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& Scientific Research
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College of education for pure
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Molecular Detection of Transposable Elements Conferring Antibiotic Resistance in *Streptococcus pyogenes* from Clinical Specimens

A Thesis Submitted to
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By

Hanan Raheem Hassooni Al-Khshali

B.Sc. Biology-College of Education for Pure Science /University of Diyala
2009
M.Sc. Biology-College of Education for Pure Science/University of Diyala
2016

Supervised by

Professor
Dr. Abbas Abood Farhan

2020 A.D

Professor
Dr. Hameed Majeed Jasim

1441 A.H

2. Literature Review

2.1 Description and Classification of Genus *Streptococcus*

Strepto and coccus are Greek words referring to “string of” and “round”, which explain the typical semblance in the *Streptococcus* genus (Barnham and Holm, 1997). *Streptococcus* is a genus that belongs to the Streptococcaceae family, within the order Lactobacillales in the phylum Firmicutes (Ryan *et al*., 2004). These organisms are gram-positive coccus or spherical bacteria, catalase negative, nonmotile, nonsporeforming and many are facultative anaerobes (Lal *et al*., 2011). Theodor Billroth was the first person who described the *Streptococcus* genus in 1874, but the historical significance of Streptococci was identified in 1879 when the microorganism was isolated from the women with puerperal fever by Louis Pasteur (Alouf and Horaud, 1997). The streptococci are a large and heterogeneous group bacteria. There are several classification systems for streptococci, so the understanding of the way of the classification is as a key to understand their medical importance (Brooks *et al*., 2013). The first endeavors to classify streptococci were made ~100 years ago; this trial was based on the ability of lysis (the red blood cells) on blood agar plates (Stevens and Kaplan, 2000). In 1903, Hugo Schottmuller made an important step to distinguish between streptococci when he used blood agar plates and implanted the streptococci in it (Ferretti *et al*., 2016). As a result, three classes of hemolytic patterns surrounding a colony on a blood agar were specified: Alpha hemolytic (α), a green zone of discoloration which is the characteristic of the *viridans streptococci*, beta-hemolytic (β) which is a clear zone of a complete hemolysis, is found in the *S. haemolyticus* type organisms such as *Streptococcus pyogenes* and gamma hemolytic (γ) show no change in the medium, this is the characteristic of the *Enterococcus* and *S. faecalis* organisms (Köhler, 2007; Miyanohara *et al*., 2013). On the other hand, Dochez, Avery,

and Lancefield began to employ immunological approaches to prove that the differences could be evinced between strains of *S. haemolyticus* (Ferretti *et al*., 2016). Lancefield used in 1933 surface antigen differences between streptococci to divide them into other groups designated by the letters A through X (Köhler, 2008).

2.2 *Streptococcus pyogenes (S. pyogenes)*

S. pyogenes is a β-hemolytic bacterium that belongs to Lancefield serogroup A. Also it is referred to as group A streptococci (GAS) (Westbroek *et al*., 2010). *S. pyogenes* are parts of the skin and pharynx normal flora (Konar *et al*., 2016). Their numbers are commonly limited by a contest from the nasopharyngeal microbial ecosystem and by nonspecific host protection mechanisms. But diseases can occur as a result of poor defense mechanisms or the acquisition of new strains that alter normal flora (Kayser *et al*., 2005). *S. pyogenes* is the major human pathogen connected with local or systemic infestation and post-streptococcal immunologic disorders (Brooks *et al*., 2013). These bacteria colonize the throat or skin and cause a number of purulent infections involving pharyngitis, necrotizing fasciitis, impetigo and streptococcal toxic shock syndrome (Walker *et al*., 2014; Beye *et al*., 2017).

Pharyngitis is one of the most common diseases infected children and is caused by *S. pyogenes* (Bingen *et al*., 2004). This disease can be distinguished by several signs including throat congestion, fever, and discharge from the pharynx and tonsils (Dietrich and Steele, 2018). The latter regarded as one of the most important characteristics for the diagnosis of group A streptococci as illustrated in figure (2-1). In addition, *S. pyogenes* may stimulate autoimmune diseases like rheumatic fever, acute post-streptococcal glomerulonephritis and rheumatic heart disease (AL-Kareem *et al*., 2014). Globally, more than half a million deaths occur yearly due to these diseases (Walker *et al*., 2014).

S.pyogenes produces large (1cm in diameter) zones of β-hemolysis around colonies and it is greater than 0.5mm in diameter. Genomic and molecular analyses have now distinguished a large number of group A streptococci virulence determinants. Many of these overlap and increase of adhesion and colonization, innate immune opposition, and the ability to support the tissue barrier degradation and diffusion within the human host (Dietrich and Steele, 2018).

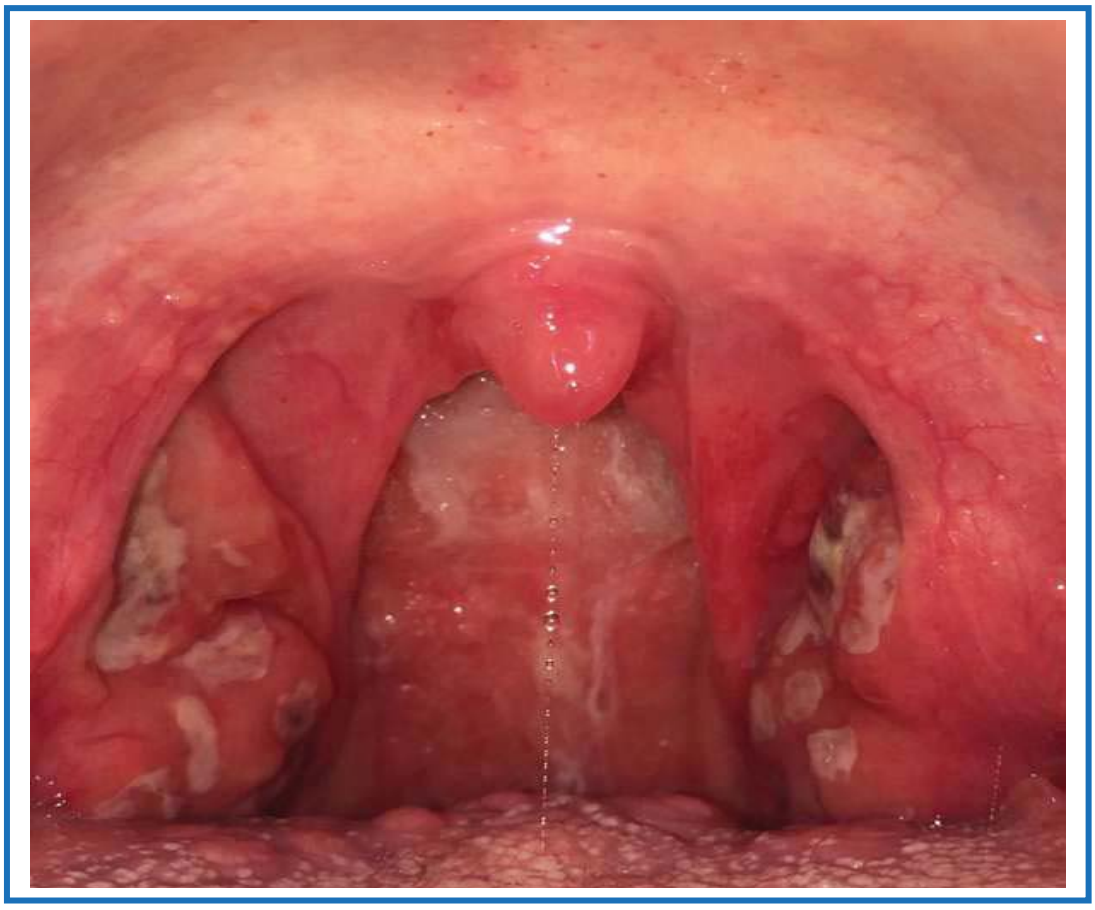

Figure (2-1): Pharynx and tonsils discharge (Dietrich and Steele, 2018)

Most group A streptococci produce capsules containing hyaluronic acid, they impede phagocytosis, The hyaluronic acid capsule probably plays a major role in virulence (Brooks *et al*., 2013). In addition, *S. pyogenes* were further

subdivided according to the presence of a surface protein called M protein into various antigenic types; Lancefield identified the M protein as the main virulence factor of *S.pyogenes* by its antiphagocytic property (Ferretti *et al.*, 2016). On the other hand, hair-like pili stand through the capsule of group A streptococci, the pili composed partly of M protein and are enveloped with lipoteichoic acid. The latter is important in the touch of streptococci to epithelial cells (Kayser *et al*., 2005). *S.pyogenes* that lack M protein are not virulent, when M protein is present, the streptococci are virulent and in the loss of M type-specific antibodies, they are capable to resist phagocytosis by polymorphonuclear leukocytes by inhibiting activation of the alternative complement pathway (Brook,2013). Lancefield was able to explain over 50 various M-types of group A streptococci during her career. More than 200 M-types have been recognized using serological and molecular typing methods since that time (Schleiss, 2009).

2.3 Virulence factors

S. pyogenes has several virulence factors that enable it to attach to host tissues, evade the immune response, and spread by penetrating host tissue layers. In *S.pyogenes*, there is a carbohydrate based capsule composed of hyaluronic acid, protecting it from phagocytosis by neutrophils (Starr and Engleberg, 2006). In addition, the capsule and several factors embedded in the cell wall, including M protein, lipoteichoic acid, and protein F (SfbI) facilitate attachment to various host cells (Buchanan *et al*., 2006). M protein also inhibits opsonization by the alternative complement pathway by binding to host complement regulators (Mora *et al*., 2005). The M protein found on some serotypes is also able to prevent opsonization by binding to fibrinogen (Golińska *et al*., 2016). However, the M protein is also the weakest point in this pathogen's defense, as antibodies produced by the immune system against M

protein target the bacteria for engulfment by phagocytes (Williamson *et al*., 2014). M proteins are unique to each strain, and identification can be used clinically to confirm the strain causing an infection (Ferretti *et al*., 2016).

Furthermore, *S. pyogenes* includes more of virulence factors, which can be illustrated as follows:

1) Streptolysin O: An exotoxin, one of the bases of the organism's beta-hemolytic property which causes an immune response and detection of antibodies to it (Walker *et al*., 2014).

2) Streptolysin S: A cardiotoxic exotoxin, another beta-hemolytic component, not immunogenic and O_2 stable. A potent cell poison affecting many types of cell including neutrophils, platelets, and subcellular organelles (Darmstadt *et al*., 2000).

3) Streptococcal pyrogenic exotoxin A (SpeA) and Streptococcal pyrogenic exotoxin C (SpeC): Superantigens secreted by many strains of *S. pyogenes*, this pyrogenic exotoxin is responsible for the rash of scarlet fever and many of the symptoms of streptococcal toxic shock syndrome, also known as (TSLS) or toxic shock like syndrome (Terao, 2012 ; Ferretti *et al*., 2016).

4) Streptokinase: Enzymatically activates plasminogen, a proteolytic enzyme, into plasmin, which in turn digests fibrin and other protein (Engel *et al*., 2014).

5) Hyaluronidase: Hyaluronidase is widely assumed to facilitate the spread of the bacteria through tissues by breaking down hyaluronic acid, an important component of connective tissue (Starr and Engleberg, 2006). However, very few isolates of *S. pyogenes* are capable of secreting active hyaluronidase due to mutations in the gene that encode the enzyme (Ferretti *et al*., 2016). Moreover, the few isolates capable of secreting hyaluronidase do not appear to need it to spread through tissues or to cause skin lesions (Terao, 2012).

Summary

A total of 500 clinical Specimens collected from patients suffering from pharyngitis, tonsillitis, and otitis attended consulting clinic at Baqubah teaching hospital, Al-Batoul teaching hospital and private clinics in Diyala governorate, the consulting clinic at central child teaching hospital and Baghdad teaching hospital in Baghdad governorate, private clinics in Erbil governorate, and Beirut medical center / American university in Lebanon. From these clinical samples, a total of 93 bacterial isolates were obtained after culturing on (blood agar medium and β- selective *Streptococcus* agar medium) supplemented with 5% fresh human blood. Among these isolates only 22 were identified as *S.pyogenes* according to their morphological, cultural, and biochemical characteristics. Furthermore, the identification of these isolates was confirmed by using Vitek-2 system and molecular identification according to the results of amplification and sequencing of 16S rDNA gene.

Molecular serotyping of *S.pyogenes* isolates showed that these isolates were belong to group A *streptococcus* according to the nucleotide sequence of *emm* gene in each isolate and the guidelines stated by the centers for disease control and prevention (CDC). Moreover, results showed that *S.pyogenes* isolates were belong to seven serotypes (*emm*104.1, *emm* 28, *emm* 44, *emm* 63, *emm* 1, *emm* 5.103 and *emm* 90) for the *emm* patterns E2, E3, E4, E6, A-C3 and M5, respectively.

Susceptibility of *S.pyogenes* isolates to different antibiotics was examined, results showed that the isolates were variable in their susceptibility as the most (90.9%) were resistant to erythromycin, then to minocycline (68.1%), lincomycin (59%), tetracycline (54.5%), clindamycin (50%), azithromycin (36.3%), clarithromycin (36.3%), doxycycline (31.8%) and oxytetracycline (27.2%).

6) Streptodornase: Most strains of *S. pyogenes* secrete up to four different DNases, which are sometimes called streptodornase (Darmstadt *et al*., 2000). The DNases protect the bacteria from being trapped in neutrophil extracellular traps (NETs) by digesting the NETs' web of DNA, to which are bound neutrophil serine proteases that can kill the bacteria (Williamson *et al*., 2016).

7) C5a peptidase: C5a peptidase cleaves a potent neutrophil chemotaxin called C5a, which is produced by the complement system (Ferretti *et al*., 2016). C5a peptidase is necessary to minimize the influx of neutrophils early in infection as the bacteria are attempting to colonize the host's tissue (Engel *et al*., 2014). C5a peptidase, although required to degrade the neutrophil chemotaxin C5a in the early stages of infection, is not required for *S. pyogenes* to prevent the influx of neutrophils as the bacteria spread through the fascia (Terao, 2012).

8) Streptococcal chemokine protease: The affected tissue of patients with severe cases of necrotizing fasciitis is devoid of neutrophils (Ferretti *et al*., 2016). The serine protease ScpC, which is released by *S. pyogenes*, is responsible for preventing the migration of neutrophils to the spreading infection (Mora *et al*., 2005). As shown in figure (2-2), ScpC degrades the chemokine IL-8, which would otherwise attract neutrophils to the site of infection (Golińska *et al*., 2016).