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IDENTIFICATION OF STREPTOCOCCUS PYOGENIUS IN THROAT CULTURES OF PATIENTS WITH THROAT PAIN

Abstract

Background: Streptococcus are gram-positive bacteria that cause various respiratory and systemic pathologies. They are the cause of upper respiratory problems which are usually manifested with clinical signs such as Sore throat, temperature, cough, and difficulty in breathing.

Materials and Methods: To carry out this study, biological samples were taken from the throat of patients who referred symptoms. These samples were subjected to laboratory microbiological examination of the throat culture on the Agar-blood medium. Then, to identify the colonies, differential biochemical tests were performed such as Gram stain, Bacitracin test, and ASO test.

Results: 200 patients aged from 4 to 79 years participated in the study.

After culture in Blood Agar and biochemical differential tests, the following results were obtained:

- 83 cultures (41.5%) were positive for pathogens of the Micrococcacea family, of which 44 males (53.01%) and 39 females (46.99%).
- 50 patients (60.24%) tested positive for β hemolytic streptococcus type A (streptococcus pyogenus). Average age 34.18 years. All patients had high ASO titers.

Conclusions: This study found an increase in cases of respiratory infections with pyogenic streptococcus in the period January-March in different age groups. The high titer of ASO testifies to the presence of systemic infections in these patients

Keywords: streptococcus pyogenic, throat culture, gram stain.

Introduction

Bacteria of the genus Streptococcus are gram-positive heads typically located in the form of a chain (2–30 cells), they are catalase-negative and have a fermentative metabolism (they produce acid from the fermentation of carbohydrates).

Blood Agar (sheep) is preferred for their growth, which provides important information on the degree of hemolysis. The colonies are small, ranging from

the exact size to 2 mm in diameter, and they may be surrounded by an area where erythrocytes suspended in agar are hemolyzed. When the area is clean, this condition is called β -hemolysis. Streptococci grow better in aerobic or anaerobic conditions, so they are (facultative).

When the area is cloudy with a green agar stain, it is called α -hemolysis. The type of hemolysis and some biochemical reactions are important for

differential diagnoses and as an indication of what subsequent taxonomic tests should be performed. Streptococci are metabolically active for various carbohydrates, proteins and amino acids. Fermentation of glucose produces mainly lactic acid.

This genus includes more than 100 species and their classification is done according to 3 classification schemes.

- antigenic properties (Lancefield Classification;
- Hemolytic models (complete beta hemolysis, partial alpha hemolysis, without gamma hemolysis;
- biochemical properties.

For convenience in practice, streptococci are divided into 2 groups:

- Beta hemolytic streptococci that are classified by the Lancefield group;
- Hemolytic alpha and gamma streptococci that are classified according to biochemical tests.

Rebecca Lancefield discovered the serological classification scheme in 1933. This scheme is used today for a few species of *Streptococcus*. According to this classification, streptococci are divided into groups A-U where the most popular are groups A-G and from this group, group A or pyogenic streptococcus is the most important.

Group A Streptococci (*Streptococcus pyogenes*)

Group A streptococcus is the most common cause of bacterial pharyngitis. Group A streptococci (GAS) usually appear in purulent lesions or in fluid cultures as spherical or ovoid cells in chains of short to medium length (4–10 cells).

Culture on Blood Agar. On blood agar plates, colonies are usually compact, small, and surrounded by a 2 to 3-mm zone of β -hemolysis, which is easily seen and clearly demarcated. β -hemolysis is caused by one of two hemolysins, streptolysin S and the oxygen-labile streptolysin O, both of which are produced by most strains of group A. Strains lacking streptolysin S are β -hemolytic only under anaerobic conditions because streptolysin O the remainder is inactive in the

presence of oxygen. This feature is of practical importance, because such strains would be absent in clinical laboratories if cultures were incubated only aerobically.

The virulence of the pyogenic streptococcus is related to the antigenic structure and the exotoxins released by it.

Antigenic structure

1. The cell wall is built on a peptidoglycan layer that provides rigidity. Also, part of the structure is the M protein and lipoteichoic acid (LTA) that are attached to the cell wall, Group A Streptococci are divided into more than 100 serotypes based on antigenic differences in the M protein.

2. Protein M has a chemical structure similar to myosin. Due to its positioning, this protein acquires an immunogenic role and serves as a binding domain for other molecules such as Fibrinogen, serum H protein or Immunoglobulins.

3. Other molecular structures that affect pathogenicity are also an **F protein and fibronectin-binding LTA**, both on the surface of streptococcus that, due to binding to the Fc part of Ig, affect optionism.

4. Hyaluronic acid in the capsule is an important element for phagocytosis.

The antigenic structure is important for the pathogenesis of the infection.

1.1 Exotoxins

Exotoxins are substances related to the virulence as well as the clinical manifestations of this bacterium. The most important toxins are:

1. Streptolysin O

Streptolysin O is a pore-forming cytotoxin that lyses leukocytes, tissue cells, and platelets. This toxin is resistant to oxygen. Streptolysin O is antigenic, and the number of antibodies against it is the basis of a serological test called anti-streptolysin O (ASO).

2. Super antigenic streptococcal toxins

Over many decades, these toxins have been assigned a variety of names related to their association with scarlet fever (erythrogenic toxins) and streptococcal toxic shock (streptococcal pyrogenic exotoxins [Spe]).

3. Other extracellular products

Most GAS strains produce a variety of other extracellular products including streptokinase, hyaluronidase, nucleases, and C5a peptidase. Peptidase is an enzyme that destroys the complement protein C5a, the main factor that attracts phagocytes to sites of complement deposition. Other enzymes play a role in tissue damage. Some of them have also been used as serological tests, eg Streptokinase.

1.2 Group a Streptococcal Infections:

Group A streptococcus is responsible for several pathologies:

Noninvasive pathology

- Pharyngitis;
- Impetigo;
- Cellulitis.

Invasive diseases:

- septicemia;
- bacteremia;
- Streptococcal Toxic Shock Syndrome.

Non-suppurative sequelae:

- Glomerulonephritis;
- Rheumatic Fever.

This study aims to identify streptococcus as the primary cause of pharyngitis in patients of different ages

2. Materials and methods

For the realization of this study, throat cultures were performed on patients of different age groups who were referred to the same clinic:

- disorders of the respiratory tract;
- cough with sputum;
- sore throat;
- difficulty in swallowing;
- temperature;
- shoulder pain.

In this time period, January-March 2023, 200 throat cultures were performed.

2.1 Laboratory sample

Sampling was done with a sterile swab from the back of the tonsils or posterior larynx. Patients must be healthy and not have taken antibiotics or other medications. Oral hygiene should also be avoided.

2.2 Culture on Blood Agar

Throat cultures were collected on Blood Agar, and incubated in a 37 °C thermostat for 24 hours and the morphology of the colonies was studied (small, grey colonies with the presence of beta hemolysis).



Figure 1. View of the field with culture of streptococcus

After culture in blood agar, differential and biochemical tests were performed to identify the group.

2.3 Bacitracin test

This test is based on the sensitivity of streptococcus group A to Bacitracin. The principle of this test consists in placing an antibiotic disk saturated with bacitracin, which after 24 hours of incubation in a thermostat at a temperature of 37 will inhibit the growth of group A streptococcus only.

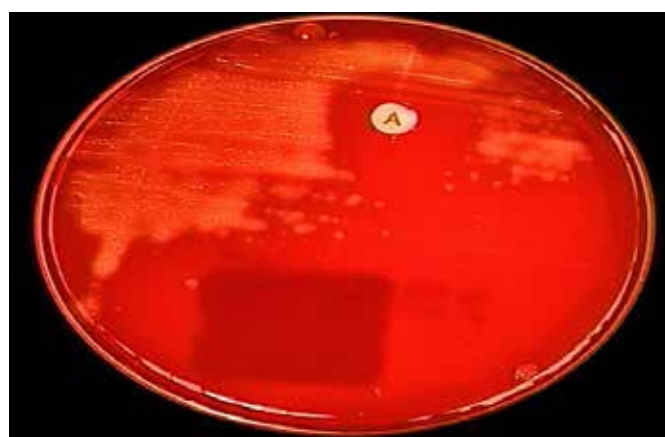


Figure 2. Bacitracin Sensitivity Test

After incubation, the developed colonies were identified and differentiated. Out of 200 throat

cultures, 83 (41.5%) of them were positive for the coccus genus.

The differential diagnosis within the type of streptococci was carried out with biochemical tests such as:

- bacitracin test (beta-hemolytic streptococcus type A);
- optokinetic test (streptococcus pneumonia).

After performing this test, 50 patients (60.24%) were found to have β -hemolytic streptococcus type A (streptococcus pyogenus).

For the diagnosis of post streptococcal consequences as evidence of a previous infection, serological tests such as the ASO test are used.

This test consists of the identification of antibodies created by the organism against Streptolysins O. This test is qualitative, but for patients who have a positive result, dilutions of the serums are made, accurately determining the concentration of the antibodies. In this case, the test takes the nature of a quantitative test and is important in diagnosis.

These antibodies show the immune response following a repeated infection and for this reason they are of the IgG type.

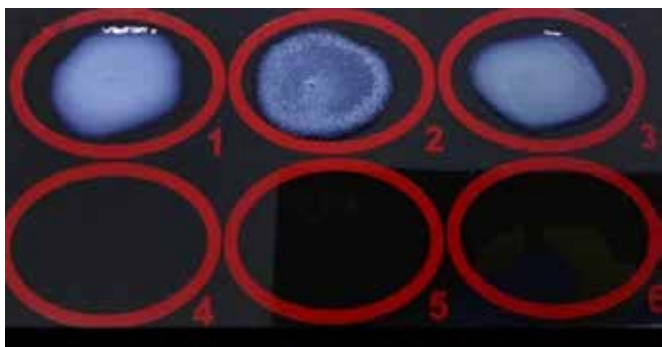


Figure 3. The ASO test

The high titer of IgG indicates an existing systemic infection that does not have a specific localization, which brings difficulties in the media treatment of these patients, both in the type of antibiotic and in the duration of treatment, thus negatively affecting the quality of life of the patient.

For patients who tested positive for Streptococcus β hemolytic type A, venous blood was taken by venipunctures in gel tubes (Clot activator) with a 21G vacuum system and centrifuged in the CAPP CENTRIFUGE centrifuge at 3500 RPM for 15 minutes. The serum was tested for the presence or absence of IgG antibodies (qualitative test). Serums that were positive with IgG were diluted and their titer was measured, which turned out to be high. This shows that we have the presence of systemic infections.

4. Conclusions

Streptococcus pyogenus is one of the most frequent causes of upper respiratory tract infections, mainly pharyngitis in children aged 5 to 15 years. According to this study, a touch of patients in the age group older than 15 years is observed, which shows a large spread of these pathogens for various reasons such as: poorly processed foods, poor hygiene.

The second discussion is related to the high titer of the ASO serological test observed in these patients. This test is a drop-down indicator to show that this infection is already systemic, so antibiotic treatment will be even more difficult.

The third discussion is related to antibiotic resistance, a current global problem. The treatment of infection should not be symptomatic and broad-spectrum antibiotics should be taken, but microbiological laboratory tests combined with antibiograms should be performed in order for the treatment to be as efficient as possible and to further avoid resistance to antibiotics.

The fourth discussion is related to the medical awareness of patients who should be more aware and not avoid routine laboratory examinations since the quality of their life depends on these simple tests.

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