

## Characterization of *Streptococcus pyogenes* Isolated from Throat Swabs in Baghdad Children Patients

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### Abstract

The study aimed to determine immune status of patients with respect to *Streptococcus pyogenes*. The present study was conducted to determine antibody against Group A *Streptococcus* (GAS) in serum of children patients suffer from tonsillitis and pharyngitis. A total of 260 samples (197 blood samples and 260 throat swab) was collected from 260 patients, 169 (65 %) of them were males and 91 (35%) were females suffering from Tonsillitis and pharyngitis. The results indicated that most age groups 2- 13years being susceptible for tonsillitis in children, were among the recurrent tonsillitis and the males were more susceptible and response to infection compared with females. The study included two main parts. The first one was the bacterial diagnosis based on relied diagnostic procedure (classical and advance which involved Api 20 Strep and Vitek 2 system). The second part was the detection of antistreptolysin O (ASO) by using a latex agglutination test. The throat swabs culture from patients conducted its Lancefield group to suggest group A. The results observed that GABH (3.07%) *Streptococcus pyogenes*, while the rest other bacteria 252 (96.92 %) group as other bacteria from total study samples (260).

**Key words:** - *Streptococcus pyogenes*, Vitek2 system, Bacitracin Test

### الخلاصة

تهدف الدراسة الى تحديد الوضع المناعي في المرضى المصابين بالعقدية المقيحة. وقد أجريت الدراسة لقياس الاجسام المضادة للاطفال المصابين بالنوع (A) من التهاب اللوزتين و البلعوم. تم جمع 260 عينة (197 عينة دم و 260 مسحة للبلعوم)، من مجموع المسحات الكلي كان عدد الذكور 169 (65%) و عدد الاناث 91 (35 %) يعانون من التهاب اللوزتين و البلعوم. المسحات تم جمعها من الاطفال الذين نقلوا الى مستشفى حماية الطفل و مستشفى بغداد التعليمي و مستشفى الجراحة التخصصي و مستشفى الطفل المركزي خلال الفترة من مارس الى يوليو 2014. وتضمنت الدراسة قسمين الاول كان التشخيص البكتيري على اساس الفحص الكلاسيكي و الفحص المتقدم أما الجزء الثاني يشمل الكشف عن الاضداد (ASO) بأستخدام اختبار التراص اللاتكس. وتم إجراء فحص Lancefield group لتمييز صنف (A) و لوحظ من خلال النتائج ان العقدية الفيحية تشكل 3.07% في حين ان بقية البكتريا الاخرى 96.92% من باقي أنواع البكتريا. . وقد اشارت النتائج الى ان المعرضين لالتهاب اللوزتين في الذكور اكثر عرضة من الاناث.

الكلمات المفتاحية: بكتريا *Streptococcus pyogenes*، نظام Vitek2، اختبار الـ Bacitracin

### I. Introduction

Streptococci are considered one of the predominant flora which colonize the respiratory tract of human (Todar, 2004). One of these *Streptococcus pyogenes* is an obligate human pathogen. It is a cause of major human morbidity and mortality worldwide. Streptococcal infections have been documented in all races, sexes and age groups. Group A streptococci (GAS) cause 18 million cases of severe diseases resulting in

517,000 deaths each year. GAS is responsible for a number of diseases ranging from common clinical illnesses such as pharyngitis, impetigo, cellulites and scarlet fever to severe invasive infections such as puerperal sepsis, streptococcal toxic shock syndrome (STSS) and post infectious sequelae such as rheumatic fever and acute glomerulonephritis (Cho and Caparon, 2008). School- age children (5-15 years) are considered as the major reservoir of group A beta-hemolytic Streptococci (Al-Kareem *et al.*, 2014).

GAS is further subdivided into different serotypes based on highly variable N terminal sequences of the cell surface M protein as described by Dr. Rebecca Lancefield. Currently, there are over 80 types of M-protein genes (*emm*) identified in GAS (Maripuu *et al.*, 2008). GAS produces several surface-associated and secreted components that have been implicated in internalization. M protein and hyaluronic acid (HA) capsule are two major virulence factors of GAS that inhibit phagocytosis and enhance virulence in animal models (Cunningham, 2000).

*Streptococcus pyogenes*, also release two important cytolytic toxins known as Streptolysin O (SLO) and Streptolysin S (SLS) (Schleiss, 2009). SLO is a potent cytolytic toxin which has a toxic effect on a variety of cells and is able to induce apoptosis of macrophage (Timmer *et al.*, 2009). SLO is an antigenic and produced by almost all Group A beta hemolytic streptococci (GABHS) strain (Bisno *et al.*, 2003). Antibodies to SLO are generated by the humoral immunity and can be quantified as Anti-Streptolysin O Antibodies, it appears in serum from 1 week to 1 month after the onset of streptococcal infection. The ASOT test used as an indicator of recent infection (Shet and Kaplan, 2002). SLS is one of the most potent cytotoxins capable of lysing leukocyte, platelets and subcellular organelles. SLS responsible for Beta- hemolysis characters on blood agar medium (Schleiss, 2009).

Although the M protein is the major virulence factor for the bacteria that acts as a barrier to the host immune system which tries to engulf the antigen. Hence the surface protein, M protein was extracted and purified to trigger the immune response in mice in a controlled release technique. This was done by limited pepsin digestion and purified by ion-exchange chromatography on DEAE-cellulose, followed by gel filtration. Purified M protein was found to be homologous on SDS-PAGE with the molecular weight of 28 KDa. (Aparna and Selvaraj, 2013).

The study aimed to determine immune status of patients with respect to *Streptococcus pyogenes*.

## II. Materials and methods:

### • Swab collection:

The throat swabs were collected from children patients who were admitted to Child protection hospital, Baghdad teaching hospital, specialist surgery hospital, and Central child hospital during the period between march to July 2014.

- **Swab culture**

The swabs taken from the tonsils and post pharyngeal were inoculated on blood agar plates and azide agar plates. The plates were incubated at 37° C for 18 to 24 hours under 5 – 10 % CO<sub>2</sub>, then diagnosis.

- **Blood Samples**

The samples were collected after phlebotomy in evacuated tubes (Without anticoagulant) and were centrifuged at 4000 rpm for 5 min after the blood had clotted and the sera used for determination of specific streptococcal antibody titer by ASO test.

- **Colonial morphology and microscopic examination**

A pure colony was taken from positive culture. Its identification depended on the morphology properties include colony size, hemolysis around colony, color, shape, translucency, edge, and elevation of texture and then investigated by Gram stain to observe shape bacterial cells and arrangement of cells (Holt *et al.*, 1994).

- **Catalase Test**

A drop of 3% hydrogen peroxide solution was placed on a slide, and small amount of the bacterial growth was mixed with the solution. The formation of gas bubbles indicated a positive result. Failure to produce effervescence or weak effervescence is interpreted as a negative result (Forbes *et al.*, 2007).

- **Bacitracin Sensitivity Test**

Bacitracin test were carried out according to (Forbes *et al.*, 2007).

- **Diagnostic by advanced technique Vitek 2 system**

The Vitek 2 System was used to confirm the biochemical test; the assay had been performed according to the manufacturer's instructions. Recently, this kit was used for detection *Streptococcus pyogenes* in rapidly (Biomérieux-France).

- **Immunological tests**

- **Qualitative Lancefield test Strep method to detect group A**

The strep A rapid test device (throat swab) is a qualitative, lateral flow immunoassay for the detection of streptococcus group A carbohydrate antigen in a throat swab in this test. The antibody specific to strep A carbohydrate antigen is coated on the test line region of the test (Abon-U.K.).

- **Serological tests**

- **Anti Streptolysin O Titer (ASOT) assay**

The ASO-latex is a slide agglutination test for the qualitative and semi-quantitative detection of anti-Streptolysin-O in human serum. Latex particles coated with Streptolysin O are agglutinated when mixed with serum of patients, which containing ASO (Spenreact, Spanish).

1- **Qualitative test**

- Allow the reagent and serum to reach room temperature.
- Place 50µl of the serum and one drop of each positive and negative control into circle on the slide test.

- Mix the ASO – latex reagent on a vortex mixer before using and add one drop of serum.
- Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.
- Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator.
- The presence of agglutination indicates positive result, while the absence of agglutination indicates negative results.

## 2- Semi-quantitative test

- Make serial two fold dilutions of the sample in 9 ml/l saline solution.
- Proceed for each dilution as in the qualitative method.
- Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator.
- The presence of agglutination indicates positive result, while the absence of agglutination indicates negative results (Spenreact, Spanish).

## III. Results and Discussion:

In this study, 260 serum samples were collected from 260 children patients. By using latex agglutination test, the ASO titer results were positive only 5 samples (1.96%) from total sample, While 255 samples (98.07 %) were negative results. These results were showed in table-1. In order to recognize between positive and negative result, the titer less than 200 U considered as normal value, these results were documented by Danchin *et al.*, (2005).

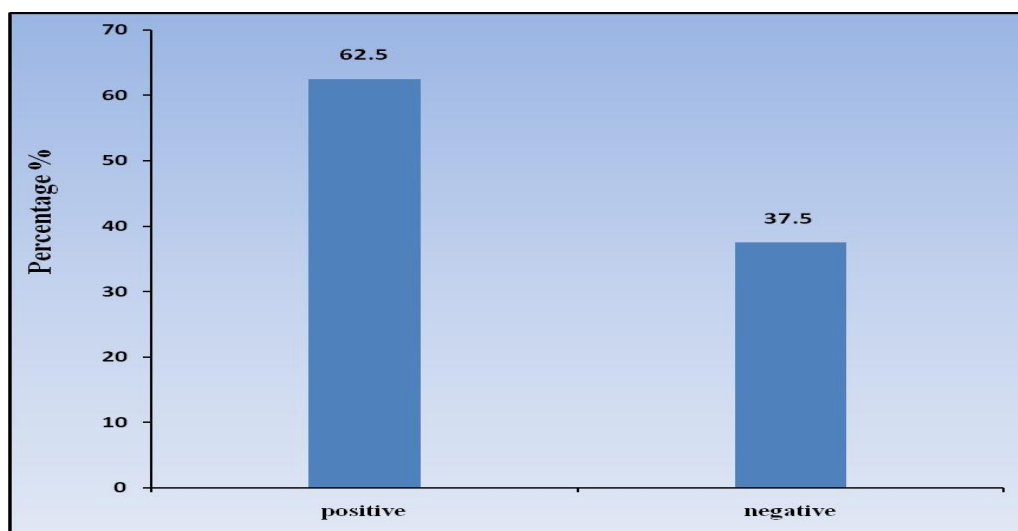
**Table (1):- The percentage of positive and negative ASO test.**

Antistreptolysin O test	No. of isolates	%
Positive	5	1.93
Negative	255	98.07
Total	260	100

Antistreptolysin O titer (ASO) is a measure of the blood serum levels of antistreptolysin O antibodies used in tests for the diagnosis of a streptococcal infection or indicate a past exposure to streptococci. The ASOT helps direct antimicrobial treatment and is used to assist in the diagnosis of scarlet fever, rheumatic fever, and post infectious glomerulonephritis.

The antibodies level starts to rise in 1-3 weeks after streptococcal infection, peaks in 3-5 weeks, and then goes back to insignificant level over 6-12 months, so a positive test can indicate current but more recent group A, C, and G streptococcal infection and may support the diagnosis of post streptococcal infection complication. Rising titers over time are more indicative of infection (Kumar *et al.*, 2007).

The ASOT according to the type of reaction shown in figure-1. this study documented that 8 samples diagnostic with Vitek2 system, but when applying ASOT only 5 (62.5%) samples out of 8 give high titer of ASOT more than (200 U), the reminder 3(37.5 %) samples gives negative results less than (200U). ASO titers may be negative in up to 20% of patients who develop acute rheumatic fever (Hilario and Terreri, 2002).



**Figure (1) Distribution of GABH with the ASO test**

The ASO titer according sex shown in table -2, revealed that the males group were 3(1.77%) gives high titer of ASOT more than 200 U., while female group shows less count 2(2.19 %) from total study count 3 samples.

**Table (2):- distribution ASO results patient according to gender of patient**

ASO-Test result	Male	Female	Total
Positive	3(37.5 %)	2 (25 %)	5 (62.5%)
Negative	2(25%)	1(12.5%)	3 (37.5%)
Total	5 (62.5%)	3 (37.5%)	8 (100%)

The results of ASO test by qualitative method appear positive or negative results, while semi quantitative method appear more accuracy titer shown in table -3.

Table (3): Deference between qualitative and quantitative for ASO test.

No. of patients	ASO test	
	Qualitative	Quantitative
1	positive	1/320
2	negative	-----
3	negative	-----
4	positive	1/640
5	positive	1/320
6	negative	-----
7	positive	1/320
8	positive	1/320

This result of this study was compatible with Saleh (2009) who reported the gender was found of no value as a risk factor in increasing or decreasing the prevalence rate of infection but this study was in disagreement with Al-Hababy (2010) who reported the females were more susceptible and response for infection compared with males to ratio (3:1).

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