; the samples were inoculated onto sheep blood agar and incubated for 24 hours. Group A and Group C Streptococci were identified by beta hemolytic colonies, bacitracin sensitivity, catalase negativity test and latex agglutination test (Oxoid Streptococcal Grouping kit). ASO (Antistreptolysin O) test was also performed from serum samples, for patients with positive cultures. The ASO titer which peak 2-4 weeks after acute infections is commonly elevated after pharyngeal infections. If high levels persist after 6 weeks or even increase is recorded, a relapse will be suspected [5, 6].

Sample processing

The samples were collected from patients, previously instructed not to brush their teeth, eat or drink anything, with a sterile cotton swab applicator by rubbing the tonsillar and pharyngeal mucosa avoiding the surrounding tissues. The swabbing was done by circular moves, whipping the tonsils, the pharynx and any surrounding swollen, ulcerated area or tonsillar exudates.

The samples were cultured on blood agar plates (Columbia agar with 5% ram blood- Bio Rad, Marnes-la-Coquette, France or bioMerieux - Marcy l'Etoile, France) and incubated at 37°C for 24 hours using CO₂ 5% incubator. Based on cultural characters, we looked for the presence of small sized (0.6-1µm), typically dry or matt colonies surrounded by a clear wide zone (2-4 mm wide) of β- hemolysis. After 24h of incubation, from the primary culture plate, we touched with a sterile straight wire the tops of each suspected colonies with similar appearance and prepared the inoculums. Using a sterile forceps, we placed on the inoculated plate the Bacitracin disc, and for the sensitivity test, we placed Penicillin discs. Following the 24 hours incubation at 37°C in CO₂ incubator the appearance of

a zone of inhibition surrounding the Bacitracin disc is indicative of GAS (group A streptococcus).

The latex agglutination test (Oxoid Streptococcal Grouping kit- Oxoid Ltd, Wade Road, Basingstoke, Hants, UK) for the identification of streptococcal groups showed that the majority of pathogenic streptococci possess specific carbohydrate antigens which permit the classification of streptococci into groups; their presence can be demonstrated with latex particles previously coated with group-specific antibodies.

VITEK 2 Systems was used to identify the types of cultures. The GP (Gram-Positive) identification card is based on established biochemical methods and developed substrates. There are 43 biochemical test measuring carbon source utilization, enzymatic activities and resistance. Final identification results are available in approximately 8 hours or less.

VITEK 2 Systems identifies an organism by using a methodology based on characteristics of the data and knowledge about the organism and reactions being analyzed. Sufficient data have been collected from known strains to estimate the typical reactions of the claimed species to a set of discriminating biochemicals. If a unique identification pattern is not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database [7].

A small quantity of antimicrobial substance was placed on the surface of a blood agar plate, which was previously inoculated with the tested bacteria. We can observe the diffusion of the antibiotic on the plate and the multiplication of the bacteria. In the areas where the antibiotics level is higher than the MIC (Minimal Inhibitory Concentration) the bacteria didn't grow. The circumference of the inhibition zone is established in the first hours of incubation as the geometric location of the points where the antibiotic reached the MIC at the critical moment of the culture. Thus, the diameter of the inhibition zone is inversely proportional with the MIC. The bacteria can be sensitive, if the MIC is at least 2-4 times lower than the average level of the antibiotic in the infectious outbreak. Resistant bacteria are characterized by a MIC which is higher than the average antibiotic level in the outbreak. We have another type of bacteria, the moderately sensitive (intermediate) bacteria. The last type of bacteria have a MIC value close to the average concentration of the antibiotic in the outbreak [8].

Results

A total of 1100 children from the ambulatory of „Louis Turcanu“ Emergency Hospital for Children Timisoara were examined, including 536 females and 564 males, from urban areas or countryside (Figure 1).

Figure 1. The gender and region distribution of the study group

Out of 1100 throat swabs studied, in 54 samples was identified BHS (beta hemolytic streptococcus). Only 2 groups were isolated, group A in 48 samples and group C in 6 samples (Figure 2).

Figure 2. Incidence rate of streptococcus

According to the data collected among the isolates, 38 were females (70%) and 16 were males (30%) (Figure 3).

Figure 3. Identified streptococcus within genders

Of the 1100 children, 50 were under 1 year old, 282 between 1-3 years, 482 between 4-9 years, 200 between 10-14 years and 86 between 15-18

years. Most of the positive cultures were identified in children with ages between 4 and 9 years old (32 patients) (Figure 4).

Figure 4. The distribution of patients by age and infection with BHS

From the positives tested patients only 22% were symptomatic, most of them being diagnosed with pharyngitis (Figure 5).

Figure 5. The distribution of patients based on the symptoms

The patients with positive cultures were also tested for ASO, 28 patients (52%) had high levels, while 26 patients (48%) had normal levels (Figure 6).

Figure 6. The distribution of patients according to the ASO values

On all positive patients an antibiogram was made and we observed that most of the isolates in Group A and all in Group C were sensitive to the antibiotics used. A few isolates in Group A were resistant, as following (Figure 7):

- 2 to Cefepime;
- 12 to Clindamycin;
- 14 to Erythromycin;
- 2 to Tetracycline;

Figure 7. Antibiotic sensitivity

Discussions

Streptococcus pyogenes (Lancefield Group A *Streptococcus*), lives with humans as the only known reservoir. While *S. Pyogenes* represents a significant cause of human disease, this organism survives primarily in an asymptomatic carrier state located on the skin or in the upper respiratory tract and it may persist for years without any related disease developing [9, 10]. However, group A and group C streptococci, being recognized as colonizers of human skin and mucous can produce several infections like scarlet fever, pharyngitis, acute otitis media, erysipelas, cellulitis, impetigo, necrotizing fasciitis, pneumonia, septic arthritis, bacteremia and toxic shock syndrome, and others.¹¹ Their impact is not only quantitative, *S. pyogenes* is a known global pathogen, that accounts for >100 million cases of skin infections and >600 million cases of pharyngitis, each year; but it is also qualitative, in regards to the severity of myositis, puerperal sepsis, the toxic shock syndrome and necrotizing fasciitis [10, 12].

One of the most common pediatric infectious disease, that is responsible for >10 million office visits to primary care physicians annually, is pharyngotonsillitis [13, 14]. It develops most often in children, ages between 5 and 15 years, who are the most susceptible and is more prevalent in temperate climates during the spring and winter months. The usual cause of Pharyngotonsillitis are viruses, but GABHS (Group A Beta Hemolytic Streptococci) is the most common bacterial cause, being responsible for 15% - 30% of cases in children and 5% -10% of cases in adults [13,15].

In addition, group A beta hemolytic streptococci (GABHS) have an important role in the development of post-streptococcal infection sequelae, such as acute rheumatic fever, acute glomerulonephritis and reactive arthritis. Interactions between the host and the pathogen with *S. pyogenes* are extremely complex and it can result in a broad spectrum of outcomes that can be chronic or acute, invasive or superficial, or entirely asymptomatic [10, 16].

We compared the study with two studies from different cities in Nepal, with the same subject as ours. In the first study, in Pokhara Valley, the prevalence of GABHS (Group A Beta Hemolytic Streptococci) was 7.2%, in the study we conducted GABHS was 4% and in the other study, in Kirtipur, BHS (Beta Hemolytic Streptococcus) was 9%. Regarding the age categories, as in our study, in Pokhara Valley, GABHS had the highest rate among children ages 3-10 years old [4, 17].

Unlike the two studies from Nepal, were GABHS (Group A Beta Hemolytic Streptococcus)

was higher in males then in females, the data we collected revealed GABHS was present in 70% of the females and in 30% of the males, without significant difference between the two in the patients lot.

In the study from Kirtipur, the antibiogram was performed, and like in our study all isolates were sensitive to penicillin, corresponding with the reported medical data. In the same study, they evaluated their patients with ASO test, all of them having results within normal range (<200 IU/mL). From the data we gathered, only 48% of the positive patients had normal ASO levels, while the rest (52%) had high ASO levels, corresponding for most of them with clinical symptoms [4].

Conclusions

The study we conducted presents the prevalence of BHS (Beta Hemolytic Streptococcus), especially GABHS (Group A Beta Hemolytic Streptococcus) which was 4%, in children from a tertiary care hospital in Timisoara, Romania; which is comparable to the findings of similar studies from different parts of the world, for example Nepal.

In spite of existing primarily in an asymptomatic carrier state, GABHS (Group A Beta Hemolytic Streptococcus) represents a worldwide health problem because of the complications that can occur once the disease develops. Thus, it is important to identify the presence and type of BHS (Beta Hemolytic Streptococcus) in time to start treatment if needed, avoiding the complications as aforementioned. The objectives of the treatment is to eradicate the organism, improve the clinical symptoms and signs and prevent the transmission of the disease.

Considering the limited data found on our subject, further epidemiological studies on streptococcal disease complex are needed. Interactions between the host and the pathogen, beta hemolytic strptococcus are extremely elaborated and it can result in a broad spectrum of outcomes that can be chronic or acute, invasive or superficial, or entirely asymptomatic.

References

1. Anon. Nosocomial group A streptococcal infections associated with asymptomatic health-care workers - Maryland and California. MMWR 1997
2. Cynthia S. Hayes, Harold Williamson. Management of Group A Beta-Hemolytic Streptococcal Pharyngitis. Am Fam Physician. 2001 Apr 15;63(8):1557-1565
3. Roxana Moldovan. Practical Bacteriology. Mirton. Timisoara.1998

4. Manandhar A, Shah Y, Shrestha J. Study on the Prevalence of Beta Haemolytic Streptococcus Among School Children. *J Nepal Paediatr Soc.* 2013;33(1):45-47
5. Patterson MJ. Streptococcus. *Medical Microbiology*. 4th edition. Galveston , 1996.
6. M.S.Sahin, M.U.Yalcin, D.Kocyigit. Prevalence of rheumatic heart disease in patients with recurrent tonsillitis and elevated anti-streptolysin O titers. *International Journal of Pediatric Otorhinolaryngology*. October 2016; 89:133-135
7. Delia Muntean, Florin-George Horhat, Luminița Bădițoiu, Victor Dumitrașcu, Iulia-Cristina Bagiu, Delia-Ioana Horhat et. al. Multidrug-Resistant Gram-Negative Bacilli: A Retrospective Study of Trends in a Tertiary Healthcare Unit. *Medicina* 2018;54(6), 92
8. Tsegahun Asfaw, Demissew Shenkute, Mihret Tilahun, Nigus Zegeye. Evaluation of Antistreptolysin O (ASO) Titer in Rheumatoid Heart Disease Debre Berhan Referral Hospital, Ethiopia. *Clinical Medicine Research* 2018; 7(1): 26-29
9. Shaikh N, Leonard E, Martin JM. Prevalence of Streptococcal Pharyngitis and Streptococcal Carriage in Children: A Meta-analysis. *Pediatrics*. 2010;126(3):557-564.
10. Hurst JR, Kasper KJ, Sule AN, McCormick JK. Streptococcal pharyngitis and rheumatic heart disease: the superantigen hypothesis revisited. *Infect Genet Evol.* 2018;61(March):160-175.
11. Bonofiglio L, Gagetti P, García Gabarrot G, et al. Susceptibility to β -lactams in β -hemolytic streptococci. *Rev Argent Microbiol.* 2018
12. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. *Lancet Infect Dis.* 2005;5(11):685-694.
13. Brook I. Brief Report A Pooled Comparison of Cefdinir and Penicillin in the Treatment of Group A beta-hemolytic Streptococcal Pharyngotonsillitis. 2005:3-10.
14. Woodwell DA, Cherry DK. National Ambulatory Medical Care Survey: 2002 summary. *Adv Data.* 2004;(346):1-44.
15. Bisno AL, Gerber MA, Gwaltney, Jr. JM, Kaplan EL, Schwartz RH, Infectious Diseases Society of America. Practice Guidelines for the Diagnosis and Management of Group A Streptococcal Pharyngitis. *Clin Infect Dis.* 2002;35(2):113-125.
16. Cunningham MW. Pathogenesis of group A streptococcal infections. *Clin Microbiol Rev.* 2000;13(3):470-511.
17. Shrestha L, Khattri J B K , Brahmadathan K N , Nagra J S, Prevalence Of Streptococcal Pharyngitis Among School Children Of Pokhara Valley, Nepal, *Journal of Nepal Medical Association*, 2001:41:253-257