SEROGROUPING OF β-HAEMOLYTIC STREPTOCOCCI ISOLATED FROM THROAT INFECTION

THESIS

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BY

MANAL MOHAMMED YASSIN MOUSTAFA

M.B., B.Ch.

UNDER SUPERVISION OF

Prof. Dr. FATMA ABDEL FATTAH

Professor of Microbiology and Immunology

Faculty of Medicine

Ain Shams University

Dr. SAMEER IBRAHIM ABDEL HADI

Lecturer of Microbiology and Immunology

Faculty of Medicine

Ain Shams University

FACULTY OF MEDICINE

AIN SHAMS UNIVERSITY

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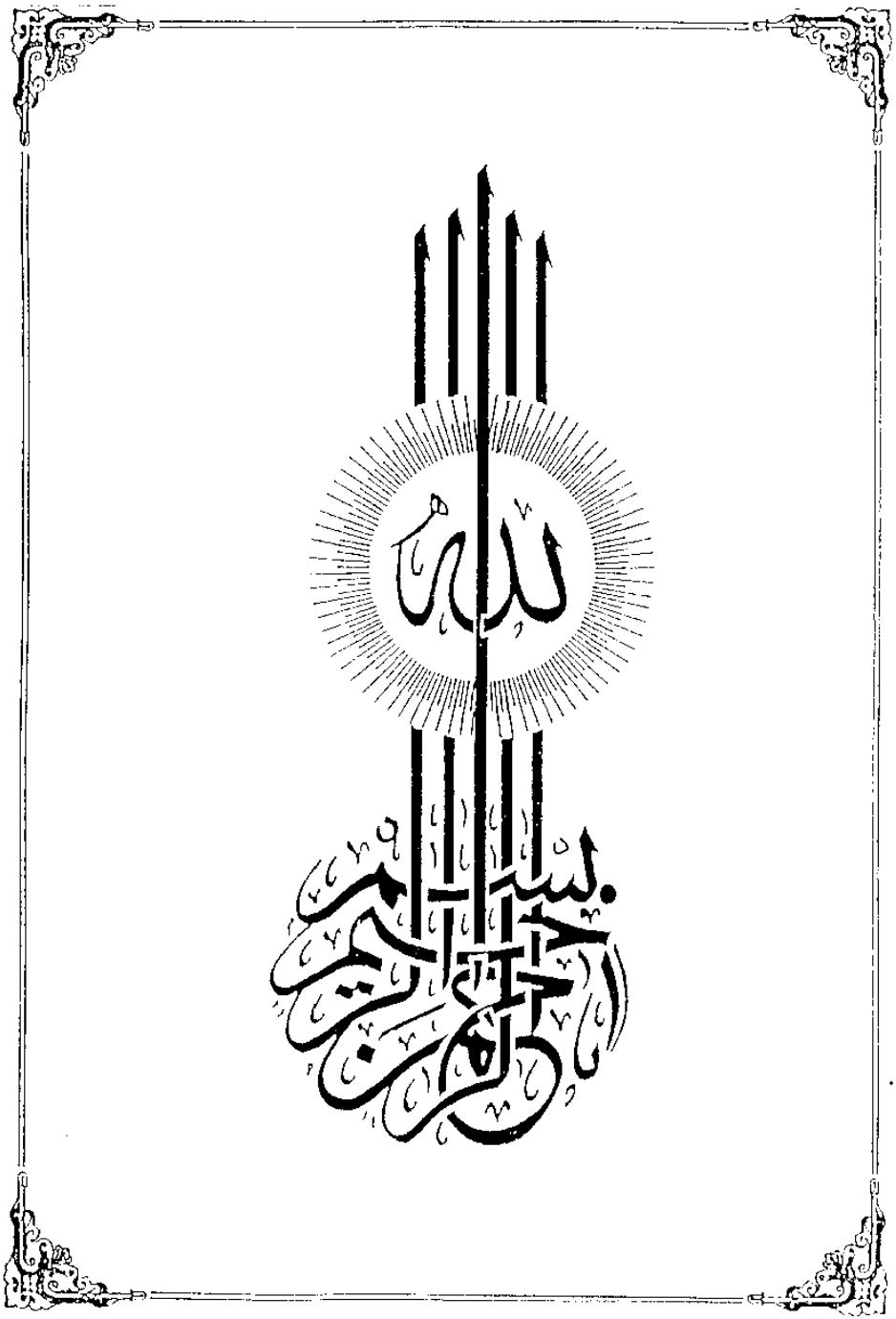

CONTENTS

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION

Streptococci are widely distributed in nature and form an important part of the normal bacterial flora of man and animals. Among the haemolytic streptococci, the most important pathogen for man is Streptococcus pyogenes (the group A streptococcus). Group A streptococci are the most usual cause of acute upper respiratory tract disease, scarlet fever, and a variety of septic lesions and the two important non-suppurative events that follow acute streptococcal infection: acute rheumatic fever and acute glomerulonephritis (WHO, 1968).

The reliable and rapid tests for the identification of streptococci are critical from both clinical and epidemiological view points. The correct grouping of an isolate may suggest the source of infection and lead to prompt treatment (Wellstood, 1982), and the early therapy of streptococcal throat infections can shorten the duration of the illness, provide symptomatic relief, and reduce the period of infectivity (Gerber, 1986).

Various methods for the early detection of Streptococcus pyogenes and other streptococci from throat swabs have been described. Non serological grouping includes

bacitracin sensitivity disks for detection of any zone of inhibition (Facklam et al., 1974). Serological grouping can be performed by extracting the group-specific carbohydrates and producing a precipitate against a group-specific antiserum. These techniques include fluorescent antibody test and latex agglutination test (streptex) (Watson et al., 1975; and Gerber et al., 1984).

AIM OF THE WORK

The aim of the present work is to serogroup different isolates of beta-haemolytic streptococci from acute throat infection (acute follicular tonsillitis) using latex agglutination technique by streptex kit.

REVIEW OF LITERATURE

STREPTOCOCCI

The term streptococcus was first applied by Billroth and Ehrlich (1877) to a chain-forming coccus that they saw in infected wounds (Parker, 1984).

Streptococci are widely distributed in nature. Some are members of the normal human flora, others are associated with important human diseases attributable in part to infection by streptococci, in part to sensitization to them (Jawetz et al., 1987).

MORPHOLOGY :

Streptococci are more or less spherical in shape and are arranged in chains. Growth occurs by elongation on the axis parallel to the chain, and division is at right angles to this, often giving rise to an appearance of pairing within the chain. In some streptococci, the longer dimension of the cell is at right angle to the axis of the chain but in others - such as the enterococci and the pneumococci - it is coincident with it. A few rod-like forms may be found in many streptococcal cultures. The length of the chain varies; it depends to some extent on the medium on which organism is grown, but some streptococci characteristically form long chains, whereas others

are mainly diplococcal (Parker, 1984). The cells forming the chain are connected by a bridge of cell wall material and cannot be separated by shaking but chains may be partly disrupted without killing many of the cocci by sonic oscillation for short periods (Slade and Slamp, 1956). Chains do not elongate indefinitely, in some cases this is because the streptococci produce a "de-chaining" enzyme, but this may be inhibited by the union of surface antigen with specific antibody (Ekstedt and Stollerman, 1960). Some streptococci, such as Streptococcus pyogenes and the pneumococcus, give rise to filamentous mutants that form extremely long tangled chains. All streptococci are non motile, except for some of the enterococci. Capsulation is not a regular character of streptococci, but some form a capsule of hyaluronic acid in the early phase of growth. Streptococci stain readily with the ordinary dyes and are almost always frankly gram positive (Mc Candless et al., 1968).

Various types of extracellular filaments have been described in streptococci. Those seen by Henriksen and Henrichsen (1975) in Streptoccus sanguis appear to be true fimbriae. Handley and Carter (1979) observed three morphological types of filamentous appendages in Streptococcus mitior. The whole cell surface of Streptococcus

pyogenes is covered by a mat of short, fine filaments (Swanson et al., 1969).

CULTURAL CHARACTERS :

Parker (1984) stated that all the streptococci are aerobic and facultative anaerobic, but some of the strictly anaerobic chain-forming cocci may prove to belong to the genus. The growth of some streptococci, including most strains of Streptococcus mutans, many strains of Streptococcus milleri is poor or absent unless in the presence of 5 percent CO_2. The optimum temperature for growth is 37°C (range from 22° - 42°C). Growth on ordinary nutrient media is generally poor, but is better on blood or serum-agar. The colonies are small, semitransparent, low convex and discrete (0.5 - 1 mm diameter) with matt surface when freshly isolated and after subculture, the surface may become smooth or glossy with change from S --> R form. Mucoid colonies may occur when strain is obviously capsulated. The growth is granular with powdery deposit in broth culture. There is poor growth and no liquifaction in gelatin.

CHANGES ON BLOOD AGAR MEDIA BY STREPTOCOCCI :

Marmorek (1895) observed the ability of some streptococci to lyse red blood corpuscles. Emphasis was laid on

the importance of studying deep colonies in pour plates rather than surface colonies on streak plates. However, most bacteriologists find that they are able to rely on the appearances around surface colonies.

The various types of change produced in blood agar by streptococci were described by Smith and Brown (1919):

Alpha (∝) haemolysis :

An indistinct zone of partially lysed red cells surrounds the colony, frequently accompanied by a greenish discoloration, which is best seen around sub-surface colonies.

Beta (β) haemolysis :

A sharply defined, clear, colorless or slightly pink zone surrounds the colony. This is best seen in deep colonies in a pour plate. Surface colonies, on the other hand may appear as alpha or non-haemolytic due to inactivation of one of the haemolysins, streptolysin O, which is oxygen-labile and streptolysin S, which is oxygen-stable haemolysin may be present in only small amounts in these strains showing poor surface haemolysis.

Gamma (γ) haemolysis :

Colonies show no apparent haemolysis or discolouration

in either surface or subsurface colonies.

Alpha-Prime (ά) haemolysis :

The zone of clearing around the zone of discoloured erythrocytes is wider and visible to the naked eye. It may be mistaken for β-haemolysis when examined only macroscopically.

The size and character of the haemolytic zone is influenced not only by the composition of the basal medium but also by the type of blood used. In the United States, 5 percent sheep blood agar was used because this inhibits the growth of haemolytic colonies of Haemophilus para-influenzae. Sheep blood agar has the disadvantage that it is somewhat less susceptible to lysis by some strepto-cocci of the pyogenic group than is horse blood agar when incubated in air. It is therefore necessary to ensure some sub-surface growth by making stabs into the medium in the area of the primary inoculum. Human blood is inferior to both horse and sheep blood (Parker, 1984).

CLASSIFICATION OF STREPTOCOCCI :

According to Brown (1919) streptococci are classified on the basis of their ability to haemolyse red blood cells, into beta-haemolytic (β-haemolysis), alpha-haemolytic

(∝-heamolysis, ∝́- "∝ prime- " haemolysis) and gamma-haemolytic (no haemolysis).

In 1919, Dochez et al. established the existence of types among the haemolytic streptococci pathogenic for man by means of agglutination tests.

Griffith (1926, 1928, 1934) distinguished 27 types by means of agglutination, but later it was found that four of them were types of group C or group G streptococci.

Lancefield (1928) found that hot-acid extracts of group A streptococci contained not only the group polysaccharide but also a type-specific protein that could be detected in precipitation tests with antiserum from which group antibody had been removed by absorption with a streptococcus of heterologous type. Lancefield used many of Griffith's type strains for the production of typing sera, and the type numbers of the Griffith and Lancefield systems were often the same.

The specific serologic types of group A streptococci are based not on carbohydrate, but on protein components, designated M antigens, which determine the production of protective antibodies specific for each type (Lancefield,

1928; Lancefield and Todd, 1928). However the organisms usually responsible for human infections are designated as group A on the basis of a serologically specific carbohydrate contained in the cell wall (Lancefield, 1933).

The most widely accepted general classification of streptococci is that of Sherman (1937), who recognized four main divisions :

(1) THE PYOGENIC STREPTOCOCCI, which are usually beta-haemolytic, have a polysaccharide group antigen, are not heat resistant and do not grow at extremes of temperature or pH, do not have strong reducing activities and usually hydrolyse arginine.

(2) THE ENTEROCOCCI, which are variable in haemolysis, have the group D antigen, are somewhat heat resistant, grow over a wide range of temperature and pH, are strongly reducing and hydrolyse arginine.

(3) THE LACTIC STREPTOCOCCI, which grow at a low temperature but are rather less tolerant of other extreme environmental conditions.

(4) THE VIRIDANS STREPTOCOCCI, which are seldom beta-haemolytic, grow at 45°C but do not hydrolyse arginine.