BETA - HAEMOLYTIC STREPTOCOCCI: GROUP SPECIFICITY IN RELATION TO CLINICAL MANIFESTATIONS OF INFECTION AND ANTIMICROBIAL SUSCEPTIBILITY OF THE VARIOUS STREPTOCOCCAL GROUPS AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA.

SUBMITTED AS A DISSERTATION FOR THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY) OF THE UNIVERSITY OF ZAMBIA.

1988

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DECLARATION

I hereby declare that this Dissertation is based on my own research and that it has not been previously submitted for a Degree of The University of Zambia or of any other University.

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APPROVAL

This Dissertation of Cyama Upul Perera is approved as having satisfied the examiners in accordance with the requirements for the Degree of Master of Science (Microbiology) of the University of Zambia.

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ABSTRACT

A total of 239 isolates of beta-haemolytic streptococci were grouped serologically using the "Streptex" rapid latex test kit (Wellcome Reagents Limited, England). The age, sex, type of the clinical specimen, and clinical diagnosis of the patients from whom the isolates were obtained were recorded, and the serologic groups of the beta-haemolytic streptococci in relation to clinical manifestations of infection determined. The antimicrobial susceptibility pattern of the isolates was determined using the comparative method of disc diffusion.

On serological grouping of 239 isolates of beta-haemolytic streptococci, all isolates were found to belong to one of groups A, B, C, D, F and G. The highest number 152 (64%) were group A streptococci. Clinical manifestations of infection due to group A streptococci included upper respiratory tract infections, pyoderma, osteomyelitis, infected wounds and abscesses. Thirty two of the isolates (13%) were group B streptococci. Clinical manifestations of infections due to group B streptococci were genito-urinary tract infections (mainly in females), and neonatal meningitis. Group B streptococci were also present as part of the normal flora of the genito-urinary tract in females. Groups C (14), D (17) and F (12) were found to cause infections of the genito-urinary tract and also formed part of the normal flora of the genito-urinary tract. Groups C, F and G were isolated from cases of upper respiratory tract infections. Groups D and G also contributed to infections of wounds.

On comparing the results of this study with those of studies in Britain and the United States, the percentage of streptococcal groups isolated from the various clinical sources and clinical conditions were similar except that in the U.T.H., there was a higher percentage of group A streptococci in infected wounds and group B streptococci were absent in the upper respiratory tract and in infected wounds.

A study of the antimicrobial susceptibility pattern of 217 beta-haemolytic streptococcal isolates, showed that streptococci of groups A, B, C, F and G were 100% sensitive to penicillin, whereas 25% of group D enterococci were resistant to penicillin but consistently sensitive to ampicillin. All streptococcal groups A, B, C, D, F and G showed high resistance to co-trimoxazole and tetracyclines. Group A showed 51% resistance to tetracyclines while groups B, C, D, F and G showed 90-93% resistance to tetracyclines. Groups F and G showed 30-50% resistance to co-trimoxazole while groups A, B, C and D showed 73-87% resistance to co-trimoxazole.

ACKNOWLEDGEMENTS

I wish to express my appreciation and thanks to Prof. Denys Morgan for his advice and guidance during the course of this work, to Prof. Nancy Pearsall for helpful criticism of the manuscript, Dr. Faika Salem and Dr. Nkandu Luo for aid and suggestions, Dr. B Nkowane and Mr. N. Ngandu for help with the tables, figures, and statistical analysis in this Dissertation, Dr. A. Ansary and his team in the Medical Illustration Unit for the preparation of all the plates and prints of tables and figures in the Dissertation, and Ms Jane Zuba for typing the manuscript.

I wish to thank my husband Mr. Nihal Perera for helping to procure the test kits for my practical work and for his continued support and encouragement throughout the course of this work.

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CHAPTER ONE

INTRODUCTION

The streptococci belong to the family streptococcaceae which consists of gram positive, cytochrome-negative, catalase- negative, coccoidal bacteria characteristically arranged in chains. Members of the genus Streptococcus are widely distributed in nature largely as parasites of man and animals. Some are members of normal human flora, while others are associated with important human diseases, due to infection by streptococci and also due to sensitization to them. They produce a variety of extracellular substances and enzymes. Their ability to haemolyze red blood cells to various degrees is one important basis of classification.

1.1 Historical Review:

The term "streptococcus" was first used by Billroth and Ehrlic (1877) to describe a chain-forming coccus that they observed in infected wounds. "Streptus" meaning pliant, and "coccus" meaning a grain or berry. Fehleison (1883) described a similar coccus as the causative organism of erysipelas. Rosenbach (1884) gave the name Streptococcus pyogenes to cocci that grew in chains, isolated from suppurative lesions in humans. The account by Pasteur et al, (1881) of a septicaemic infection produced in rabbits after inoculation with human saliva is probably the earliest reference to the pneumococcus followed later by the description of the organism in 1886 by Fraenkel. In 1887 Nocard and Mollereau reported the development of

mastitis in the cow and goat by inoculation into the udder of a streptococcus from the milk of a cow with mastitis. Schutz (1887) described streptococci isolated from the lesions of equine pneumonia and strangles (in sheep).

Marmorek (1895) first noted the ability of some streptococci to lyse erythrocytes and Schottmuller (1903) proposed that the ability to cause haemolysis in vitro should be used as means of classifying streptococci. The various types of changes produced in blood agar by streptococci were well described by Smith and Brown (1915).

Lancefield (1933) introduced the classification of beta-haemolytic streptococci into specific groups on a serological basis, according to the results of the anti-C precipitin test. The group antigens, which she extracted from streptococci using hot hydrochloric acid, gave a specific precipitate with anti-serum made by the injection of formalinized streptococci into rabbits. These findings became significant when it was found that the distribution of group antigens corresponded closely to the animal host and to the type of disease from which the streptococci had been isolated. Results of her study showed that groups A is composed mainly of strains of human origin, group B of strains causing mastitis in cows and from normal milk of cows, group C from various animals (guinea pigs, cattle, horses), group D from cheese, and the few strains in group E from milk. Classification by serological means was found to agree with groupings made on the basis of biochemical and cultural characteristics. The group specific

substance found in strains of human origin was identified as carbohydrate in nature. Lancefield (1940, 1943) also showed that two classes of protein antigens were responsible for type specificity in group A streptococcus.

1.2 Characteristics of the Genus Streptococcus:

(a) Morphology and Cultural Characters

Streptococci are spherical or oval in shape and are arranged in chains. The length of the chain varies depending partly on the medium in which it is grown, usually being greater in liquid media, but some streptococci characteristically form long chains whereas others are mainly diplococcal. All the streptococci are non-motile except for some members of group D.

Capsulation is not a regular feature of the beta-haemolytic streptocci, but the majority of groups A, B and C form a capsule of hyaluronic acid in the early phase of growth and the alpha-haemolytic pneumcocci have capsules composed of type-specific polysaccharides. Streptococci stain readily with conventional dyes and are gram-positive. They are not acid fast.

Growth of streptococci on ordinary nutrient media is generally poor. On media enriched with blood, serum or glucose growth is more rapid. In broth, strains that form long chains generally

give a granular growth with a powdery or flocular deposit and a clear supernatant fluid. Growth in liquid media is increased by the addition of glucose but the pH falls rapidly and growth stops. In buffered glucose media such as Todd-Hewitt broth (Todd and Hewitt 1932) or in media with a high glucose content to which alkali is added continuously during growth, a heavy yield of streptococci is obtained.

Most of the streptococci are aerobic and facultatively anaerobic, but the genus Peptostreptococcus is anaerobic. The growth of some streptococci including most strains of Streptococcus mutans, Streptococcus pneumoniae, and occasional strains of Streptococcus pyogenes is poor unless 5-15% of carbodioxide is added to the atmosphere.

The range of temperature over which growth occurs in vitro for most streptococci is 20-42°C, the optimum temperature being 37°C. However, the enterococci are able to grow in vitro at temperatures between 10-45°C. (Topley and Wilson 1975).

The most important cultural characteristic for the recognition of streptococci is their haemolytic action on the red blood cells (RBC's) in the medium. The size and the character of the haemolytic zone are influenced by the composition of the basal medium and also the type of blood used (Facklam and Carey 1985). For example, 5% horse blood agar is found to produce good haemolytic zones and 5% sheep blood agar has been found to be useful as it inhibits the growth of haemolytic colonies of Haemophilus haemolyticus. Sheep blood lacks sufficient amounts of pyridine nucleotides

(V factor) to support the growth of Haemophilus haemolyticus. However it has the disadvantage that it is less susceptible to lysis by some strains of pyogenic streptococci than horse blood agar when incubated in air. It is therefore necessary to ensure subsurface growth by making stabs into the medium in the area of the primary inoculum. Human blood is not as satisfactory as either horse blood or sheep blood in media for the identification of beta-haemolytic streptococci.

In beta-haemolysis the colony is surrounded by a sharply defined clear and colourless zone in which RBC'S are completely lysed. In alpha-haemolysis a greenish discolouration of the blood 1 - 3 mm in width surrounds the colony. The margin of the zone is indistinct and usually consists of a narrow zone of clearer haemolysis; the zone of discoloured erythrocytes is wider and visible to the naked eye. This may look like hazy clearing and may be mistaken for beta-haemolysis. The term "haemolytic" is commonly applied to streptococci only when they are beta-haemolytic. The term "non-haemolytic" or "gamma haemolytic" refers to streptococci that produce no haemolysis on blood agar.

The beta-haemolysis produced by pyogenic streptococci on blood agar plates incubated in air is usually due to the action of an oxygen-stable haemolysin "S". Improvement in haemolysis by streptococci of groups A, C, and G by anaerobic incubation of sheep or rabbit blood agar plates is due to the additional action of the oxygen - labile "O" haemolysin. This difference is less obvious on horse blood which contains O-antilysin.

Haemolytic enterococci give clear beta-haemolytic zones due to a substance that is also a bacteriocin. Streptococcus pneumoniae which show alpha-haemolysis both aerobically and anaerobically form an oxygen sensitive haemolysin. All strains of Streptococcus agalactiae whether haemolytic or not produce a substance which completes the lysis of sheep or ox red blood cells by staphylococcal betalysin. This forms the basis of the CAMP test (Christie et al, 1944, Facklam et al, 1979).

Typically after 18-24 hours of incubation on blood agar the colonies of group A streptococci are about 0.5mm in diameter, either transparent or translucent and domed. They have a smooth surface and an entire edge. They are surrounded by a well-defined zone of complete haemolysis, usually 2-4 times the diameter of the colony. The appearance of the colonies depend on the medium used and also the atmosphere of incubation. The appearance of beta-haemolytic group C or G streptococcal colonies differ sufficiently from group A colonies to be useful in identification. Group B colonies may be larger than group A colonies and are surrounded by a much smaller zone of complete haemolysis while some strains are non-haemolytic. Some strains of group B streptococci form dull brick-red pigmented colonies after anaerobic incubation. Group D colonies are larger than group A colonies (0.5 - 1.0mm). They are less opaque and some strains are glossy white, resembling staphyococcal colonies on blood agar. Group D streptococci may produce beta or alpha, or no haemolysis. The beta zones produced by the haemolytic action of group D streptococci are usually larger than the beta zones

produced by other streptococci. Group F streptococci generally form minute colonies. Zones of haemolysis similar in size to those produced by group A streptococci surround these minute colonies. However, this feature has no diagnostic value since some strains of group C and G and even A also form minute colonies.

The viridans streptococcal colonies vary in size from pin point (0.1mm) to equal to or larger than the size of group A colonies (0.5mm). They may be mucoidal and translucent or glossy and opaque. The colonies may be surrounded by a small zone of alpha-haemolysis or no haemolysis. Under anaerobic incubation, viridans streptococci are usually non-haemolytic. Pneumococcal colonies are round with entire edges, mucoid and about one mm in diameter. When the culture has been incubated in an atmosphere of 5-10% carbon dioxide, the colonies are surrounded by a fairly large zone of alpha-haemolysis. Young pneumococcal colonies are raised (like alpha-haemolytic streptococci) but in old culture the colonies become flattened and the central part may be depressed.

b. Biochemical Reactions.

Tests for fermentation of carbohydrates may be carried out in meat extract broth or in serum sugars. All the streptococci ferment glucose and maltose and most ferment lactose and sucrose. Streptococci give a negative catalase reaction except a few members of group D (Jones et al, 1964). These may, under certain conditions, produce a little gas from hydrogen peroxide. This is not due to a catalase reaction

because the organisms do not possess a cytochrome system and therefore give a negative reaction in the benzidine test (Deibel and Evans, 1960, Facklam and Carey, 1985).

Nitrate reduction occurs in only a few of group D streptococci, Streptococcus milleri, and a few other non-haemolytic streptococci (Colman 1970).

A combined test for bile tolerance and aesculin hydrolysis is often used in the identification of group D streptococci (Rochaix, 1924). The 6.5% salt-tolerance test differentiates enterococci from non-enterococcal group D streptococci (Facklam et al, 1979). Liquifaction of gelatine occurs only in the liquifaciens variety of Streptococcus faecalis but several other streptococci have some proteolytic activity. On agar plates containing various proteins and observed for clearing around individual colonies, about half the Streptococcus pyogenes strains hydrolize casein. Group D streptococci hydrolize albumin and globulin as well. Groups B and C are inactive in these respects (Sherwood et al, 1954).

All strains of Streptococcus pyogenes and the enterococci hydrolize a substrate known as PYR (L-Pyrrolidonyl-beta-naphthalamide). Other streptococci are unable to hydrolize PYR. An agar medium and a broth medium containing PYR have been described (Bosley et al, 1983). When the PYR reagent (N.N-dimethyl aminocinnamaldeyde) is added to the surface growth of an organism on PYR agar (incubated over-night at 35°C, at normal atmosphere) a red colour develops if Streptococcus pyogenes or enterococci are present. All other streptococci show a yellow colour or no colour change within one minute.

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Streptococcus faecalis is able to reduce tellurite and therefore grow as black colonies on tellurite agar.

1.3 Toxins and Enzymes of the Pyogenic Streptococci:

More than twenty extracellular toxins are produced by group A streptococci and also by some of the other pyogenic streptococci, including the following:

Streptokinase (fibrinolysin) is produced by many strains of beta-haemolytic streptococci. Filtrates of some streptococci were found to cause the dissolution of a fibrin clot by means of streptokinase (Tillett and Gardner 1933). The enzyme transforms the plasminogen of human serum into plasmin, an active proteolytic enzyme, that digests fibrin and other proteins (Christenson 1945). It is formed by most group A streptococci, in amounts varying in different strains (Report 1947), and also by some strains of groups C and G. Group A streptococci produce at least two immunologically different streptokinases (Dillon and Wannamaker 1965). A skin test with streptokinase-streptodornase is positive in adolescents and adults with normal cell mediated immunity. Streptokinase is given intravenously for treatment of pulmonary emboli and venous thromboses.

Nucleases - All strains of Streptococcus pyogenes form both deoxyribonuclease (streptodornase), and ribonuclease (McCarty 1948, Tillett et al 1949). Dnase is also produced by groups B,

C,G, and L streptococci (Deibel 1963) but usually in smaller amounts. StreptoDNase depolymerizes DNA. The viscosity of purulent exudates is due to the deoxyribonucleo-protein. Mixtures of streptoDNase and streptokinase are used in "enzymatic debridement". They help to liquify exudates, facilitate removal of pus and necrotic tissue, to enable better access to antimicrobial drugs. An antibody to Dnase develops after streptococcal infections, especially in pyoderma.

Hyaluronidase - Many streptococci, including those strains capable of producing hyaluronic acid capsules, form hyaluronidase but capsulation and hyaluronidase production seldom co-exist. Certain M types of Streptococcus pyogenes in which capsulation is rarely seen, form large amounts of hyaluronidase (Crowley 1944). The formation of hyaluronidase occurs also in groups C and B, in the large colony form of group G, in groups R, S, T, in pneumococci, and in some strains of non-haemolytic streptococci. Hyaluronidase is antigenic, but the enzyme produced by group A strains is immunologically distinct from that of streptococci of groups C and G. following infection with hyaluronidase-producing organisms, specific antibodies are found in the serum. Purified streptococcal hyaluronidase has been used in medical therapy to facilitate the spreading and absorption of fluids injected into tissues.

Erythrogenic toxin was first detected when filtrates of

cultures of group A streptococci were injected intradermally into healthy people (Dick and Dick 1924a, b) resulting in an erythematous reaction. Erythrogenic toxin is soluble and is destroyed by boiling for one hour. It produces the rash that occurs in scarlet fever (scarlatina) which is caused only by streptococcal strains producing this toxin. Non-toxigenic streptococcal strains are converted to erythrogenic toxin producing strains by lysogenic conversion. Erythrogenic toxin is antigenic, giving rise to the formation of specific antitoxin that neutralizes the toxin. Persons with antitoxin (following infection with erythrogenic toxin producing strains) are immune to the scarlet fever rash but susceptible to streptococcal infection. Susceptibility to erythrogenic toxin can be demonstrated by the Dick test, when 0.1 ml of standardized, diluted erythrogenic toxin (broth culture filtrate) is injected intradermally, similar material, heat inactivated is used as a control. If there is no significant concentration of antitoxin in the blood, a positive Dick test is seen, consisting of the appearance of erythema and oedema measuring over 10mm in diameter. This test was used in the past to test for susceptibility to scarlet fever.

Diphosphopyridine Nucleotidase-(DPNase) or Nicotinamide-adenine Dinucleotidase (NADase): This enzyme is produced by some strains of group A streptococci and may be related to the ability of the organism to kill leucocytes (Bernheimer et al, 1957): Antibodies to this enzyme appears in man following streptococcal infection.

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Proteinases - These enzymes are formed by most strains of Streptococcus pyogenes under some conditions, and attacks proteins, including casein, fibrin and gelatin, although they are not responsible for fibrinolysis or gelatin liquifaction (Elliott 1945, Deibel 1963). They destroy several proteins formed by streptococci, including streptolysin 0, streptokinase, hyaluronidase and M antigen.

Haemolysins - Many haemolytic streptococci form two distinct haemolysins (Todd 1932, 1938a). One is haemolytic in the reduced form and the other is oxygen - stable and apparently soluble in serum. Todd (1938a) named them streptolysin O (oxygen labile) and Streptolysin S (serum soluble). Streptolysin 0 is formed by most group A streptococci and by many strains of groups C and G but not by members of other groups. It is haemolytic and lethal only in the reduced form but is antigenic in both the oxidized and reduced forms. Antibody to it can be detected by inhibition of haemolysis by reduced toxin (Todd 1932) and this is the basis of the AntiStreptolysin 0 Test (ASOT). The O-lysins of groups A, C and G streptococci are immunologically identical (Todd 1939). The lysin is in the oxidized form when in contact with air, and is rapidly reduced by sodium hydrosulphite or cysteine. It is stable when frozen, but rapidly and irreversibly inactivated at room temperature probably by the action of streptococcal protease. Streptolysin 0 has a cytotoxic action on leucocytes (Todd 1942) and lyses platelets (Bernheimer and Schwartz 1965a). Streptolysin S is responsible for the beta-haemolysis around

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colonies of many streptococci on aerobically incubated blood agar plates. Oxygen - stable streptolysin of the S type are formed by most streptococci of groups A, C and G and also by members of a number of other groups. Todd (1928 b) showed that this haemolysin was not inactivated by oxygen. Streptolysin S is sensitive to heat and acid and can be preserved by storage at very low temperatures. It lyses erythrocytes more slowly than does streptolysin 0. It is not antigenic. However sera of human and animals frequently contain a non-specific inhibitor of streptolysin S.

Serum Opacity Factor - Keogh and Simmons (1940) showed that certain serotypes of group A streptococci gave rise to opacity in serum broth. According to Krumwiede (1954) this is due to an enzyme associated with the cell-wall that liberates lipid from serum alpha-lipoprotein. Gooder (1961) observed that certain M types for which satisfactory M antisera were difficult to prepare and strains not identifiable with existing M antisera often gave a positive result. Top and Wannamaker (1968 a, b) showed that the opacity reaction was specifically neutralized by streptococcal antisera and that the specificity of this neutralization coincided with the typing pattern of the streptococci. The neutralization test is therefore of value in streptococcal typing (Maxted et al 1973 b) and can be used to identify members of some M types for which precipitating antisera are not available. The serum opacity factor probably forms part of the M-protein complex.

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1.4 Antigenic structure and Serological Grouping of Beta-haemolytic Streptococci:

The cell wall of the streptococci is composed mainly of the mucopeptide, peptidoglycan. In general mucopeptides of all streptococci are similar in composition, but there are some differences between the streptococci in the amino-acid composition of the interpeptide bridges (Gooder 1970). Many streptococci have cell wall polysaccharide antigens known as C substances or C carbohydrates that are attached to the mucopeptide and form the criteria of the Lancefield grouping system.

1.4.a Group Antigens:

The chemical basis of the antigen specificity of some of the group carbohydrates is known. The serological specificity of the C carbohydrates is determined by a glycosamide. The Group A, C-carbohydrate is composed of N-acetyl glucosamine and rhamnose and its antigenic determinant is the terminal glycosamine, N-acetyl glucosamine attached through a beta linkage to a series of rhamnose molecules (Schmidt 1952, Krause 1963). The group C, C-carbohydrate has a terminal glycosamine N-acetyl galactosamine attached to the rhamnose. For group F the glycosamide is glucopyranosyl N-actyl galactosamine. The determinant of the group L carbohydrate is also N-acetyl glucosamine attached to a rhamnose oligosaccharide which accounts for partial cross reaction between groups A and L, but the linkage

of the terminal sugar to the rhamnose sugar appears to be different in the two groups (Karakawa et al, 1971).

Streptococci may also have teichoic acid antigens which include the group antigen of group D streptococci (Wicken et al, 1963) and the group antigen of group N streptococci.

1.4.b Type Antigens:

Streptococci of some Lancefield groups also possess type specific protein antigens. In group A streptococci the type antigens are M, T, and R antigens. The M antigens are resistant to heat and acid, soluble in alcohol and are destroyed by trypsin. They are associated with virulence of group A streptococci and occur mainly in organisms producing mucoid colonies. Repeated passage on artificial media may result in loss of M protein production which may be restored by rapidly repeated animal passage. M protein interferes with phagocytosis of virulent streptococci. M antigen can also aid the attachment of streptococci to human epithelial cells (Ellen and Gibbons 1972). By electron microscopy the M antigen appears as a series of hair like fimbriae on the surface of the organism (Swanson et al , 1969). There are more than 60 M types in group A.

The T antigens are less resistant to heat and acid, insoluble in alcohol and resistant to trypsin. They do not contribute to virulence. Williams and Maxted (1955) suggested a combined typing system in which T antigens were first identified by the agglutination of trypsinized

suspensions and precipitation tests were then performed with M antisera for the types known to carry the T antigens that were detectable.

R antigen is a trypsin-resistant, pepsin sensitive protein, which is also not related to virulence. It occurs in some types of Streptococcus pyogenes and also in streptococci of groups B, C and G (Maxted 1949).

Procedures for the Serological Grouping of Beta-haemolytic Streptococci:

The carbohydrate antigens or group specific antigens of beta-haemolytic streptococci can be demonstrated by a variety of techniques. Group specific precipitating, agglutination, and fluorescent-antibody (FA) sera, which can be used with extracts, cell suspensions and spent broth media are commercially available. The use of groups A, B, C, D, F, and G antigens is recommended for the identification of beta-haemolytic streptococci (Facklam and Carey 1985).

Procedures available for the Extraction of Streptococcal Group Antigens:

These procedures include the Lancefield hot-hydrochloric acid, hot formamide, autoclave, nitrous acid and Streptomyces albus - lysozyme extraction procedures. Each extraction method has certain advantages and disadvantages. The Lance-field hot acid technique (Lancefield 1933) is the standard method for streptococcal grouping. It is the only technique available for extracting the protein type specific antigens

(of groups A and B) as well as the carbohydrate (group A, B, C, F and G) and teichoic acid (groups D and N) antigens. However it is somewhat more complicated and time consuming than are other methods.

The hot formamide technique (Fuller 1938) is also relatively complex and time consuming. Like the Lancefield method, it can be used to extract all group antigens. However this technique cannot be used for typing groups A and B streptococci because it destroys their protein type specific antigens. The autoclave technique is relatively simple and can be used for grouping. When used with the Centres for Disease Control (CDC) antisera for the identification of groups A, B, C, D, F and G streptococci, the hot acid, formamide, and autoclave techniques are equally effective (Facklam and Carey 1985). The Streptomyces albus enzyme extraction technique (Maxted 1984) although it is easy to perform, can be used to group only A, B, C, F and G streptococci. The group D antigen is not extracted satisfactorily by the Streptomyces albus enzyme.

The pronase B enzyme extraction technique is also easy to perform but does not extract group D or F antigen as satisfactorily as does the hot-acid, formamide, autoclave or Streptomyces albus-lysozyme technique. The Streptomyces albus-lysozyme enzyme technique however, extracts the group antigens of groups A, B, C, D, F and G streptococci. The reagents for this technique are more expensive than are those for any of the other techniques.

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Methods for Serologic Grouping

Capillary Precipitin Test for Detecting Streptococcal Group Antigens - This test involves the reaction of antigen (streptococcal extraction with antibody (specific antisera) to form a white precipitate, and depends largely on the potency of the antisera used. Control streptococcal strains should be used to test each new lot of commercial antiserum. The choice of extraction technique is influenced by the cost, complexity of procedure, time requirements and efficiency of extraction. The Lancefield precipitin technieque originally involved layerin the extract under the antiserum, a method that was satisfactory for hyperimmune antisera. However, since commercial antisera are usually not as potent as those specified for the Lancefield procedure, the extract is layered over the antiserum.

Identification of Groups A and B Lancefield's Group Antigens by Immunofluorescent Staining (IF)

Immunofluorescent staining has some advantages over conventional identification techniques. These advantages include rapidity of test (several hours rather than several days), sensitivity (detection of small numbers of streptococci in mixed culture) and detection of non-haemolytic group B streptococ (often missed with conventional techniques) (Moody et al 1963). The Immunofluorescent reagent, or conjugate is composed of appropriate dilutions of anti-group carbohydrate immunoglobulin attached to florescein isothiocyanate. Non-specific staining of Staphlococcus aureus or members of other streptococcal groups which cannot be distinguished from group A streptococci

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morphologically, can be avoided by the addition to the conjugate of unlabelled non-immune serum to cause a "blocking" of non-specific staining and prevent the non-specific binding of the IF reagent. The group B streptococcal IF procedure is less widely used because the specific reagent is not commercially available (Romero et al, 1974).

Slide Agglutination Tests

Two slide agglutination tests have been described in which carrier particles are used for the group specific antisera. The reagents for these tests are available commercially. In the Co-Agglutination test (COA) specially prepared protein A-rich staphylococcal cells conjugated to group-specific streptococcal antisera are used. In the latex agglutination test (LA), latex particles conjugated to group specific antisera are used. The carrier particles in these tests, (staphylococcal cells and latex particles) are so large that agglutination can be seen without magnification.

The more distinctive feature of these reagents is that they shorten the identification time over that of conventional extraction and capillary precipitation tests and are equally accurate. These reagents can be used to detect streptococci from spent broth medium. For accurate results the manufacturers' instructions should be followed strictly.

The COA and LA reagents have been used in combination with the micronitrous acid extraction procedure to identify group A streptococci directly from throat swabs (Slifkin and Gil, 1982, 1984, Miller et al 1984). The COA and LA reagents have

also been used to identify beta-haemolytic colonies from primary throat culture blood agar plates (Castle et al, 1982). In some cases single colonies have been used for grouping (Slifkin and Interval 1980). When these direct testing procedures fail to identify the beta-haemolytic colonies, 4 hours and overnight broth cultures and culture supernatants have been used as antigens. Most of these reagents are of high quality and the choice is one of personal preference (Slifkin and Pouchet-Melvin, 1980, Burdash et al, 1981).

As potential errors may arise when group D streptococci are identified with the COA and LA tests, it is recommended that two physiological tests be carried out to aid in the identification of these streptococci. Also, since enterococcal and non-enterococcal group D streptococci differ in their susceptibility to antibiotics it is recommended that they be differentiated by carrying out the Bile-Aesculin test (positive for all group D streptococci) and the salt tolerance test (positive for enterococci only) (Facklam et al, 1974).

A recent report on comparison of "Meritec - Strep" with "Streptex" for direct colony grouping of beta-haemolytic streptococci from primary isolation and subculture plates (Hamilton 1988) showed that "Meritec - Strep" provided faster results than "Streptex" by eliminating the time and manipulation of antigen extraction. However, group D is not identified by "Meritec - Strep".

Several miniaturized and rapid identification systems for the streptococci are now available commercially. The

API 20S system (Analytab Products, Ayerst Laboratories, Plainview, N.J.) and the API Rapid strep system (DMS Laboratories, Inc., Flemington, N.J.) products are similar. They are both essentially qualitative micromethods, in which chromogenic substrates are used for the identification of clinically isolated streptococci. Tests are based on microbial degradation of specific substrates by enzymes, fermentation of sugars by microbes and detection by indicator systems, or addition of reagents. In both the API 20S system and the API Rapid Strep system, there are twenty tests and although the tests in the two systems are not exactly the same, they are read in a similar fashion. Although by the use of these two test systems, the beta-haemolytic streptococci are also identified (Colman and Ball, 1984, Appelbaum et al 1984, Appelbaum et al 1986, Pontrel and Ryniewicz 1984), their advantage is questionable since convenient rapid methods already exist for the grouping of these strains.

CHAPTER TWO

PATHOGENESIS AND CLINICAL SIGNIFICANCE OF THE VARIOUS SEROLOGICAL GROUPS OF BETA-HAEMOLYTIC STREPTOCOCCI

2.1 Host-parasite Interaction

A variety of distinct disease processes are associated with streptococcal infections. The pathologic picture produced depends on the biologic properties of the infecting organisms, the nature of the host response, and portal of entry of the infection (Jawetz et al, 1987). The streptococci are part of the endogenous microbial flora of the nasopharynx, skin, vagina and rectum. Their numbers are usually limited by competition from the microbial ecosystems at these sites. They may also be secondary exogenous invaders following viral disease or disturbances in the normal bacterial flora. In the normal host, non-specific defence mechanisms prevent organisms from penetrating beyond the superficial epithelium of the upper respiratory tract. Also if surface epithelial cells have IgA antibodies on their surface, attachment of the micro-organism may be prevented. Usually micro-organisms are not capable of penetrating intact skin, but many can enter sweat glands, sebaceous glands, and hair follicles and establish themselves at these sites. Sweat and sebaceous secretions have anti-microbial properties because of their acid pH and the presence of substances such as fatty acids. Lysozyme, an enzyme that lyses bacterial cell walls, is also

present in the skin. However, the cell walls of the group A streptococci are resistant to attack by lysozyme. In the adult vagina, an acid pH is maintained by lactobacilli which are present in the normal vagina and this interferes with the establishment of some micro-organisms. The host phagocytic system is a second line of defence against pathogens. Opsonization of organisms can be facilitated by activation of the classical or alternate complement pathways or by specific immuno-globulin binding. IgA in the respiratory secretions and serum IgG are the important classes of protective anti-body to streptococci. Protective immunity results from the development of type-specific antibody to the M protein. This antibody, which is produced following respiratory and skin infections, is persistant. Antibody to the erythrogenic toxin is also long lasting (Patterson 1982).

2.2 Group A Streptococci - Streptococcus pyogenes

Streptococcus pyogenes is found almost exclusively in man. It is a known cause of streptococcal pharyngitis with occasional complications of peritonsillar or retropharyngeal abscess, otitis media, suppurative cervical adenitis, and acute sinusitis. Rarely this organism causes meningitis, pneumonia or bacteraemia with metastatic infection in distant organs. Strepto-coccus pyogenes is also a cause of impetigo, erysipelas, puerperal sepsis, wound and burn infections,

and scarlet fever (Ryan, 1984).

Following an acute infection with group A streptococci after a latent period of 1 - 4 weeks acute glomerulonephritis or rheumatic fever occasionally develops. The latent period suggests that these post-streptococcal diseases are not attributed to the direct effect of disseminated bacteria but are the result of an immunological mechanism caused by similarities between streptococcal and human tissue antigens (Unny and Middlebrooks, 1983). Nephritis is more commonly preceded by infection of the skin, rheumatic fever by infection of the respiratory tract.

2.2.1 Diseases due to invasion of tissues by Streptococcus pyogenes

The main clinical picture depends on the site of entry of the infection. In each case however there is a rapidly spreading cellulitis that involves the tissues and extends along the lymphatics. The infection spreads rapidly to the bloodstream, leading to bacteraemia with consequent septicaemia, meningitis and rarely, metastatic infection in organs, e.g acute endocarditis.

Examples of invasive infections due to Streptococcus pyogenes include:

Erysipelas

When the site of entry of infection is the skin, erysipelas results. This condition is usually brought under complete control within 48 hours by treatment with penicillin.

Puerperal fever

If the beta-haemolytic streptococci enter the uterus after delivery puerperal fever may develop. Formerly, prior to the introduction of proper methods of sterilization, disinfection, and antiseptic procedures, puerperal sepsis due to Streptococcus pyogenes was very common. Today puerperal sepsis in rare but an occasional case due to Streptococcus pyogenes is reported. Nieburg et al, (1975) reported a case of puerperal and neonatal sepsis in mother and twins, due to Streptococcus pyogenes, where the probable source of infection was the mother herself.

Neonatal sepsis and meningitis

Before the 1940's prior to the introduction of antibiotics, neonatal sepsis was commonly caused by group A streptococci. Today apart from sporadic cases and occasional outbreaks in nurseries or in the community, group A streptococci are an uncommon cause of septicemia or meningitis and have been replaced by group B and other streptococcal groups (Nyhan and Fonsek 1958, Parker 1977, Coulter et al, 1984). The rarity of group A streptococcal infections in the neonate now, may be partly due to placental transfer of group A type-specific antibody conferring passively acquired immunity to the neonate (Zimmerman and Hill 1969).

Group A beta-haemolytic streptococcus is an uncommon cause of bacterial meningitis but it may occur in all age groups with about half the cases occuring during the

neonatal period (Murphy 1983). Streptococcal meningitis is usually associated with otitis media, sinusitis, infection of the respiratory tract or head injury (Applebaum and Abler, 1958, Breese, 1960, Murphy 1983). Other associated findings are congenital dermal sinus of the nose (Shadravan et al, 1976), necrotizing fasciitis (Nutman et al, 1979), erysipelas (Dillon 1966), and pyogenic gingival cyst (Patamasueon et al, 1980). Puerperal transmission is suggested by positive maternal cervical culture in the case of an infant who developed streptococcal meningitis (Nutman et al, 1979).

Wound and burn infections

Although less common than in the past, group A streptococcal infections of wounds and burns can develop and spread rapidly to adjacent tissues, with the risk of bacteremia and sepsis. Surgical wound infection varies from trivial local inflammation to acute fatal generalised infection. If the organisms are introduced into deep tissues e.g. peritoneal cavity at the time of operation, a severe infection, sometimes without fever or localising signs may develop within a few hours. However, symptomless colonization of wounds is also found to occur, especially if the organism is introduced sometime after infliction of the wound. Burn infections may occasionally cause septicemia and are associated with failure of skin graft (Jackson et al, 1951). Streptococcus pyogenes may also cause a necrotizing type of local infection, described by Meleney (1924) as streptococcal gangrene. It may or

may not be associated with trauma. After an initial cellulitis dusky areas appear, sometime with haemorrhagic bullae which ulcerate, exposing an extensive area of gangrene beneath. Necrosis may extend widely along tissue planes and metastatic abscesses may occur. The fatality rate is high even with penicillin treatment (Paine et al, 1963; Beathard and Guckian, 1967; Buchanan and Haserick, 1970). Acute necrotising fasciitis due to streptococcal infection in a new born infant, reported by Nutman et al (1979) probably represents a similar lesion. Acute cellulitis of the perianal region (Amren et al 1966) and acute purulent vaginitis have been reported in children and appear to be associated with dissemination of the bacteria from streptococcal infection of the respiratory tract in the patient or a family contact. Several cases of perianal cellulitis have been reported to occur in the same family (Hirschfield, 1970).

2.2.2 Diseases due to local infection with Group A Streptococci and their products

Streptococcal pharyngitis

Group A Streptococcal pharyngitis is usually self limiting. Typically the fever is gone by the third to the fifth day and other manifestations subside within a week. Occasionally the infection may spread beyond the pharynx to produce peritonsillar or retropharyngeal abscess, otitis media, suppurative cervical adenitis, and acute sinusitis. Rarely, more extensive spread may

occur producing meningitis, pneumonia or bacteraemia with metastatic infection in distant organs.

Following an attack of acute streptococcal pharyngitis, after a latent period of 1 - 4 weeks, acute rheumatic fever or glomerulonephritis occasionaly develops.

Pathogenesis of pharyngeal Infection

Initially in group A streptococcal infection of individuals with intact epithelia attachment takes place to host cells, usually of the pharynx. This attachment is accomplished by the fimbriae, which contain both M-protein and Lipoteichoic acid (LTA). Although there is no attachment of M-protein negative mutants, currect evidence favours LTA as the primary mediator of this process (Swanson et al, 1969, Ellen et al, 1972, Beachy and Ofek, 1976). Upon attachment, local epithelial damage occurs. In many Streptococcus pyogenes infections however, invasion occurs through epithelial breaks. The hyaluronic acid capsule may resist phagocytosis. Strains containing M-protein can resist phagocytosis by polymorphonuclear neutrophils and grow in tissue fluids and serum. Strains lacking M-protein do not have these features and are avirulent. Antibody to M-protein provides a type-specific immunity that last for many years, because opsonised Streptococcus pyogenes are killed rapidly when ingested by phagocytic cells. The exact role of other factors in the pathogenesis of infections is uncertain, but the combined effect of streptokinase, DNA ase, and hyaluronidase may prevent effective localisation of the infection, while the streptolysins produce tissue injury (Ginsburg 1972). Antibodies against all of these components are formed in the course of a streptococcal infection, but

immunity is M-type specific (Ryan 1984). A recent study has revealed that certain group A streptococci have surface receptors that bind selectively to the fibrinolytic enzyme plasmin. Bacterium bound plasmin is found to retain its ability to cleave synthetic substrates and to hydrolize a fibrin clot. These findings may contribute to further explanation of the pathogenesis of group A beta-haemolytic streptococci (Lottenburg et al, 1978).

Scarlet fever

Infection with strains that produce the erythrogenic toxin may produce the signs of scarlet fever together with streptococcal pharyngitis or any other infection due to Streptococcus pyogenes in patients with no circulating antibody to the toxin. For unknown reason scarlet fever is less severe than previously (Ryan 1984).

Streptococcal pyoderma

Local infection of superficial layers of skin by group A streptococci is known as impetigo. It is often caused by "nephritogenic" strains. The serotypes of group A streptococci responsible for impetigo are different from those that cause tonsillitis (Parker et al, 1955, Top et al, 1967). In general these "skin" types do not appear to be responsible for clinical infections of the respiratory tract but they may be isolated from the throat in areas where impetigo is common (Dill on et al, 1967). Strains of Streptococcus pyogenes

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causing uncomplicated impetigo were found to be different from those causing impetigo followed by acute glomerulonephritis (Dill on et al, 1967).

2.2.3 Delayed non-suppurative sequelae of infections due to Streptococcus pyogenes

2.2.3.a Rheumatic fever

Attacks of rheumatic fever typically begin three weeks (range 1 - 5 weeks) after a group A streptococcal pharyngitis, and in the absence of anti-inflammatory treatment, last 2-3 months. The age of those affected is normally 5-15 years. The most serious manifestation of rheumatic fever is carditis. Acute rheumatic fever also has a marked tendency for recurrences with subsequent streptococcal infections. Repeated attacks lead to progressive damage to the endocardium and heart valves with scarring and valvular stenosis or incompetence (rheumatic heart disease). Rheumatic fever does not follow non-respiratory infections or infections with streptococci other than Streptococcus pyogenes. Although some strains may be more likely to cause initial attacks, the events involved in recurrent rheumatic fever and the subsequent cardiac damage indicate that pathogenesis of the disease is group rather than type specific, because recurrences can be caused by many M-protein types. Prophylactic penicillin therapy is used to prevent recurrent rheumatic fever by preventing subsequent streptococcal

infection (Stollerman and Rusoff, 1952; Chamovitz et al, 1954; Stollerman et al, 1955).

Aetiology of Rheumatic Fever

Several theories have been advanced to explain the role of group A streptococci in rheumatic fever. Description of rheumatic lesions began to appear in the early 18th century (Lancici, 1707). Several other workers described the manifestations of rheumatic fever and its relation to streptococcal infections in the 18th and 19th centuries (Pulteney, 1761; Bouilland, 1837; Aschoff, 1904; Geipel, 1905). Historically the parallel between the epidemiology of rheumatic fever and acute glomerulonephritis, and of streptococcal infections formed one of the strongest lines of evidence which established them as a sequelae to streptococcal infection.

Evidence for the group A streptococcal aetiology of rheumatic fever

Four major lines of indirect evidence, i.e. clinical, epidemiological, immunological, and prophylactic, established the group A streptococcus as the sole infective agent causing initial and recurrent attacks of rheumatic fever (Lancefield, 1941, Stollerman and Rusoff, 1952, Alban et al 1964, Unny and Middlebrooks, 1983).

Various streptococcal antigens have been studied to find out which ones played a major part in the pathogenesis of rheumatic fever. The most important components were found to be somatic antigens such as M-protein, complexes of mucopeptide and polysaccharide, persisting degraded streptococci, and

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streptococcal antigens cross-reactive with tissues.

Role of M-protein

M-protein is one of the most important factors affecting the virulence of group A streptococci. It has been identified as a part of the cell wall structure.

There is evidence that many human sera contain antibodies to a streptococcal antigen that is closely associated with M-protein but is not type-specific, i.e. the M-associated protein (MAP) (Widdowson et al, 1971).

A variable proportion of normal human sera contain antibodies to MAP as do sera from most patients with recent streptococcal infection, acute rheumatic fever (ARF), or post streptococcal glomerulonephritis. Highest titres of antibodies to MAP have been found in patients with ARF (Beachy et al, 1973 Epidemiological and bacteriological evidence suggests that group A streptococci may vary in their potential to cause rheumatic fever. Strains may be "rheumatic" or "non-rheumatic".

In studies of outbreaks of streptococcal pharyngitis it has been found that certain serotypes, such as type 5, frequently give rise to ARF whereas others, such as type 4 and most other opacity -factor positive sero-types, generally do not do so (Rothbard et al, 1948).

It is believed that the M-protein of group A streptococci functions as a major virulence factor due to its anti-phagocytic property. Peterson et al, (1979) provided evidence to support the concept that in the absence of type-specific opsonic antibodies (non-immune serum) M-protein inhibits

phagocytosis. This is achieved by interference with the process of bacterial opsonization, probably via the alternate complement pathway. Bisno (1979) carried out studies which suggested that the M-protein retards interaction of alternate pathway components with structures present on the streptococcal cell surface.

Role of complexes of mucopeptide and polysaccharide

Mucopeptide - polysaccharide complexes of group A, C and G streptococci induced myocardial and valvular lesions in rabbits and mice when inoculated into these animals by the intravenous and intraperitoneal routes (Cromartie and Craddock, 1966). The lesions obtained in mice were claimed to be similar to the cardiac lesions characteristic of rheumatic carditis in humans. Localisation of the cell wall components was also found to occur in the reticuloendothelial system.

Persisting, degraded streptococci

There appears to be a direct relationship between the persistence of streptococcal components in macrophages and the maintenance of a chronic inflammatory process in the tissue of experimental animals (Ginsburg, 1972). However, there is not much evidence of prolonged persistence of streptococci in human tissue.

Streptococcal antigens cross-reactive with tissue

There is evidence to support the existence of cross-reacting antigenic structures common to both streptococcal

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and mammalian tissue (Kaplan and Frengley, 1969). Such cross-reactions have been demonstrated between the streptococcal wall and protoplast membrane and the heart and skeletal muscles, smooth muscles of blood vessels, kidney basement membrane, mucopolysaccharide of heart valves, connective tissue elements, and with some transplantation antigens. Studies have shown the persistence of elevated levels of antibody to the streptococcal group A carbohydrate (anti-A antibody) in patients with chronic rheumatic valvular disease (Dudding and Ayoub, 1968). The C polysaccharide of streptococci was found to be cross-reactive with valvular glycoprotein (Goldstein and Trung, 1968). Antibodies which bind to the sarcolemma and subsarcolemmal sarco plasm of cardiac and other striated muscle, as well as to the smooth muscles of vessel walls and endocardium, have been found in rabbit antisera to group A streptococci (Zabriskie et al, 1966). Suggested streptococcal cross-reactive antigenic components include the cell-wall (closely associated with type-specific M-protein) or protoplast membrane or both.

Pathogenesis of rheumatic fever

Stollerman (1975) has listed a few absolute requirements for the development of rheumatic fever:

I The involvement of group A streptococci

II A streptococcal antibody response, indicating that actual recent infection has occured.

III Persistence of the streptococci in the pharynx for a sufficient length of time.

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IV Location of the infection in the upper respiratory tract.

There is considerable evidence for the involvement of immunological mechanisms in the pathogenesis of rheumatic fever. The average interval between streptococcal sore throat and onset of rheumatic fever is about 3 weeks, which fits well with the time required for the development of an antibody response. On testing serum samples of patients for antibody response to several extracellular products of group A streptococci from the start of infection and at periodic intervals after that throughout the course of the disease, it was found that the antibody titres rose sharply at the time of first appearance of symptoms of rheumatic fever and reached their peak 1 or 2 weeks afterwards (Wannamaker et al, 1951).

Also in support of the role of immunological mechanisms in rheumatic fever is the finding that the mean antibody response to a number of different streptococcal antigens is higher in patients who develop rheumatic fever than in patients in the same epidemic who have uncomplicated streptococcal infection (Wannamaker et al, 1951).

It was shown that effective penicillin therapy of established streptococcal sore throat would prevent rheumatic fever. Streptococci are eliminated by effective chemotherapy which also usually suppresses the antibody response to streptococcal antigens. The risk of rheumatic fever in a susceptible patient is reduced when the antigenic stimulus is removed (Wannamaker et al, 1951). Less direct evidence to support immunological mechanisms in the pathogenesis of

rheumatic fever has been obtained from certain analogies.

Allergic theories

Rheumatic fever is similar to serum sickness in that both conditions are produced by circulating immune complexes and both produce arthritis. However rheumatic fever differs from serum sickness in many aspects. Unlike rheumatic fever serum sickness is associated with angioneurotic oedema and acute glomerulonephritis. The latent period of ARF does not decrease with repeated attacks as would be expected with repeated bouts of serum sickness (Stollerman, 1975). Serum sickness can result from immune complex disease due to many causes, but acute rheumatic fever (ARF) follows streptococcal infections only. Arteritis and fibrinoid degeneration of collagen occur in both ARF and serum sickness, but serum sickness never elicits the formation of Aschoff bodies in the myocardium.

Experimental evidence for the allergic theory comes from many sources. For example, immune complexes were detected in the sera of patients with ARF and carditis or chorea by Williams et al, (1980). There is data to indicate that these immune complexes in rheumatic fever contain streptococcal antigens (Friedman et al, 1982).

It has been demonstrated that ARF patients have an increased delayed hypersensitivity skin test reactivity to streptococcal antigens when compared with non-rheumatic controls (Humphrey and Pagel, 1940). It has been noted that ARF does not tend to occur in children below the age

of 3 - 4 years, consonant with the suggestion that disease occurs only after repeated infections (Rantz et al, 1951). ARF patients who were free of intercurrent infection for five years after the initial attack rarely developed a second attack (Stollerman 1954). All these observations indicate that cellular reactivity to streptococcal antigen might also play an important role in the pathogenesis of ARF and carditis (Reid et al, 1980).

Lymphocyte-mediated cytotoxicity for the myocardium may play an active role in the pathogenesis of rheumatic myocarditis (Friedman et al, 1971, Yang et al, 1977, Hutto and Ayoub, 1980). Neither antibody nor complement appears to play a part in this activity in vitro, a fact that does not exclude their participation in vivo.

Autoimmune theory of the pathogenesis of rheumatic fever

Two major lines of evidence are concerned with the demonstration of anti-heart antibodies in rheumatic fever patients and with cross-immunity of streptococcal antigens with heart tissue.

The frequency of occurence of heart antibodies is higher in rheumatic fever patients who develop carditis than in those who do not (Stollerman 1975). However, autoantibody has been found as a general response to tissue injury, such as burns. It has been shown that cardiac damage by rheumatic, traumatic, or ischaemic heart lesions leads to antigenic

changes which are manifested by the production of autoantibodies (Enrenfield et al, 1961, Hess et al, 1964). Therefore heart antibodies in rheumatic carditis may represent the result of tissue injury rather than its cause.

The theory of the pathogenesis of rheumatic fever with the most experimental support is the autoimmune mechanism caused by similarities between streptococcal and human tissue antigens. Patients with rheumatic fever have higher levels of various anti-streptococcal antibodies than those with streptococcal infection that do not develop rheumatic fever. As mentioned earlier, some of these antibodies have been shown to react with both heart tissues and streptococcal antigens.

The most important of these cross - reactions according to recent evidence appears to be due to an antibody to a polypeptide fragment of type 5 M-protein which reacts with sarcolemmal membranes of human heart muscle (Dale and Beachey, 1982). Cross-reaction between group A polysaccharide and a glycoprotein isolated from heart valves has also been described (Goldstein and Trung, 1968).

These observations correlate with the long known increased humoral immune response of patients with rheumatic fever to a variety of streptococcal products. Such patients also show greater cell-mediated immunity to streptococcal antigens. A cellular reaction pattern consisting of lymphocytes and macrophages aggregated around fibrinoid deposits is found in human hearts. This lesion known as the "Aschoff body"

is characteristic of rheumatic carditis.

Use of experimental models

Murphy and Swift (1949) carried out studies of streptococcal rheumatic carditis and many related studies have been done since then (Murphy, 1952, Becker and Murphy, 1980). This work demonstrated that when rabbits are infected by group A streptococci they develop focal cardiac lesions characterized by acute and chronic inflammation; that certain genetic strains of rabbits are more susceptible than others; and that repeated infections with certain serological types by the intradermal route are more likely to produce lesions (Thomas 1972). However, it has not been unequivocally proved that the group A streptococcus was responsible for producing the lesions in rabbits. It is suggested that a similar study should be conducted to determine the effects of repeated exposure of rabbits to other varieties of bacteria (Thomas 1972). Models using immunological procedures, in experimental animals, to prove the allergic and autoimmunization theories have not yielded any concrete evidence for the pathogenesis of rheumatic fever.

The possibility that infectious agents other than group A streptococci may be involved in the aetiology of rheumatic fever has been considered by some research workers (Thomas 1972).

Many cases of chronic valvular disease occur without a prior history of ARF, and a lack of serological evidence (Burch et al, 1970). Burch and co-workers used coxsackie

viruses to infect mice and cynomolgus monkeys and showed that coxsackie viruses produce myocarditis, pericarditis and valvulitis (Sun et al, 1967). The evidence that this virus has a predilection for the valves as well as other parts of the heart suggests that the experimental pathology of rheumatic fever needs further study.

Genetic factors are also probably important in rheumatic fever. Attack rates are highest among those of lower socio-economic status and vary among those of different racial origin. The gene for an alloantigen found on the surface of B-lymphocytes appears among rheumatic fever patients at a frequency four-fold to five-fold greater than the general population. Localization of this gene in a region associated with immune response further suggests a genetic predisposition to hyperreactivity to streptococcal products (Patarroyo et al, 1980). Studying the mechanism of HLA - disease associations would help in acquiring new knowledge of the pathogenesis of rheumatic fever.

Decades of research in experimental pathology to reproduce rheumatic-like cardiac lesions have not produced conclusive information on the mechanism of this disease (Unny and Middlebrooks, 1983).

2.2.3b Acute glomerulonephritis (AGN)

Post-streptococcal glomerulonephritis may follow either respiratory or cutaneous group A streptococcal infection; however, it is more often associated with

streptococcal pyoderma than with infections of the respiratory tract (Dill on et al, 1967, Poon-King et al, 1967). It involves only certain strains known on epidemiological grounds as nephritogenic. Nephritogenic strains are of a few M types only, such as type 12 (respiratory) and type 49 (skin) (Dill on, 1967; 1979). The average latent period between infection and glomerulonephritis is ten days from a respiratory infection, but generally about 3 weeks from skin infection (Wannamaker, 1970). AGN is primarily a disease of childhood, mainly of pre-school children (Dill on, 1968). The clinical course is usually mild with spontaneous healing over weeks to months, but occasionally a progressive course leads to renal failure and death. Second attacks require infections with nephritogenic strains of another M type. This event is rare however and recurrences are rare.

Aetiology and pathogenesis of AGN

As in rheumatic fever, the pathogenesis of AGN appears to involve immunologic mechanisms. Immunoglobulins, complement components and antigens that react with antibodies against group A streptococci have been identified in the diseased glomerulus (Micheal et al, 1966, Andres et al, 1966). The renal injury may be caused by reactions between renal tissue and anti-streptococcal antibodies or simply by deposition in the glomerulus of preformed antigen - antibody complexes. Such complexes have been identified in the serum during acute disease and together with complement as discrete deposits in the glomerulus (Wannamaker and Matsen, 1972).

2.2.4. The antibody response to Streptococcus pyogenes

Antibodies to many different consituents of Streptococcus pyogenes are formed as a result of infection. Their production has been studied not only an aid to diagnosis, but also for explaining the epidemiology and pathogenesis of streptococcal disease as discussed earlier.

The extracellular products formed by all or most strains of Streptococcus pyogenes are streptolysin O, streptolysin S, erythrogenic toxin, proteinase, hyaluronidase, DNases and DPNases. of these, Streptolysin S is not antigenic and proteinase is poorly antigenic. Antibodies to the other extracellular products can be detected by testing for neutralization of their specific biological activity.

In streptococcal infections except impetigo, the pattern of antibody response to streptolysin O, hyaluronidase, DNase B, and DPNase is similar. With this exception and if each antibody is considered separately, between 50 - 80% of all patients who suffer from clinical infection have a raised titre. Titres in rheumatic fever tend to be somewhat higher than in uncomplicated tonsillitis, but is not clear cut and many patients fail to give a significant response to each antigen. Because low titres of individual antibodies occur in a random fashion the use of two or three tests will increase the proportion of patients

giving a significant antibody response to one or more antigens to almost 100% (Wannamaker and Ayoub, 1960). In streptococcal pyoderma there is little or no ASO or antiDPNase response even when infection is complicated by AGN. However, titres of antiDNase B are usually high (Bascon et al, 1967, Kaplan et al, 1970, Wannamaker 1970, and Widdowson et al, 1974); hence the importance of detecting antiDNase B in AGN.

In general, titres in AGN do not differ in comparable but uncomplicated streptococcal infections. In infections of the respiratory tract, the titre of ASO usually begins to rise towards the end of the first week of illness and increases during the next 2 - 4 weeks. It then falls slowly and in the absence of reinfection tends to approach its original value in 6 - 12 months. However, there is much individual variation. The titre tends to increase for a longer period and stay up longer if rheumatic fever develops, and the response is on average poorer in infants than in older children and adults.

Early treatment with penicillin in adequate doses and for a sufficient period of time reduces both the frequency and titre of the antibody response (Wannamaker et al, 1951).

2.2.5 Epidemiology of infections due to Streptococcus pyogenes

Humans are the natural hosts for the group A Streptococcus pyogenes. Transmission from person to

person is normally associated with close contact with a patient infected with Streptococcus pyogenes or an asymptomatic carrier who is colonized in the nasopharynx, skin, vagina or rectum.

Group A streptococcal pharyngitis is spread by respiratory secretions. It may occur by direct contact with the mucosa or secretions or through large droplets produced by coughing, sneezing or even conversation. Droplet transmission is most efficient at short distances (2 - 5 ft). Spread is common in families, and is increased by crowding in institutions such as schools and military barracks. Food, although a less common vehicle of transmission has been the source of many explosive outbreaks in a large number of people. Three epidemics of milk-borne streptococcal sore throat due to Streptococcus pyogenes affected 835 airmen, 120 of whom had scarlet fever (Taylor et al, 1959). Boiled eggs were incriminated as the vehicle of transmission in an epidemic of group A streptococcal pharyngitis in over one thousand cadets (Hill et al, 1969[1]). The acute case is the most important source of the organism. Without treatment the organism is often present for 1 - 4 weeks after symptoms have disappeared. Asymptomatic carrier rates are usually low. Many of the long-term sources are nasal carriers who include patients with sinusitis or with infected excoriations of the anterior nares. Patients with an ear discharge or extensive skin lesions secondarily infected with Streptococcus pyogenes may occasionally be responsible for outbreaks of streptococcal tonsillitis (Loosli et al, 1950). Environmental

sources and fomites are not important means of spread, although group A streptococci may survive for many weeks in dried secretions and dust (Rammalkamp et al, 1958).

Impetigo caused by Streptococcus pyogenes has an earlier age incidence than streptococcal pharyngitis, peaking between 2 to 5 years, as compared with 5 to 15 years for pharyngitis. Streptococci causing impetigo are acquired mainly from skin lesions in other persons (Basset, 1972). Clinical impetigo is often preceded by skin colonization which is favoured by poor hygiene (Dudding et al, 1970). Lesions occur mostly on unclothed parts of the body and their distribution often appear to correspond with that of insect bites. Scabies lesions may sometime determine the site and frequency of streptococcal impetigo (Rumao and Sant, 1971, Svartiman et al, 1972). Transmission involves direct contact or shared fomites such as towels. Impetigo is most common among low socio-economic groups, in hot climates, and at times when bites are frequent. Certain flies which have been shown to act as vectors feed on moist skin lesions, become contaminated with streptococci and convey the organisms mechanically from one lesion to another (Taplin et al, 1965, Basset, 1967).

Group A streptococci were once a leading cause of nosocomial wound and puerperal infections. The primary mode of transmission from patient to patient was by the hands of physicians or other medical personnel and through poor hygienic practice. Infections may be acquired from staff or patients ill with pharyngitis or carrying the organisms in the

pharynx or nose. Contaminated particles or epithelial cells from non-respiratory carriage sites can also be a source of infection e.g. nosocomial infections of Streptococcus pyogenes involving eleven patients have been traced to an anal carrier in an operating theatre (McKee et al, 1966). An epidemic of surgical wound infections caused by Streptococcus pyogenes affecting eighteen patients in a community hospital has been traced to a vaginal carrier (Stamm et al, 1978).

To evaluate the possibility of streptococcal carriage in tracing sources of epidemics of nosocomial and other infections, it is necessary that besides nose, throat and skin cultures, anal and vaginal cultures should also be performed. For tracing the source of infection in common source outbreaks, serotyping group A streptococci according to their cell wall M and T antigens is helpful.

2.3 Infections due to other groups of beta-haemolytic streptococci

2.3.1 Group B streptococci (Streptococcus agalactiae)

For over 80 years group B streptococci (Streptococcus agalactiae) have been recognised as a cause of mastitis in cattle. The fact that similar organisms could be found in the human vagina has been known for over 40 years (Lancefield and Hare, 1935). However its importance as a cause of serious neonatal disease has been realized only recently. In 1937 Colebrook and Purdie first described the isolation of these organisms

from a blood culture in a case of puerperal endocarditis. From 1958 onwards reports of neonatal disease due to group B streptococci became frequent (Parker, 1977). In 1968 Jones and Howells reported two cases of neonatal meningitis; this was the first account of serious neonatal disease due to group B streptococci in the British literature.

Neonatal disease was described as being of two clinical types (Franciosi et al, 1973). Early onset disease (in the first ten days of life) might be either septicaemic or meningitic, was often associated with a long interval between rupture of membranes and delivery, tended to affect infants of low birth weight and had a high mortality despite prompt treatment. The infecting organism could nearly always be isolated from the mother's vagina. Late onset disease was exclusively meningitic, affected apparently healthy infants after normal labour and had a lower mortality if adequate treatment was given. Vaginal carriage in the mother was not a constant feature.

Lancefield (1934, 1938) classified group B streptococci into four serotypes possessing the polysaccharide antigens Ia, Ib, II and III respectively. Later, another type- Ic was added, characterized by the presence of a protein antigen in some strains (Wilkinson and Eagen, 1971). The capsular sialic acid has been associated with virulence and shown to prevent activation of the alternate complement pathway (Ryan, 1984). Differences in the frequency of these types among strains from different categories of infection have been reported. Group B streptococci isolated from cases of

late-onset neonatal meningitis were found to be type III (Franciosi et al, 1973). A predominance of type III in both 'late' and 'early' neonatal meningitis and 'late' septicaemic cases was also observed (Baker and Barret, 1973 and 1974). The difference in type distribution in meningitic and septicaemic disease in the USA has been attributed to qualitative differences in the pathogenicity of individual streptococcal strains (Parker, 1977). The source of infection for neonates is usually the female genital tract (Franciosi et al, 1973; Hoog Kamp - Korstanje et al, 1982). Carriage rate in the female genital tract shows a wide variation probably due to variations in technique (Baker and Barret, 1973). Vaginal carriage in late pregnancy appears to be unstable (Ferreiri et al, 1977). Studies have shown that the bowel is the main carriage site of the organism (Hoog Kamp Korstanje et al, 1982). Franciosi et al (1973) observed in pregnant women a vaginal carriage rate of 10.2% and a rectal carriage rate of 17.9% and of 142 women with group B streptococci in the anal swab, only 61 had a positive vaginal swab. On investigating the role of maternal antibody in neonatal group B streptococcal infection, it was found that the sera of women whose infants suffered from invasive type III infection did not have antibodies against this type, but sera from 22 of 29 pregnant, type III vaginal carriers whose infants were healthy contained antibody with a prevalence significantly different from that in women delivering infants with type III disease. These data suggest that transplacental

transfer of maternal antibody protects infants from group B streptococcal infection with type III strains (Baker and Kasper, 1967).

Colonization of newborn infants by group B streptococci also occurs via cross colonization in the newborn nursery. Epidemiological data suggest that the mode of nosocomial transmission of group B streptococci among infants was cross colonization via personnel contact rather than carrier or environmental reservior (Parades et al, 1977). Howard and McCracken (1974) reported some unusual forms of group B streptococcal disease in infants under three months of age. Asymptomatic bacteraemia in four infants, septic arthritis in three infants and osteomyelitis ethmoiditis with orbital cellulitis, pneumonia with empyema, facial cellulitis and conjuctivitis in one infant each.

Group B streptococci may also colonize the throat in children and adults in whom they have been associated with a variety of pyogenic infections at non-respiratory sites. It is usually an opportunistic pathogen in adults who are immunocompromised for a variety of causes including diabetes mellitus, alcohol abuse, malignancy and others on corticosteroid treatment etc. The infection caused in these adults are post-partum infections, bacteraemias, osteomyelitis, endocarditis (Duma et al, 1969, Lerner et al, 1977) pneumonia, empyema, pyelonephritis, septic arthritis (Laster and Michels, 1984) and meningitis (Felton et al, 1984).

In puerperal sepsis the source of infection is usually the female genital tract. Bacteraemia due to group B

streptococcal infection was reported in two male patients following prostatic infection where the probable source of infection was the male genital tract (Duma et al, 1969). As indicated by the species name agalactiae, these organisms cause mastitis in cattle. However, pasteurization of milk has eliminated this source of infection of humans.

2.3.2 Group C Streptococci

The group C streptococci are primarily veterinary pathogens but are known to cause infection in humans. The four species of group C streptococci, Streptococcus equisimilis, Streptococcus zooepidemicus, Streptococcus equi and Streptococcus dysgalactiae, can be differentiated by their biochemical properties, their hosts and their pathogenicity for humans.

Streptococcus equisimilis is the cause of most cases of disease due to group C streptococci in humans and has been isolated from the throat, nose, vagina and rectum of asymptomatic carriers. Pharyngitis and tonsillitis due to group C streptococci were first reported by Griffith (1934). Hutchinson (1946) described 33 patients with tonsillitis, six of whom had fever, lymphadenopathy and necrotic debris on the tonsils. An outbreak of infection due to group C streptococci causing cellulitis in which 27 people were affected with four deaths has been reported (Portnoy and Reitler, 1944). Puerperal

sepsis complicated by endocarditis due to group C streptococci was reported by Feingold et al (1966).

Streptococcus equisimilis is reported to have caused pharyngitis with secondary bacteraemia (Appelbaum et al, 1979). A case of pharyngitis followed by bilateral cavitatory pneumonia with empyema, bacteraemia and arthritis which was fatal was reported by Stamm and Cobbs (1980). Other infections in humans caused by Streptococcus equisimilis include puerperal sepsis (Ramsay and Gillespie 1941) endocarditis (Bulloch et al, 1970) bacteraemia with secondary osteomyelitis (Asplin et al, 1979), brain abscess, (Dinn 1971) and post operative wound infection (Goldman and Breton, 1978).

Streptococcus zooepidemicus is a common cause of serious epidemic disease in domestic animals, but it is now established that this species can also cause disease in humans. Duca et al (1969) reported a milk borne epidemic of sore throat, cervical lymphadenitis and pyrexia due to Streptococcus zooepidemicus involving 85 patients, a third of whom later developed acute glomerulonephritis as a complication. Kohler and Cederverg (1976) described an isolated case of fever, cervical lymphadenitis and respiratory distress in a three year old boy. Meningitis in an adult was reported by Mohr et al (1978). Pneumonia in a young woman was described by Rose et al (1980). A milk borne outbreak of pharyngitis in five members of a family, followed by Acute glomerulonephritis in three of them has been reported by Barnham (1983). Streptococcus zooepidemicus has been shown to produce endostreptosin, a

cytoplasmic polypeptide which is important in the development of post streptococcal glomerulonephritis. The only reported case of human disease due to Streptococcus equi occured in a 57 year old woman with bacteraemia, where the source of infection was the genital tract or skin (Duma et al, 1969).

The only reported case of human disease due to Streptococcus dysgalactiae was meningitis in a preterm infant (Quinn et al, 1978).

Group C streptococci are the source of the streptokinase used as a fibrinolytic agent in the treatment of thromboembolic disease (Marder, 1979).

2.3.3 Group D Streptococci

The group D streptococci are normal inhabitants of the human gastrointestinal tract and they may spread from this site to cause bacteraemia, cholecystitis and wound infections. Approximately 10% of urinary tract infections and 20% of cases of endocarditis are caused by these organisms. The medically important group D streptococci are divided into the enterococcal species - Streptococcus facaelis, Streptococcus faecium and Streptococcus durans and the non-enterococcal species - Streptococcus bovis, biotypes I and II. When septicemia with group D streptococci occurs in the neonatal period its presentation may be identical to early onset group B streptococcal disease. Anatomical

defects of the central nervous system, neurological intervention, endocarditis and urinary tract infections with group D streptococcus may predispose the patient to meningitis which has a mortality rate of 33% (Bayer et al, 1976). Sepsis with Streptococcus bovis is associated with colonic carcinoma and the isolation of this organism may be the first indication of underlying gastro-intestinal lesions (Klein et al, 1977).

Formerly emphasis was placed on differentiation of the enterococci as it was assumed that the non-enterococcal strains were all susceptible to penicillin whereas the enterococci required both penicillin and an aminoglyocide for the treatment of serious infections. However, this distinction cannot reliably predict the susceptibility patterns of the organism because occasionally Streptococcus bovis strains are relatively resistant to the lethal action of penicillin and other antibiotics (Savitch et al, 1978).

2.3.4 Group F Streptococci

The group F beta-haemolytic streptococci and other beta-haemolytic streptococci that form minute colonies are called Streptococcus anginosus by American workers but are termed Streptococcus milleri by the British taxonomists. It has also been called "minute streptococcus" usually described as non-haemolytic but a small percentage are beta-haemolytic and some also are alpha-haemolytic (Long and Bliss 1934, Parker and Ball, 1975). They were found to be dextran positive, usually

aesculin positive, arginine positive, VP positive and had uniform "sugar reactions". Some of the Streptococcus milleri gave serological reactions identical with groups A, C, F and G but some were ungroupable (Parker and Ball 1975, Ruoff et al 1985). These streptococci have been isolated from the upper respiratory tract, gastro-intestinal tract and vagina, (Long and Bliss, 1934 Wort, 1975). They have also been isolated from tooth surfaces and dental root canals (Mejare and Edwardson, 1975). Therefore the oropharyax, gastrointestinal tract and vagina are potential sources for bacteraemia as well as suppurative infections. Streptococcus milleri appears to be an opportunistic pathogen. In a study of 712 streptococci isolated from human blood and internal organs the streptococcus most commonly isolated from purulent lesions was Streptococcus Milleri (29.3%). Clinical syndromes include abscess formation in the major organs and dentition, purulent involvement of the body cavities, bacteraemia, endocarditis and osteomyelitis (Murray et al, 1978). Streptococcus milleri has also been associated with brain abscess, meningitis, and pleural empyema (Koepke, 1965, Wort, 1975; Parker and Ball 1975).

Streptococcus milleri accounted for 75% of 70 group C isolates, 15% of 69 group G isolates, 75% of 16 non-groupable isolates and 100% of 20 group F isolates obtained from clinical specimens (Ruoff et al, 1985). The clinical significance of these isolates was not established.

2.3.5 Group G Streptococci

Group G Streptococci are pathogens of animals and man. Beta-haemolytic group G organisms were added to the original classification of Lancefield in 1935 after a study of streptococci in the vaginal flora of healthy and infected peripartum women (Lancefield and Hare, 1935). Later it was confirmed that group G streptococci may be part of the vaginal flora (Christensen et al, 1974) and may occur normally in the pharynx (Forrer and Ellner, and in the gastrointestinal tract or skin (Sherman, 1937). Serious infections due to group G Streptococci that have been reported include puerperal sepsis (Colebrook and Purdie, 1937; Duma et al, 1969) neonatal sepsis (Baker 1974, Mohan et al, 1980) otitis media (Armstrong et al, 1970) pneumonia and empyema (Foley, 1947 Duma et al, 1969) meningitis (Armstrong et al, 1979) peritonitis (Khan et al, 1975) and cellulitis of the skin (Feingold et al, 1966).

Asymptomatic pharyngeal carriage of group G streptococci has been reported in 23% of humans (Hill et al, 1969 [2]) and the organisms were found in 12% of throat cultures from patients with pharyngitis (Forrer and Ellner, 1979). Although group G streptococci have also been reported in association with three epidemics of pharyngitis in Seoul, Korea, Czechoslovakia and eastern United States, (Kang et al, 1958, Kahlich, 1964, Hill et al, 1969 [2]), definitive proof that group G organisms

cause the non-suppurative sequelae associated with group A streptococci is lacking. Bacteraemia due to group G streptococci has been reported by Duma et al, 1960, Armstrong et al, 1970, Auckenthaler et al, 1983, and Dickie et al, 1984. An association of group G streptococcal bacteraemia with malignancy has been demonstrated independently in these studies. Four cases of group G streptococcal endocarditis have been reported (Blair and Martin, 1978).

2.3.6 Group R Streptococci

Streptococci possessing R, S or T antigens are collectively known as Streptococcus suis. Although these organisms are primarily associated with disease in pigs, group R streptococci have been known to cause meningitis and septicemia in humans. Group R streptococci were first isolated in man in 1968 when two cases of meningitis and a fatal case of septicemia were reported in Denmark (Perch, 1968). From 1968 - 1974 seven cases of septicemia and purulent meningitis caused by porcine streptococci of the Lancefield group R and three cases caused by similar streptococci lacking R antigen occured in the Netherlands. Bacteria isolated from all ten patients shared the characteristics of a biochemically well-defined bacterial species. Nine of the ten patients had had close contact with live or slaughtered pigs. Half the patients complained of deafness and vertigo after the acute phase of disease was over, even with

prompt treatment (Zanen and Engel, 1975). Because most of the patients who developed meningitis due to group R streptococci were reported to have had close contact with live or slaughtered pigs in their work and most of the patients were left with residual deafness and vertigo, it has been suggested that group R streptococcal meningitis be classified as a new industrial disease (Twort, 1981). These streptococci have also been reported as a cause of endophthalmitis (McLendon, 1978). Human infections rarely occur with the beta-haemolytic streptococci belonging to groups E, L, M, U, and V although they are important veterinary pathogens.

CHAPTER THREE

LABORATORY DIAGNOSIS OF INFECTIONS DUE TO BETA-HAEMOLYTIC STREPTOCOCCI

A typical clinical picture with demonstration of the organism in the infected site is the usual means by which active Streptococcus pyogenes infection is diagnosed. In pharyngitis, a swab of the posterior pharynx and tonsils is taken. A direct gram stained smear is not helpful because of the presence of many other streptococci in the normal pharyngeal flora. Blood agar plates are inoculated and incubated at 35 - 37°C. Anaerobic incubation has a favourable influence on the demonstration of beta-haemolysis.

After overnight incubation beta-haemolytic colonies are gram stained to confirm that they are streptococci, then speciated according to groups. Although definitive speciation is mainly by immunologic techniques, non-immunologic methods can be used because of their good correlation with the definitive test. The bacitracin test is based on the extreme susceptibility of group A strains to bacitracin and the relative resistance of strains of the other groups. When a disc containing a small amount of the bacitracin (0.02 units) is placed on a plate streaked with an isolated colony of beta-haemolytic streptococci, more than 99% of group A strains show zone inhibition whereas 90-95% of non-group A strains do not. The low rate of false negative results has made this method a useful presumptive test in hospital laboratories.

Most group A and B streptococci are resistant to co-trimoxazole. The susceptibility and resistance of beta-haemolytic streptococci to co-trimoxazole and bacitracin is also helpful in the presumptive differentiation of group A and non group A streptococci in the laboratory. Beta-haemolytic, co-trimoxazole resistant, bacitracin susceptible streptococci are presumptively identified as group A, and B streptococci that are bacitracin resistant are presumptively identified as group B. Beta-haemolytic, co-trimoxazole susceptible, and bacitracin resistant strains are labelled non-group A or B beta-haemolytic streptococci (Facklam et al, 1979).

Definitive identification of streptococci requires demonstration of the group specific antigen by precipitin, immunoflorescence, or agglutination procedures. Until recently throat culture has been the main laboratory test used in most laboratories to confirm the diagnosis and guide treatment with a 24-72 hour delay. Since a delay in treatment of up to several days does not incur the risk of rheumatic fever (Cantanzaro et al 1954) and many authorities have stated that early treatment does not significantly alter the acute clinical course of the disease, (Peter and Smith, 1977, Pantell 1981) this delay has been considered acceptable. However, three well-controlled studies have shown that early antibiotic treatment of children with streptococcal pharyngitis does significantly alter the acute clinical course of

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the disease (Nelson 1984, Krober et al, 1985, Randolf et al, 1985) patients treated early with penicillin were afebrile within 24 hours and had significant symptomatic improvement.

Several commercial kits are available for rapid detection of group A streptococcal antigen from throat swabs. These kits use enzymatic or chemical methods to extract the antigen from the swab, then use Enzyme Linked Immunosorbant Assay (ELISA) or agglutination tests to demonstrate the presence of the antigen (Slifkin et al, 1982, 1984, Miller et al, 1984). The tests can be completed 1- 4 hours after the specimen is obtained. They are 90-95% sensitive and 98-99% specific when compared to culture methods. Although kit tests are rapid they are more expensive than cultures for individual determinations. Recently other newly commercially available tests that permit rapid, lab - confirmed diagnosis of streptococcal pharyngitis by detecting group A specific antigen directly on the throat swab have been studied (Radetsky et al, 1985, White et al, 1986). These tests require only minutes to perform (7 - 10 mins) and in most studies results have been shown to correlate well with the throat cultures. Also one of the rapid tests studied (White et al, 1986) was equal to the throat culture in detecting serologically, streptococcal infection in a group of 45 of those patients in whom it was possible to obtain acute and convalescent serum specimens. These new developments may soon make possible an early lab confirmed diagnosis at the initial clinic visit in patients with suspected streptococcal pharyngitis, permitting

early specific antimicrobial treatment when results are positive, and note at the same time that antimicrobials are not indicated when results are negative. In addition to reducing the duration of symptoms to 24 hours or less, early treatment should decrease the incidence of suppurative complications, limit spread of disease in the family and community, and permit an earlier return to school or work place.

Several serological tests have been developed to aid in the diagnosis of post streptococcal sequelae, due to group A streptococcal infections. A rise in the titre of antibodies to many group A streptococcal antigens can be estimated. Such antibodies include antistreptolysin 0 (ASO) especially in respiratory disease, anti DNase and antihyaluronidase especially in skin infection, antistreptokinase, anti M-type - specific antibodies and others. Of these the ASO is most widely used. Antibodies to several streptococcal antigens and enzymes are measured by the streptozyme test, which is performed by some diagnostic laboratories. The antigens are adsorbed on to sheep red blood cells on a slide and agglutination by antibodies occurs within a few minutes (Jawetz, 1987).

CHAPTER FOUR

SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS AND TREATMENT OF INFECTIONS DUE TO BETA-HAEMOLYTIC STREPTOCOCCI - PROPHYLAXIS

The group A streptococci are still universally susceptible to penicillin G. The antimicrobial agent of choice for treatment of infections due to Streptococcus pyogenes is therefore still penicillin G and it is unnecessary to perform antimicrobial susceptibility tests for these organisms unless the patient is allergic to penicillin. Patients allergic to penicillin are usually treated with erythromycin if the organisms are susceptible to it or with a cephalosporin if cross-allergy is absent. However, many strains of streptococci have become resistant to a number of antimicrobial agents other than penicillin. Sulphonamide resistant strains of Streptococcus pyogenes were first observed during the second world war and became prevalent in army camps in which the drug was used for mass prophylaxis (Francis, 1942, Report 1945).

Tetracycline resistance was confined mainly to burn wounds for several years (Lowbury and Hurst, 1956), but from about 1960 it became prevalent in the general population of the United States (Kuharic et al, 1960), Britain (Parker et al, 1962) and several other countries. Formerly erythromycin resistance was rare, demonstrated by occasional strains only (Lowbury and Hurst 1959, Baker et al, 1979). However, in Japan, erythromycin resistance of group A beta-haemolytic

Streptococci was reported as 60% (Marnyama et al, 1979), while only 16 (2.8%) of 578 isolates from several areas across the United States were shown to be resistant to erythromycin in a recent report (Arthur et al, 1984).

In 1982 Boureau et al, studied the susceptibility of recently isolated group A streptococci to penicillin, also their susceptibility to alternative antibiotics and evaluated the activity of three new beta lactam antibiotics moxalactam, cefotaxime, and N-formimidoyl thienamycin against these isolates.

They found that penicillin, on a weight basis remained the most active of the currently available antibiotics tested. Also the geometric mean minimum inhibitory concentration (MIC) of penicillin (0.0073ug/ml) has not changed significantly from previous reports over a period of thirty two years. By determining the MIC of the antibiotics likely to be selected for patients allergic to penicillin, resistance to erythromycin (MIC $>$ 5ug/ml) was observed in 3% of the isolates and resistance to tetracycline (MIC $>$ 10ug/ml) was detected in 5% of the isolates. Two of the three new beta lactam antibiotics tested, cefotaxime (geometric mean of MIC 0.014ug/ml) and N-formimidoyl thienamycin (geometric mean of MIC 0.042ug/ml) showed excellent activity against the strains tested. In contrast the geometric mean MIC of moxalactam was 1.6ug/ml. Boureau et al, concluded from this study that routine susceptibility testing of group A streptococci continued to

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be unnecessary when penicillin is the drug of choice. However, in situations in which a beta lactam antibiotic cannot be used, susceptibility tests should be performed to confirm the suitability of the antibiotic selected. Adequate penicillin treatment of streptococcal pharyngitis within ten days of onset will prevent rheumatic fever by removing the antigenic stimulus. Penicillin does not prevent the development of acute glomerulonephritis (Ryan 1984).

Penicillin G is also the drug of choice for the treatment of infections caused by the other beta-haemolytic streptococci. Usually the groups B, C, F and G streptococci are susceptible also to ampicillin, cephalothin and chloramphenicol and resistant to tetracycline. Vancomycin or erythromycin are alternative antibiotics for the penicillin allergic patient. When a beta-haemolytic streptococcus is confirmed to be the causative agent of any infective process, single drug therapy with penicillin or ampicillin is usually given. However, evidence for accelerated killing of the group B streptococci with the combination of ampicillin and gentamicin (Schauf et al, 1976) and reports of penicillin tolerant strains, may indicate the need to review single drug treatment in patients with group B streptococcal infections (Siegal, et al, 1981, Rolston et al, 1982).

The group D enterococci are the most resistant of the streptococci to antimicrobial agents. They are penicillin resistant. They require 4-16ug/ml penicillin for inhibition

and much higher concentration for bactericidal effect. The enterococci are also resistant to aminoglycosides. However, infections due to group D enterococci are treated with penicillin or ampicillin in combination with an aminoglycoside. Under these conditions, the action of penicillin on the cell wall allows the aminoglycoside to enter the cell, reach its ribosomal receptor site and kill the cell. Where there is a high level of resistance to streptomycin (MIC > 2000ug/ml), the combination of penicillin and streptomycin is unlikely to be synergistic. Penicillin and gentamicin or vancomycin and gentamicin are effective against these strains. Combinations of penicillin with tobramycin, netilmicin or sisomicin are effective in vitro against Streptococcus facaelis but not Streptococcus faecium (Moellering et al, 1979). The group D enterococci are also resistant to the cephalosporins, a fact which may contribute to the emergence of these enterococci as opportunistic pathogens in patients treated with cephalosporins. They are also often resistant to sulphonamides, tetracycline and occasionally to erythromycin and chloramphenicol. Ampicillin is the antimicrobial agent most consistently active against the enterococci. The majority of non-enterococcal group D streptococci e.g. Streptococcus bovis, are susceptible to penicillin (Thornsbery et al, 1974). However resistance of Streptococcus bovis to penicillin, vancomycin, and cephalothin has been reported (Savitch et al, 1978). Combination therapy may also be required to treat serious infections caused by these organisms.

Prophylaxis

The greatest advance in the development of penicillin preparations for treatment of patients with streptococcal pharyngitis came in 1951 with the introduction of penicillin G benzathine, a slowly absorbable compound that produced relatively low blood levels of penicillin G but levels bactericidal for all strains of group A beta-haemolytic streptococci for an extended period of up to one month (Wannamaker et al, 1951). This preparation given at monthly intervals also evolved as the treatment of choice for prophylaxis against recurrent streptococcal pharyngitis for individuals who have had rheumatic fever (Stollerman and Rusoft, 1952, Stollerman et al, 1955).

Currently penicillin prophylaxis with long-acting preparations is used to prevent recurrences of rheumatic fever during the most susceptible ages (5 - 15 years). Patients with a history of rheumatic fever or known rheumatic heart disease usually receive antimicrobial prophylaxis while undergoing procedures known to cause transient bacteraemia, such as dental extraction (Ryan 1984).

CHAPTER FIVE

MATERIALS AND METHODOLOGY

5.1 Objectives

5.1 (a) To carry out definitive identification of beta-haemolytic streptococci isolated at the U.T.H. into specific serological groups using "Streptex" latex agglutination test kit.

5.1 (b) To relate the specific groups of beta-haemolytic streptococci identified to clinical manifestations and to compare the results with those obtained from studies carried out elsewhere.

5.1 (c) To study the antimicrobial susceptibility patterns of the different groups of beta-haemolytic streptococci isolated.

5.2 Clinical Materials

Serological grouping was carried out on beta-haemolytic streptococci isolated from clinical specimens received in the bacteriology laboratory from in-patients and out-patients at the University Teaching Hospital, Lusaka during the fourteen month period from 13/8/86 - 20/10/87. The clinical specimens from which the strains were isolated included throat swabs, high vaginal swabs, urine, cerebrospinal fluids, pus swabs, sputum and blood. On obtaining a scanty, moderate, or heavy growth of beta-haemolytic streptococci after primary plating of

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the specimens, the following datat were recorded:

date of receipt of specimen

ward or clinic

name of patient, age and sex

type of specimen

clinical diagnosis

Some patients' records were followed up for additional information regarding clinical features and outcome of disease.

5.3 Bacteriological Culture

All the specimens obtained were processed according to standard bacteriological techniques (Cruickshank et al 1975[b]). For primary isolation the specimens were inoculated on a blood agar plate prepared from blood agar base (Oxoid) containing 7% human blood, and a MacConkey agar plate. Pus swabs were also inoculat into thioglycolate broth. All plates were incubated aerobically and some anaerobically at 37°C overnight (18 - 24 hours).

Blood for culture was inoculated into dextrose broth (Oxoid) and after overnight incubation was subcultured on to blood agar and MacConkey agar plates. For each blood culture three subcultures were carried out at intervals of 48 hours. For urine a semi-quantitative culture was carried out using a calibrated loop to detect significant growth (Cruickshank et al 1975[c]). After overnigh incubation all plates were examined for beta-haemolytic streptococci. The colonies were Gram stained and a catalase test was carried out.

Plate 1

Colonies of beta-haemolytic streptococci on blood agar.

Serological grouping was was carried out on 239 streptococcal isolates and not on all 632 beta-haemolytic streptococci isolated during the period of the study due to economic constraints. The number 239 was approved by collegues in the Department of Community Medicine as satisfying statistical requirements.

Antimicrobial susceptibility testing were done on 217 of the 239 streptococcal isolates that were grouped. Twenty two of the isolates were scanty growths insufficient for antimicrobial susceptibility testing. The number 217 was also approved as satisfying statistical requirements.

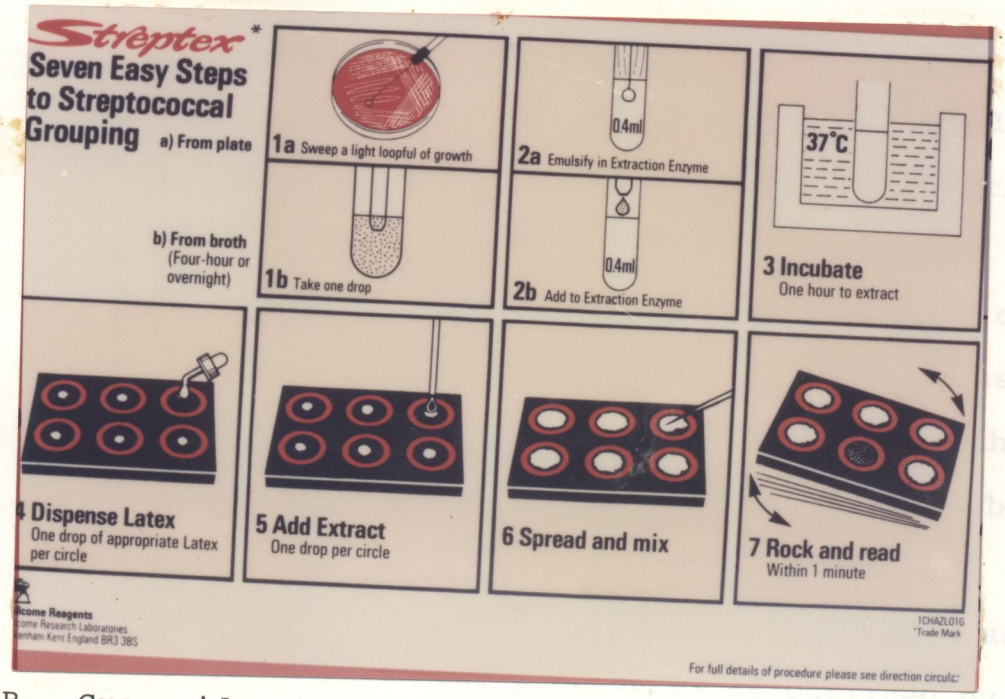
5.4 Serological Grouping

Serological grouping was carried out using Streptex (ZLOI Wellcome Diagnostics) a rapid latex kit for streptococcal grouping.

Plate 2

Streptex Rapid Latex Test Kit.

Plate 3



B. Group identification of extract

Each of the latex suspensions (A,B,C,D,F and G) were resuspended by vigorous shaking for a few seconds. The contents of the indwelling pipettes were expelled to ensure complete mixing. One drop of each latex suspension was placed on separate circles on a clean reaction tile. Using a Pasteur pipette one drop of antigen extract was added to each of the six circles on the reaction tile. The contents in each circle were mixed with a mixing stick and spread to cover the complete area of the circle. A separate stick was used for each circle and discarded after use. The tile was rocked gently until one or more of the latex reagents

showed definite agglutination or for a maximum of one minute. The tile was held at normal reading distance from the eyes.

C. Interpretation of results

1. A positive result was indicated by the developmen of an agglutinated pattern showing clearly visible clumping of the latex particles.
2. When there was obvious agglutination in one latex suspension only, the other five suspensions showing no agglutination, the group of the streptococcus being tested was taken to be the suspension with the positive result.
3. When there was a different pattern of reaction, quality control procedures as outlined by the manufacturer were carried out. These included a test of the reactivity of the latex suspensions, test for specificity of agglutination and test of enzyme extraction procedure.

Some strains of group D streptococci were found to possess in addition, group G antigen. These strains were confirmed as group D by the bile-esculin test (Facklam et al, 1974). The bile-esculin test and salt tolerance test were carried out on all group D strains.

5.5 Antimicrobial Susceptibility Testing of the Strains of Beta-haemolytic Streptococci Isolated

The comparative method of disc diffusion susceptibility testing was carried out (Cruickshank et al, 1975).

Blood agar plates were inoculated by touching several colonies of a similar morphology with a sterile loop and streaking uniformly over the entire plate to produce a semiconfluent growth. The standard organism used (the Oxford strain of Staphylococcus aureus - (National Collection of type Cultures No. 6571) was streaked on a separate blood agar plate in a similar manner.

Antimicrobial discs were applied on the plates after inoculation and the plates were incubated at 37°C aerobically for 24 hours. The discs used were multidiscs (Oxoid) containing penicillin, ampicillin, cloxacillin, co-trimoxazole, chloramphenicol and erythromycin. When the commercially prepared discs were not available, the same antimicrobial discs were prepared as individual discs in the laboratory according to standard techniques (Cruickshank et al, 1975). The efficiency of the locally prepared discs was tested after preparation using the standard Oxford strain of Staphylococcus aureus.

The zones of growth inhibition produced by each drug on the test bacteria were compared with that produced by the standard organism and the test bacteria were reported as being sensitive, moderately sensitive, or resistant to each of the antimicrobial drugs used.

CHAPTER SIX

RESULTS

6.1 Assessment of the Clinical Significance of the Isolated Streptococcus

Patient data was reviewed to assess the clinical significance of the various serological groups of beta-haemolytic streptococci isolated. For urine culture, Streptococci isolated from patients with a clinical diagnosis of urinary tract infection, increased pus cells on urine microscopy and a doubtful or significant bacteriuria were taken to be causative agent of the infection. Streptococci isolated from urine cultures for routine examination, e.g. from antenatal clinics with no clinical diagnosis of infection, with occasional pus cells and an insignificant bacteriuria were considered normal flora of the urethra.

Culture of high vaginal swabs from patients with a clinical diagnosis of vaginal discharge, pelvic inflammatory disease, or puerperal sepsis, yielding a moderate to heavy growth of beta-haemolytic streptococci, with evidence of pus cells on microscopy of smear were considered clinically significant. Scanty to moderate growth of streptococci isolated from vaginal swabs for routine examination e.g. for investigation of sub-fertility, with no evidence of infection, were included among the normal flora of the vagina.

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Beta-haemolytic streptococci isolated from normally sterile body fluids like cerebrospinal fluid, synovial fluid and blood, with clinical diagnosis of neonatal meningitis, septic arthritis and bacterial endocarditis were considered to be the causative agents of infection. The cerebrospinal fluids were also turbid in appearance, with increased white cell count and polymorphonuclear leucocytosis suggestive of bacterial infection.

Beta-haemolytic streptococci isolated from throat swabs of patients with pharyngitis and acute tonsillitis were considered to be clinically significant.

Streptococci from peritonsillar abscesses were almost certainly the causative agent of the infection. Streptococ isolated from clinical conditions like impetigo, and cellulitis were clinically significant as these are well-known manifestations of beta-haemolytic (group A) streptococcal infection.

Streptococci isolated on culture of pus from cases of osteomyelitis and other infected wounds and abscesses were also considered to be clinically significant.

6.2 Serological grouping of beta-haemolytic streptococci isolated from the various clinical specimens received in the laboratory during a fourteen-month period (August 1986 - October, 1987)

A total of 239 isolates of beta-haemolytic streptococci were included in the study. The number and percentage of beta-haemolytic streptococci isolated, and grouped, from

the various clinical specimens are shown in Table 1. The highest percentage of streptococci grouped of all culture positive clinical specimens received in the laboratory was from throat swabs (14%). The second highest from pus swabs (8%), third from high vaginal swabs (6%), sputum (3%) urine (2%), cerebrospinal fluid (2%) and blood culture (0.2%).

TABLE 1

THE NUMBER AND PERCENTAGE OF BETA HAEMOLYTIC STREPTOCOCCI GROUPED FROM THE VARIOUS CLINICAL SPECIMENS RECEIVED IN THE LABORATORY DURING A FOURTEEN-MONTH PERIOD (AUG. 1986 - OCT. 1987)

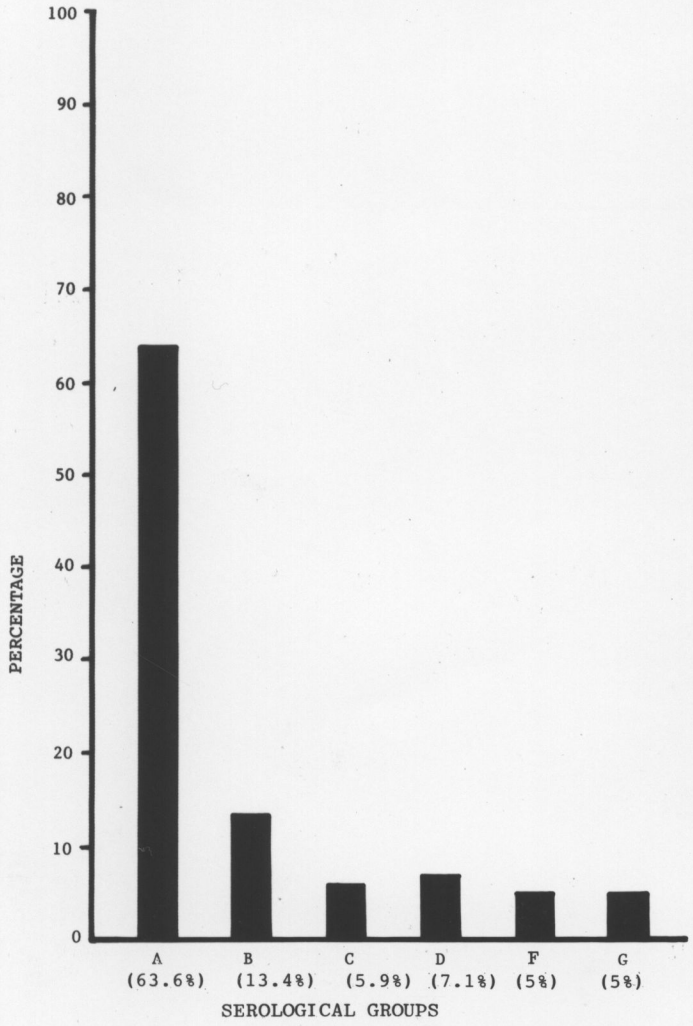
CLINICAL SPECIMEN	TOTAL SPECIMENS (CULTURE POSITIVE)	NO. BHS	PERCENTAGE BHS	NO. GROUPED	PERCENTAGE GROUPED
THROAT SWAB	199	108	54.3%	28	14%
HIGH VAGINAL SWAB	574	113	19.7%	37	6.4%
BLOOD CULTURE	441	2	0.5%	1	0.2%
URINE	1487	93	6.3%	25	1.7%
CEREBRO SPINAL FLUID	188	3	1.6%	3	1.6%
PUS SWAB	1770	288	16.3%	140	7.9%
SPUTUM	147	25	17%	5	3.4%

The results of serological grouping of 239 beta-haemolytic streptococci, the number and percentage of isolates belonging to each of groups A, B, C, D, F and G are shown in table 2 and figure I.

TABLE 2 - SEROLOGICAL GROUPING OF 239 BETAHAEMOLYTIC STREPTOCOCCI ISOLATED FROM CLINICAL SPECIMENS

	NUMBER ISOLATED (PERCENTAGE)				
GROUP A	B	C	D	F	G
152	32	14	17	12	12
(63.6%)	(13.4%)	(5.9%)	(7.1%)	(5%)	(5%)

FIG 1 SEROLOGICAL GROUPING OF 239 BETA HAMOLYTIC STREPTOCOCCI ISOLATED FROM CLINICAL SPECIMENS N=239



The highest percentage of isolates were group A ($^{125}/_{239}$) 64%. Next group B ($^{32}/_{239}$) 13%, group D ($^{17}/_{230}$) 7% group C ($^{14}/_{239}$) 6%, groups F and G ($^{12}/_{239}$) 5%.

TABLE 3

SEROLOGICAL GROUPING OF BETA HAEMOLYTIC STREPTOCOCCI ISOLATED IN RELATION TO THE VARIOUS CLINICAL SPECIMENS

	NUMBER ISOLATED (PERCENTAGE)					
	GROUP					
CLINICAL SPECIMEN	A	B	C	D	F	G
THROAT SWABS n=28	15 (53.6%)	- -	5 (17.6%)	- -	4 (14.3%)	4 (14.3%)
HIGH VAGINAL SWABS n=37	5 (13.5%)	17 (45.9%)	4 (10.8%)	5 (13.5%)	5 (13.5%)	1 (2.7%)
URINE n=25	1 (4%)	12 (48%)	4 (16%)	5 (20%)	3 (12%)	- -
PUS SWABS n=140	128 (91.4%)	- -	- -	5 (3.6%)	- -	7 (5%)
SPUTUM n=5	3 (60%)	- -	1 (20%)	1 (20%)	- -	- -
TOTAL 239*	152	32*	14	17*	12	12

*BLOOD 1 (D)
*CSF 3 (B)

Table 3 shows the Serological grouping of 239 beta-haemolytic streptococci in relation to the various clinical specimens.

From throat swabs, groups A, C, F and G streptococci were isolated, the highest percentage being group A (54%).

From high vaginal swabs all groups A, B, C, D, F and G were isolated, the highest percentage being group B (46%). The highest percentage of streptococci isolated from urine too were group B.

From pus swabs groups A, D and G streptococci were isolated, the percentage of group A being 91%.

From sputum groups A, C, and D streptococci were isolated, the highest percentage being group A (60%).

6.3 Serological grouping of beta-haemolytic streptococci in relation to clinical diagnosis

Table 4 shows the streptococcal groups in relation to the clinical diagnosis of infection.

TABLE 4

SEROLOGICAL GROUPING OF BETA HAEMOLYTIC STREPTOCOCCI IN RELATION TO CLINICAL DIAGNOSIS

CLINICAL DIAGNOSIS	NUMBER ISOLATED (PERCENTAGE) GROUP A	B	C	D	F	G
UPPER RESPIRATORY TRACT INFECTION (n=33)	18 (54.5%)	- -	6 (18.1%)	1 (3%)	4 (12%)	4 (12%)
PERITONSILLAR ABSCESS (n=11)	11 (100%)	- -	- -	- -	- -	- -
INFECTED WOUNDS AND OTHER ABSCESSES (n=110)	98 (89%)	- -	- -	5 (4.5%)	- -	6 (5.4%)
PYODERMA (n=8)	8 (100%)	- -	- -	- -	- -	- -
OSTEOMYELITIS (n=10)	10 (100%)	- -	- -	- -	- -	- -
NEONATAL MENINGITIS (n=3)	- -	3 (100%)	- -	- -	- -	- -
GENITO-URINARY INFECTION (n=29)	1 (3.4%)	16 (55.2%)	3 (10.3%)	5 (17.2%)	4 (13.8%)	- -
NORMAL FLORA (GENITO-URINARY TRACT) (n=33)	5 (15.1%)	13 (39.3%)	5 (15.1%)	5 (15.1%)	4 (12.1%)	1 (3%)
TOTAL	152*	32	14	17*	12	12

*SEPTIC ARTHRITIS 2(A)(G)

*BACT. ENDOCARDITIS 1(D)

Out of 33 beta-haemolytic streptococci isolated from upper respiratory tract infections, 18 (54%) were found to be group A, 6 (18%) in group C, 4 (12%) group C, 4 (12%) group F and one (3%) group D.

Out of 110 isolates from infected wounds and other abscesses, 98 (89%) were group A, 5 (4%) group D and 7 (7%) group G.

Out of 29 isolates from genito-urinary tract infections, 16 (55%) were group B, 5 (17%) group D, 4 (14%) group F, 3 (10%) group C, one (3%) group A.

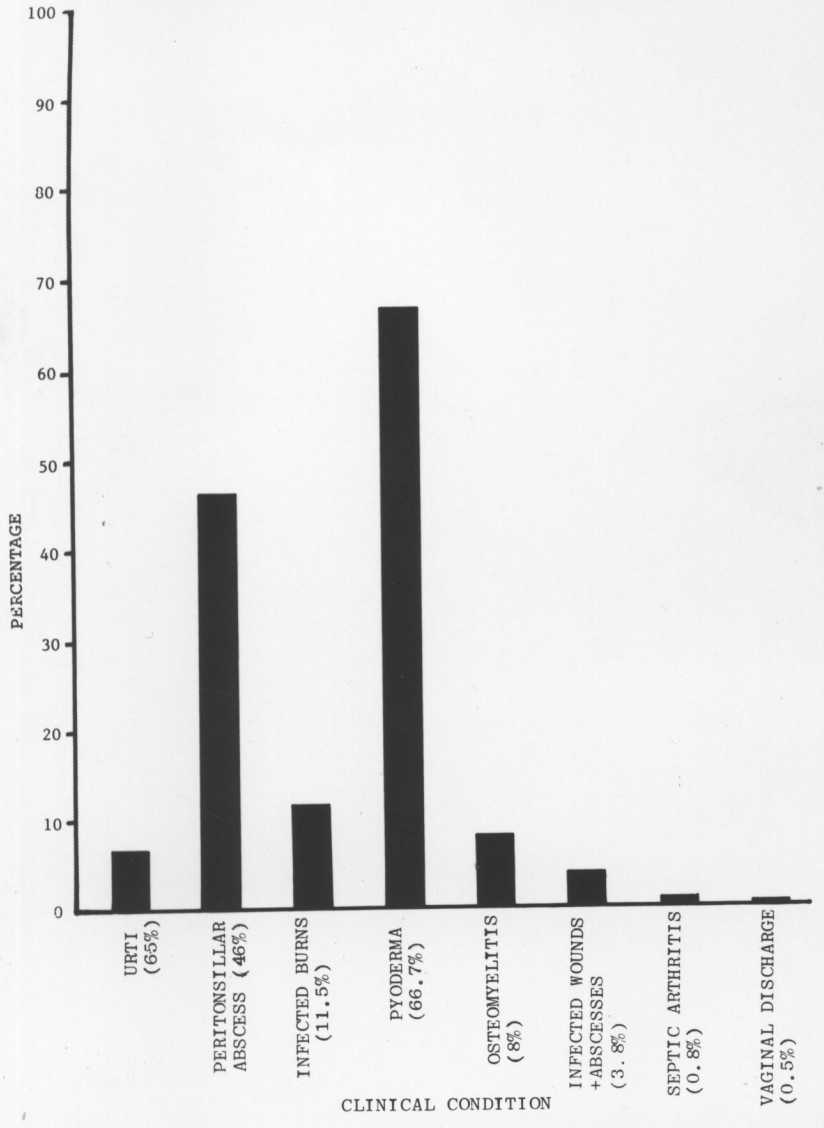
All eleven isolates from cases of peritonsillar abscess, all eight isolates from cases of pyoderma, and all ten isolates from cases of osteomyelitis were found to be group A. All three isolates from neonatal meningitis were group B streptococci.

There were 33 isolates among the specimens from the genito-urinary tract without signs of infection and thus thought to be normal flora of the genito-urinary tract, out of which 13 (39.3%) were group B, 5 (15%) in each of groups A, C and D, 4 (12%) group F and 1 (3%) group G.

There were two isolates from cases of septic arthritis which were found to be groups A and G respectively. One isolate from a blood culture of a case of bacterial endocarditis was found to be group D.

The bar diagram in figure 2 shows in detail the percentage of group A beta-haemolytic streptococci isolated from the various clinical conditions.

FIG 2 PERCENTAGE OF GROUP A ISOLATES BY CLINICAL CONDITION (N=147)



The highest percentage of group A streptococci was found in pyoderma (67%). The second highest was in peritonsillar abscess (46%), and the third highest in infected burns wounds.

FIG 3 THE NUMBER OF GROUP B BETAHAEMOLYTIC STREPTOCOCCI ISOLATED IN RELATION TO CLINICAL DIAGNOSIS N=32

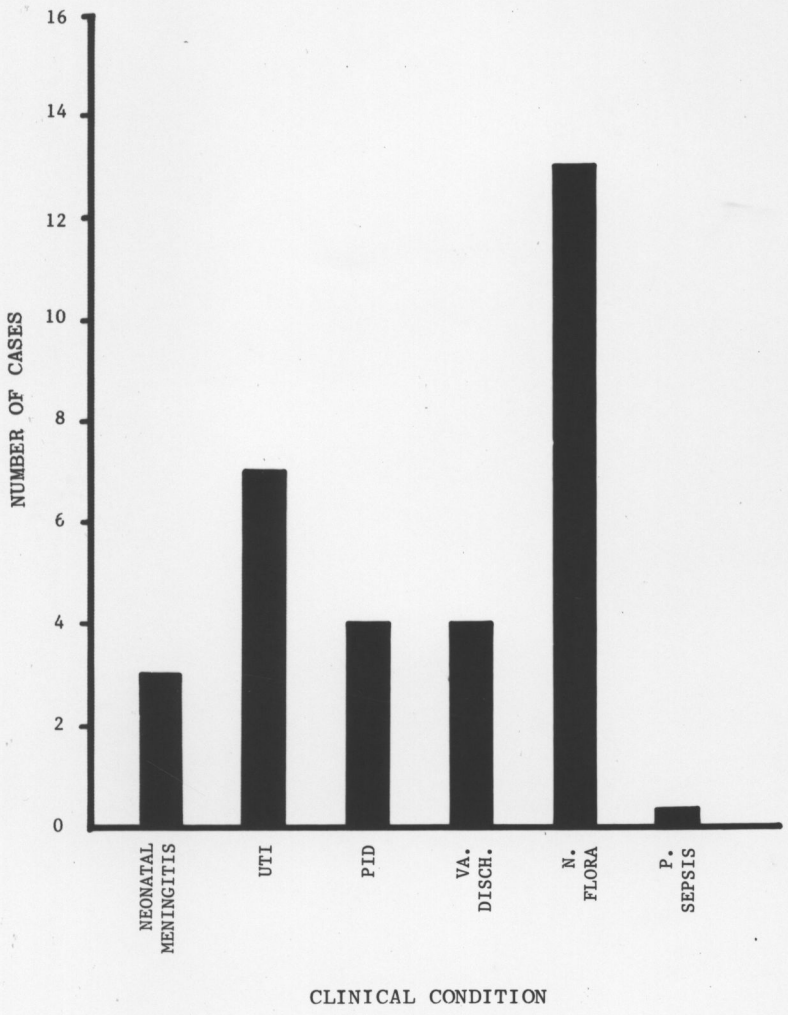


Figure 3 shows the number of group B beta-haemolytic streptococci isolated from various conditions.

The highest number of group B streptococci were found among the normal flora of the genito-urinary tract 13 (41%) next in urinary tract infection 7 (22%), pelvic inflammatory disease 4 (13%), vaginal discharge 4 (13%), neonatal meningitis 3 (9%) and puerperal Sepsis 1 (3%).

6.4 Antimicrobial sensitivity patterns of the various groups of beta-haemolytic streptococci

A total of 217 isolates of beta-haemolytic streptococci were included in the study of their antimicrobial sensitivity patterns. The sensitivity pattern of these strains to penicillin, tetracycline, co-trimoxazole, chloramphenicol, and ampicillin was studied.
Table 5 and figure 4 show the number and percentage of streptococci resistant to these antimicrobial agents and also the sensitivity/resistance pattern in relation to the various serological groups A, B, C, D, F and G.

Streptococcal groups A, B, C, F, and G were sensitive to penicillin (100%), while 4 (25%) of group D isolates were found to be resistant to penicillin. Groups A, B, C and F showed 7 - 10% resistance to erythromycin, group G, 30% and group D, 43.7%. All groups A, B, C, D, F and G showed high resistance to tetracycline and co-trimoxazole (30% - 93%).

All 12 isolates in group C were sensitive to chloramphenicol, while groups A, B, F and G showed 10 - 20% resistance and group D 43.6% resistance. In general group D isolates were found to be multi-resistant to penicillin, erythromycin, tetracycline, co-trimoxazole, and chloramphenicol. The only anti-microbial agent to which group D streptococci showed 100% sensitivity was ampicillin. Groups A, C and G also showed 100% sensitivity to ampicillin, while group B streptococci showed 3.7% resistance and group F 10% resistance.

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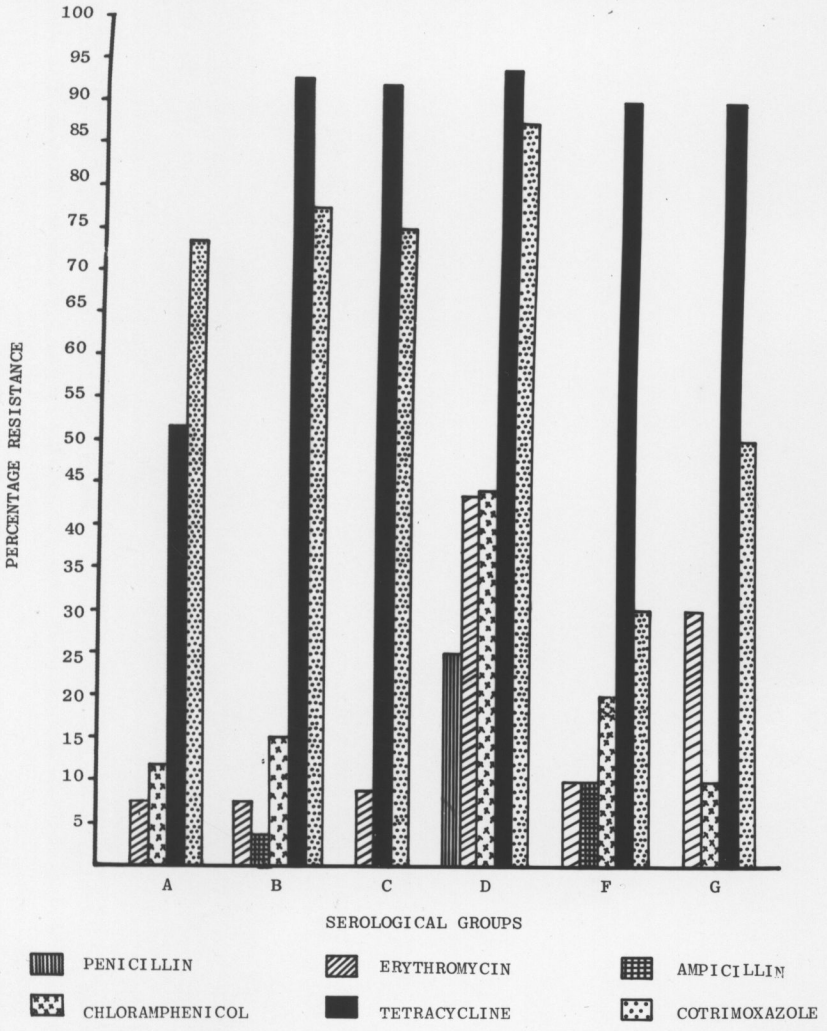
TABLE 5

ANTIMICROBIAL SENSITIVITY PATTERN OF THE VARIOUS GROUPS OF BETA HAEMOLYTIC STREPTOCOCCI

Number Resistant (percentage)

	NO. R. PENICILLIN	ERYTHROMYCIN	TETRACYCLINE	COTRIMOXAZOLE	CHLORAMPHENICOL	AMPICILLIN
GROUP A	-	11	73	104	16	-
142	-	(7.7%)	(51.4%)	(73.2%)	(11.3%)	-
GROUP B	-	2	25	21	4	1
27	-	(7.4%)	(92.5%)	(77.8%)	(14.8%)	(3.7%)
GROUP C	-	1	11	9	-	-
12	-	(8.3%)	(91.6%)	(75%)	-	-
GROUP D	4	7	15	14	7	-
16	(25%)	(43.7%)	(93.7%)	(87.5%)	(43.6%)	-
GROUP F	-	1	9	3	2	1
10	-	(10%)	(90%)	(30%)	(20%)	(10%)
GROUP G	-	3	9	5	1	-
10	-	(30%)	(90%)	(50%)	(10%)	-
TOTAL 217	4	25	142	156	30	2
TOTAL PERCENTAGE RESISTANCE[1]	(1.8%)	(11.5%)	(65.4%)	(71.9%)	(13.9%)	(0.9%)

FIG. 4 ANTIMICROBIAL SENSITIVITY PATTERN OF THE VARIOUS GROUPS OF BETA HAEMOLYTIC STREPTOCOCCI



The bar diagram in figure 4 shows the general trend of all serological groups A, B, C, D, F and G to be resistant to tetracycline and co-trimoxazole. Groups A, B, C, F and G streptococci are shown to be sensitive to penicillin, while group D streptococci show resistance to penicillin.

6.5 Statistical Analysis

A chi-square test with Yates correction was used in comparing U.T.H. results with those of Britain and the United States. A P value of less than five percent (0.05) was considered significant for all the statistical tests.

CHAPTER SEVEN

DISCUSSION

The classification of beta-haemolytic streptococci (initially based on haemolytic and biochemical properties) now requires serotypic techniques for a more definitive identification. This serotypic analysis, first described by Lancefield (1933), is based on the polysaccharide antigen present in streptococcal cell walls. Lancefield demonstrated distinctive antigenic differences in the cell wall carbohydrates of the beta-haemolytic streptococci. She related these findings to the different biological and pathogenic characteristics that allow these micro-organisms to be grouped serologically, currently into group A - U. Today, the presence of group-specific antigens has been conventionally accepted by microbiologists as the primary taxonomic criterion for those species of streptococci possessing such antigens.

Before 1940 group A streptococci were known to be the cause of most severe streptococcal infections, including puerperal sepsis, neonatal sepsis and meningitis, nosocomial wound and burn infections, and streptococcal pharyngitis, with suppurative and non-suppurative complications (Thomson and Glazebrook 1942, Coburn 1943, Nyhan and Fonsek 1958, Ryan 1984).

During the period 1940 - 1950 Rantz (1942) and Foley (1947) reported on the frequent isolation from clinical specimens of streptococci belonging to serologic groups other

than A. From 1958 onwards, group B became the principal streptococci of neonatal disease (Howard and McCracken, 1974, Parker, 1977). Group D streptococci were found to be not a infrequent cause of urinary tract infection and endocarditis (Feingold et al, 1966, Savitch, 1975). Groups C, F, G and R streptococci have also been found to be clinically significant as shown by a review of the literature.

In the Microbiology laboratory at the University Teaching Hospital, Lusaka (U.T.H.) beta-haemolytic streptococci are frequently isolated from clinical specimens. However, no serological grouping is done due to lack of reagents. In this study 239 isolates of beta-haemolytic streptococci were definitively categorised into specific groups using "Streptex" latex agglutination test kit. The "Streptex" latex agglutination test is a slide agglutination test, which has a distinct advantage over conventional extraction and capillary precipitation tests because of its simplicity and rapidity of identification with equal accuracy (Castle 1982). "Streptex" is able to identify groups A, B, C, D, F and G which are the commonest groups encountered in clinical specimens. All 239 streptococcal isolates were identified as A, B, C, D, F or G. There were no ungroupable isolates. There were a few cross reactions with groups G and D, but these were specifically identified by carrying out biochemical tests, the bile-aesculin test and the salt-tolerance test (Facklam and Carey 1985).

Out of 33 beta-haemolytic streptococci isolated from cases of pharyngitis and acute tonsillitis, 18 (55%) were

found to be of group A, 6 (18%) of group C, 4 (12%) of group G. Group A streptococci are well-known causative agents of upper respiratory tract infection, responsible also for post-streptococcal sequelae rheumatic fever and acute glomerulonephritis (Rantz et al, 1951 Siegal et at 1961, Denny et al, 1964).

Group C streptococci have been documented as a cause of pharyngitis followed by acute glomerulonephritis. Duca et al, (1969) and Barnham (1983) reported two milk-borne epidemics of pharyngitis due to group C streptococci followed by acute glomerulonephritis in some of the patients affected, in Romania and Britain respectively. Group G streptococci have been reported in association with three epidemics of pharyngitis in Seoul, Korea, Czechoslovakia and eastern United States (Kang et al, 1958, Kahlich 1964, Hill et al, 1969[2]). However, there is no report documented in the literature of post-streptococcal sequelae following infections with group G streptococci. It has been suggested that the presence of a membrane antigen in group A streptococci is responsible for glomerulonephritis and rheumatic fever (Kaplan and Svec 1964). Freimer (1963) has shown that although there are antigens present in the membranes of group A streptococci that are unique to this group, there are also cross-reacting antigens in the membranes of groups A, C and G that may cross-react with absorbed sera in the fluorescent antibody technique (FAT) for streptococcal grouping (Cherry and Moody 1965). Heart tissue reactive antibodies present in anti-streptococcal sera were found to be absorbed by membrane

antigens present in groups A, C and G streptococci (Zabriskie and Freimer 1966). The above evidence suggests that although group G streptococci have not been documented as causative agents of sequelae of streptococcal infection, they may be potential causative agents of these sequelae.

One isolate obtained here, in the UTH study was found to be in group D (3%) and 4 other isolates in group F (12%). These two groups of streptococci have not been documented in the literature as established causes of upper respiratory tract infections.

On studies of throat-swabbing of school children, it has been found that there is an increased prevalence of non-group A beta-haemolytic streptococci. El Kholy et al, (1973) found that 70% of the beta-haemolytic streptococci isolated from throat swabs of Egyptian School-Children were non-group A. In the U.S.A. the percentage of non-group A beta-haemolytic streptococci from throat swabs is lower. The studies of Quinn and Federspiel (1973) showed an increase in the percentage of strains that are non-group A from 16% in 1953 - 1955 to 37% in 1961 - 1967. In the present study carried out at the U.T.H., Lusaka (1986-87) the percentage of non-group A strains from throat swabs in upper respiratory tract infections was found to be 45%. The increase in the proportion of non-group A strains among beta-haemolytic streptococci emphasizes the importance of serological grouping and also the use of antibody tests in providing proof of infection with group A streptococci.

However, an antistreptolysin 'O' response may occur during

infection with groups A, C or G and there is insufficient information available about the group specificity of many of the other streptococcal antibody tests (Wannamaker 1979). Even antibody to the group A carbohydrate may not be entirely group specific in human sera (Aasted et al, 1979). This distinction is important in studies relying primarily on antibody responses as presumed proof of infection with group A streptococci (Bisno et al, 1977).

Ryan (1984) has published data suggesting that 80% of upper respiratory tract infection in all age groups are due to viruses. A study carried out by Glezen et al, (1967) on the causative agents of acute pharyngitis, showed that viruses were the most important cause of pharyngitis in infants, group A streptococcal infections were common in the 6 - 8 age group and Mycoplasma pneumoniae was associated with pharyngitis in early adolescence. Disease produced by viruses or by Mycoplasma pneumoniae cannot be distinguished clinically from classical streptococcal pharyngitis. A recent study on streptococcal pharyngitis (Komaroff et al, 1983) showed rises in antibody titres for mycoplasmas and chlamydiae as well as streptococci in the serum of patients with pharyngitis. During the period of the present study at the U.T.H. (1986-87), beta-haemolytic streptococci were isolated from 54% of cases of pharyngitis (Table 1). If serological grouping and antibody studies were to be done on all these streptococcal isolates, a much smaller percentage would be found to be the actual causative agents of pharyngitis. Other causative agents were not isolated, due to lack of facilities, at U.T.H.

Pharmaceutical companies and some medical workers are now advocating the use of erythromycin for the treatment of pharyngitis as it would be effective in treating infections due to mycoplasma and chlamydiae as well as streptococci.

Another important factor that should be considered in the evaluation of streptococci as causative agents of acute pharyngitis is that of asymptomatic carriers. The differentiation of an active infection from concomitant non-streptococcal pharyngitis in a streptococcal carrier is a difficult diagnostic problem. Carrier rates of streptococci in children were found to range from 10 - 40% depending on age of children studied and time of year (Cornfield and Hubbard, 1961). In a study to differentiate active infection from the carrier state in the symptomatic child (Kaplan et al, 1971) it was found that only 43% of children with paired sera from whom group A streptococci were recovered, showed a significant antibody response to either streptolysin 0 and/or streptococcal deoxyribonuclease B. In patients from whom group A was recovered, the presence of anterior cervical lymphadenitis correlated best with the development of a rise in streptococcal antibodies. In the relatively large proportion of patients who failed to show an increase in titre of antibody response, the group A streptococci isolated reflected previous rather than current infection.

Asymptomatic pharyngeal carriage of group C streptococci in humans has been found to be 2.7% (Hare 1940). Asymptomatic

pharyngeal carriage of group G streptococci has been reported in 23% of humans (Hill et al, 1969 [2]).

Streptococcal isolates from eleven cases of peritonsillar abscess were all found to belong to group A. Some of these isolates were found as mixed growths with anaerobic organisms such as Bacteroides spp. The incidence of peritonsillar abscess at the U.T.H. is currently fairly high as shown also by a study carried out by Kocka et al, 1986 (personal communication) where 62 cases were seen during a period of one year. Whereas Herzon (1984) reported 41 cases during a period of five years at the New Mexico Hospital, Albuquerque where the patient load however, may be much less than at the U.T.H. According to Schmit (1972) during the pre-antibiotic era (1934 - 1936) parapharyngeal abscesses and suppurative cervical adenitis accounted for 13% of hospital admissions for streptococcal disease. After the availability of penicillin (1948 - 1950) these illnesses accounted for only 1.4% of admissions.

TABLE 6

SEROLOGICAL GROUPS OF BETA-HAEMOLYTIC STREPTOCOCCI ISOLATED FROM THE VARIOUS CLINICAL SOURCES IN U.T.H., LUSAKA (AUG. 1986 - OCT. 1987) COMPARED WITH U.K. (DEC. 1969 - DEC. 1971)

TABLE 6

U. T. H. (AUG. 1986 - OCT. 1987)						U. K. (DEC. 1969 - DEC. 1971)					
SOURCE	SEROLOGICAL GROUPS NUMBER ISOLATED (PERCENTAGE					SOURCE	SEROLOGICAL GROUPS NUMBER ISOLATED (PERCENTAGE)				
	A	B	C	G	F + D		A	B	C	G	NG
URT n=44	18 (66%)	- -	6 (13.6%)	4 (9%)	5 (11.4%)	URT n=1773	919 (51.7%)	153 (8.6%)	363 (20.4%)	134 (7.6%)	207 (11.7%)
GUT n=62	6 (9.7%)	29 (46.8%)	8 (12.9%)	1 (1.6%)	18 (29%)	GUT n=673	29 (4.3%)	479 (71.2%)	55 (8.2%)	39 (5.8%)	71 (10.5%)
WOUNDS + EXUDATES n=129	117 (90.6%)	- -	- -	7 (5%)	5 (3.6%)	WOUNDS + EXUDATES n=1502	1188 (79.1%)	65 (4.3%)	60 (4.0%)	110 (7.3%)	79 (5.3%)

On comparing the results of serological grouping of beta-haemolytic streptococci isolated from specimens of the upper respiratory tract with those of a similar study carried out in U.K., (Pollock and Dahlgren 1974), (Table 6), the highest percentage of isolates was found to belong to group A in both studies (Table 6). Twenty nine out of 44 isolates of beta-haemolytic streptococci (66%) were identified as group A in the U.T.H. study and 916 out of 1773 isolates (51.7%) in the U.K. study. The higher percentage in the U.T.H. study may be due to the inclusion of eleven isolates found in peritonsillar abscesses.

The clinical significance of the isolates was not considered in the U.K. study. The percentage of groups C and G isolates in the two studies were more or less similar (Table 6). There were no group B isolates from the upper respiratory tract in the U.T.H. study while in the U.K. study there were 8.6% of group B streptococci. In the U.K. study, grouping of only A, B, C, and G streptococcal isolates was carried out and the 11.7% of strains that were non-groupable were found to correspond with the percentage of strains in groups F and D (11.4%) grouped by "Streptex" in the U.T.H. study.

On serological grouping of 62 beta-haemolytic streptococci isolated from the genito-urinary tract (GUT), it was found that the highest number of strains, being 29 (49.8%) belonged to group B. Of these 13 strains were isolated from amongst the normal microbial flora of the GUT of female patients (mainly from the vagina). Four strains were isolated from

patients with pelvic inflammatory disease (PID), four from patients with vaginal discharge and one from a patient with puerperal sepsis. That group B streptococci could be isolated from the human vagina has been reported since 1935 (Lancefield and Hare 1935). Baker and Barret (1973) found that up to 30% of normal adult females carried group B streptococci in the vagina. Christensen et al, (1974) found group B streptococci to be the commonest streptococci in the male urethra and the female lower GUT in patients under examination for gonorrhoea. Group B streptococci have been reported as a not infrequent cause of post-partum or post-abortal sepsis and a common cause of neonatal sepsis (Eickhoff, et al, 1964). The presence of group B streptococci among the normal flora of the vagina and as causative agents of urinary tract infections and septicaemia in female patients and in neonatal sepsis have been reported (Feingold et al, 1966, Mhalu 1976). These observations suggest that the vaginal flora are an important reservoir of group B streptococci that cause disease. However, other studies have shown that the bowel is the main carriage site of the micro organism (Hoog Kamp - Korstanje et al, 1982). According to Dillon et al, (1982) the intestinal tract appears to be a primary reservoir for group B streptococci and the likely source of vaginal or urinogenital colonization in pregnant women. The prevalence of group B streptococci in the gastrointestinal tract in patients at the U.T.H. is not known, as streptococcal cultures are not carried out on routine stool cultures for enteric pathogens.

In the present study at the U.T.H., three isolates of group B streptococci were from cases of neonatal meningitis

(Table 4). During the period of this study, these three group B streptococci (1.6%) were the only beta-haemolytic streptococci isolated out of 188 culture-positive cerebrospinal fluid (CSF) samples, (Table 1). However, four years ago, 13 beta-haemolytic streptococci were isolated from the CSF of neonates with meningitis and two from infants, a total of 15 beta-haemolytic streptococci out of 145 culture positive cerebrospinal fluids (10.3%). These beta-haemolytic streptococci were probably mostly group B streptococci, but no serological grouping was carried out at that time. The reason for the higher percentage of isolation of beta-haemolytic streptococci in 1984 as compared to the period of the present study is probably due to the fact that the number of cerebrospinal fluid samples received for culture in the laboratory in 1984 was 2370, almost double the number received in subsequent years, 1985, 1986, 1987. The decrease in the number of CSF specimens received during the last three years is probably attributable to the lack of sterile containers and other facilities required for the collection of CSF.

The emergence of group B streptococci as important pathogens of neonatal disease since 1958 is well known, (Parker 1977). Different strains of group B streptococci are found to cause either "early onset" or "late onset" neonatal meningitis (Franciosi et al, 1973). However, a predominance of type III strains in both "late" and "early" neonatal meningitis and "late" septicaemic cases has also been observed (Baker and Barret 1973). The source of infection for neonates is usually the female genital tract (Franciosi et al, 1973),

Hoog Kamp Konstanje et al, 1982). A recent report describes a simple technique for differentiating type III strains (and possibly other serotypes) isolated from infected and asymptomatically colonized infants on the basis of the growth response of the strains in medium containing high levels of phosphate. This growth response appears to be related to the levels of lipoteichoic acid (LTA) expressed by clinical isolates. By identifying carriers of potentially virulent strains of group B streptococci and selective intrapartum treatment with appropriate antibiotics, the incidence of infections caused by group B streptococci might be drastically reduced (Maurer and Mattingly 1988).

The four isolates of group B streptococci from cases of pelvic inflammatory disease and four isolates from cases of vaginal discharge, were the predominant organisms isolated on culture of specimens of these eight high vaginal swabs. Although group B streptococci are not established causative agents of PID or vaginal discharge they may play a role in contributing to these infective processess which are usually polymicrobial infectionsof exogenous and endogenous agents. In a study carried out at the U.T.H. by Hira et al, 1986 (personal communication) it was found that six out of 39 (11.6%) of bacterial isolates from cases of PID were beta-haemolytic streptococci. Although no serological grouping was carried out most of these isolates were probably group B streptococci.

TABLE 7

SEROLOGICAL GROUPS OF BETA-HAEMOLYTIC STREPTOCOCCI ISOLATED IN RELATION TO CLINICAL DIAGNOSIS AT U.T.H., LUSAKA (AUG. 1986 - OCT. 1987) COMPARED WITH U.S.A. (APR. - AUG. 1966)

TABLE 7

U. T. H. (AUG. 1986 - OCT. 1987)							U. S. A. (APR. - AUG. 1966)						
CLINICAL DIAGNOSIS	SEROLOGICAL GROUPS NUMBER ISOLATED (PERCENTAGE)						CLINICAL DIAGNOSIS	SEROLOGICAL GROUPS NUMBER ISOLATED (PERCENTAGE)					
	A	B	C	D	F	G		A	B	C	D	F	G
UTI	-	8	1	4	2	-	UTI	1	12	1	27	-	-
n=15	-	(53.3%)	(6.7%)	(26.7%)	(13.3%)	-	n=41	(2.4%)	(29.3%)	(2.4%)	(65.9%)	-	-
INFECTED WOUNDS + EXUDATES n=129	117	-	-	5	-	7	INFECTED WOUNDS + EXUDATES n=95* L-1*	53	1515	9	8	1	9
	(90.6%)	-	-	(3.6%)	-	(5%)		(55.8%)	(15.8%)	(9.5%)	(8.4%)	(1.1%)	(9.5%)

Out of 15 beta-haemolytic streptococci isolated from urinary tract infection, eight were found to belong to group B, (55.3%), seven of which were from female patients. Four isolates were in group D (26.7%). In a study carried out at the Massachusetts General Hospital in the U.S.A. (Feingold et al, 1966), 12 out of 41 streptococcal isolates (29.3%) were group B, while 27 isolates were group D (65.9%) (Table 7). Eleven of the twelve isolates of group B streptococci were from female patients. On comparing the results of the two studies (U.T.H. Lusaka and U.S.A.) there was no significant difference between the number of group B isolates from urinary tract infections (P=0.117). However, there was a significantly smaller number of group D isolates from urinary tract infections in the U.T.H. compared with the U.S.A. (P=0.0021).

The reason for this is probably that group D streptococci may be alpha, beta or gamma (non-haemolytic) and in the U.T.H. study only beta-haemolytic streptococci were grouped serologically, while in the U.S.A. study all streptococci were grouped.

On comparing the results of serological grouping of streptococcal isolates from the GUT at the U.T.H., Lusaka with those of a study carried out in the U.K. (Pollock and Dahlgren 1974) (Table 6), it was found that 46.8% of the isolates were group B and significantly different from the U.K. result (71.2%) (p=0.0001). The reason for this may be that many more specimens from the female genital tract were included in the U.K. study. However, the sex and clinical condition of the patients were not considered in the U.K. study.

In the current study at the U.T.H., small numbers of groups A, C, G and F were isolated from the GUT both among the normal flora and as causative agents of infection (2.13%) These results were similar to the results of the two studies in the U.K. and U.S.A. (Table 6 and 7) except that group F was absent in urine cultures in the U.S.A. study and group F was not grouped in the U.K. study.

During the period of this research 288 beta-haemolytic streptococcal isolates were obtained from 1770 culture-positive pus swabs (16.3%) as shown in Table 1. This represents a slight increase in the percentage of beta-haemolytic streptococ isolated according to the results of a study carried out by Pearsall and Perera (1985) where the percentage isolated was found to be 6% in 1980 and gradually increasing to 11% in 1984. The increase in percentage of beta-haemolytic streptococcal isolates may reflect the general deterioration of hospital infection control practices due to the lack of disinfectants and antiseptics.

The 11.5% of group A streptococci isolated from burn wounds (Fig. 2) is fairly high. According to most authors, Streptococcus pyogenes was formerly very commonly isolated from burn wounds and other infected wounds, which are mostly nosocomial infections, but now due to their high susceptibility to penicillin they are no longer a problem (Dineen 1972, Ryan 1984). According to a study on burn microbiology done by Pruitt et al, (1984) out of the bacteria isolated, members of the genus Staphylococcus persisted at a more or less constant level in the burn wound flora, but individual strains

of staphylococci and members of the gram negative flora showed a marked variation in prevalence. Beta-haemolytic streptococci were not isolated. Biopsy sampling of burn wounds has been recommended as the best available means of monitoring the microbial status of a burn wound and diagnosing the presence of infection in such wounds (Pruitt and Foley 1973). Although in the present study at the U.T.H., only surface cultures were done, the presence of Streptococcus pyogenes is still significant as they may cause rejection of skin grafts on such wounds. There is no separate Burns Unit at the U.T.H. In a study carried out in a surgical ward at the U.T.H., it was found that 19% of the ward staff were carriers of beta-haemolytic streptococci (probably Streptococcus pyogenes). In a study by Ndinya-Achola and Mbalu (1987) on the pattern of burn wound infection in the first year of operation of a newly opened Burns Unit in Kenyatta National Hospital, Nairobi, 12.9% of the bacterial isolates were Streptococcus pyogenes.

In the present study cultures of pus swabs from all sites yielded mixed growths. From burn wounds the commonest combination of organisms was Streptococcus pyogenes with Staphylococcus aureus. Members of the Enterobactericeae were also found as mixed growths with Streptococcus pyogenes.

All ten streptococcal isolates from cases of acute and chronic osteomyelitis were group A streptococci. The causative agents of osteomyelitis are usually age-related, with the commonest organism isolated being Staphylococcus aureus in any age group (Ray, 1984. Infection due to Streptococcus pyogenes is usually found after trauma or surgery. In the U.T.H. too,

Staphylococcus aureus is the commonest organism isolated from cases of osteomyelitis. The group A streptococci isolated were 8% of all culture positive pus swabs from cases of osteomyelitis (Fig. 2). Many of them were from cases of chronic osteomyelitis and the group A streptococci were often found in mixed growth with Staphylococcus aureus. Some of these infections at the U.T.H. may be nosocomial infection of wounds in cases of chronic osteomyelitis.

One isolate from a case of septic arthritis was found to be group A. The causative agents of septic arthritis are also usually related to the age of the patient and infection due to Streptococcus pyogenes is usually found in children of one month - 4 years of age (Ray 1984); however the patient in this study was an adult female. The other streptococcal isolate from septic arthritis was group G. This patient was an HIV-positive patient presenting with Kaposi's sarcoma. Septic arthritis due to group G streptococci has been reported previously in a chronic alcoholic (Fujita et al, 1982). An association of group G streptococcal bacteraemia with malignancies has been demonstrated in some studies (Duma et al, 1960, Auckenthaler et al, 1983 and Dickie et al, 1984). It may be that the immuno-compromised state of the HIV-positive patient contributed to bacteraemia with group G streptococci, resulting in septic arthritis.

The highest percentage of group A isolates of all culture-positive swabs was from cases of pyoderma (67%) (Fig. 2). Streptococcal pyoderma usually occurs in the 2-5 year age group (Dillon 1979). In the present study all eight isolates

from cases of pyoderma, including cases of superficial cellulitis and impetigo were group A streptococci and the age incidence ranged from 3 years to young adult. Most streptococc isolates were found in combination with Staphylococcus aureus. In research carried out by Mhalu (1973), 52% of bacterial isolates from superficial skin infections in Tanzanian children were beta-haemolytic streptococci, 92% of which were group A, 2% group C and 6% ungroupable. In Mhalu's study, Streptococcus pyogenes was often found together with Staphylococcus aureus in mixed cultures.

On comparing the results of the U.T.H. study with those in the U.K. and U.S.A. (Table 6 and 7), serological grouping of streptococcal isolates from infected wounds and exudates showed that in the U.T.H. 91.4% of isolates were group A, while 79.1% were group A in the U.K. study and 55.8% were group A in the U.S.A. study. In all three studies, the highest percentage of streptococcal isolates was group A, but the percentage in the U.T.H. study was significantly higher than in either the U.K. ($P=0.0007$) or U.S.A. study ($P=0.0001$).

The reason for this could be that in the U.T.H., being a hospital in a developing country, due to the lack of a continuous supply of disinfectants and antiseptics, and the lack of other facilities for hospital infection control, the nosocomial infections due to Streptococcus pyogenes may be higher

Also on comparing the results of the three studies, it is seen that groups B and C streptococci are absent in wounds and exudates at the U.T.H. while they are present in the other two studies 4.3% and 4% in the U.K. study, and 15.8% and 9.5%

in the U.S.A. study. Group B streptococci are frequently isolated from wounds in ischaemic regions, or in peripheral vascular disease (Eickhoff et al, 1964, Feingold et al, 1966). Together with group D they are involved in wounds in the perineal area. Their absence from wounds in the U.T.H. study may be due to the fact that the type of lesion from which group B streptococci are usually isolated was absent.

On studying the antimicrobial sensitivity pattern of 217 isolates of beta-haemolytic streptococci, it was found that groups A, B, C, F and G streptococci were 100% sensitive to penicillin G. That the beta-haemolytic streptococci of group A have remained highly sensitive to penicillin G with a minimum inhibitory concentration (MIC) of 0.0073 ug/ml over a period of thirty two years was reported by Boureau and Campos (1982). The continued susceptibility to penicillin G of groups B, C and G has also been reported (Jacobs et al, 1982, Rolston et al, 1982).

In a study of streptococcal pharyngitis, (Gastanaduy et al, 1978), the recovery of group A streptococci of the same serologic type was documented on follow-up throat cultures in 20% of patients treated with intramuscular benzathine penicillin in doses recommended by the American Heart Association. After second and third courses of therapy even higher frequencies of treatment failures were observed. These group A streptococci were very sensitive to penicillin in vitro. These failures may be due in part to the inclusion of some chronic carriers of group A streptococci who presented with intercurrent non-streptococcal infection, and it is well known that the group A

streptococcal carrier state may be difficult or impossible to eradicate by treatment with antibiotics (Wannamaker 1979).

Resistance of group A streptococci to erythromycin was found to be infrequent (3%) by Boureau and Campos (1982). At the U.T.H., the resistance of group A streptococci to erythromycin was found to be 8%. Resistance to erythromycin is known to be associated with M types 4 and 12 of group A streptococci (Youngs 1984, Walker et al., 1984). Rapid increases in resistance to erythromycin were recorded between 1971 and 1974 in some areas of Japan (Miyamato et al., 1978) and resistance as high as 60%, also in Japan (Marnyama et al., 1979). Increase in resistance of group A streptococci to erythromycin was also reported in Canada (Dixon and Lipinski 1974). In the United States resistance to erythromycin was reported as 3% (Arthur et al, 1984) and in Britain, 0.3% (Youngs 1984). The transduction of plasmid-mediated resistance to erythromycin, between group A and group G streptococci (Skjold and Wannamaker, 1986) provides a possible explanation for the increasing prevalence of erythromycin-resistance among different serological groups and among multiple serological types of group A streptococci in some countries. The incidence of group A streptococcal M types resistant to erythromycin may also increase under selective pressure from this drug.

Resistance of groups A and B streptococci to co-trimoxazole is well known and is used in the laboratory for the presumptive identification of these two groups from other beta-haemolytic streptococci (Facklam et al, 1979). However at the U.T.H., besides groups A and B which showed 73-78% resistance, the other

groups of beta-haemolytic streptococci, C, D, F and G also showed a high percentage of resistance to co-trimoxazole.

Streptococcal group A isolates at the U.T.H. showed 51% resistance to tetracyclines while all the other groups B, C, D, F and G showed 90-95% resistance. Tetracycline resistance became prevalent in the United States from about 1960 (Kuharic et al, 1960), also Britain (Parker et al, 1962) and several other countries. Resistance of group A streptococci to tetracycline reflects the indiscriminate use of this agent.

Group D enterococci were found to be multi resistant to all antimicrobial agents studied, penicillin G, erythromycin, tetracycline, co-trimoxazole and chloramphenicol, except ampicillin, to which it was 100% sensitive. This antimicrobial sensitivity pattern of group D enterococci has been documented also in other studies. Combination therapy with penicillin and an aminoglycocide is given in serious infections due to group D enterococci and sometimes even in non-enterococcal group D infections, Savitch et al, 1978, Moellering et al, 1979).

Four percent of group B streptococci were resistant to ampicillin. Reports of penicillin tolerant strains of group B streptococci (Siegal et al, 1981) may indicate combined therapy also in treatment of serious infections due to group B streptococci.

Resistance to antimicrobial agents may be chromosomal or plasmid-mediated. Resistant strains originate from a small proportion of members of a species before the introduction of an antimicrobial agent and the proportion is increased by the use of the antimicrobial. Resistance is

also due to mutation or recombination in the nucleic acid complex. The origin of plasmid-mediated determinants of resistance may have existed before the clinical use of the antimicrobial agent, playing a role in nature to protect an organism against another that produced the agent, or the cell from its own antibiotic. Some may have been chromosomal genes transposed to plasmids, and some plasmid genes that mutated to provide altered specificity. The increase in resistant strains is due mainly to the selective effect of the use of antimicrobials in therapy and prophylaxis, antimicrobial contamination of the environment in human and veterinary hospitals and antimicrobial processing factories, and the use of antimicrobials in animal feeds. The increase and spread of resistant strains of bacteria is also due to spread of infectio in human societies, the transfer of bacteria from animal microbial populations to man, and the ability of plasmids to cross bacterial species lines (Sherris 1984).

Some of the strategies to be used in the control of resistant strains of streptococci and other bacteria in the U.T.H. would be the conservative and specific use of antimicrobials in therapy, with adequate dosage and duration of therapy to eliminate the infecting organism and reduce the risk of selecting resistant variants. The prophylactic use of antimicrobials only in situations in which it has been proved valuable and for the shortest possible time to avoid selection of a resistant flora; avoidance of environmental contamination; application of aseptic and hand-washing procedures to help prevent spread of resistant organisms; restriction of the use

of therapeutically valuable antimicrobials for non-medical purposes; and lastly, when information is available on local antimicrobial use and bacterial resistance, the results should be included in a hospital antibiotic policy (Phillips 1979). A Sterilization and Disinfection Policy, would help in preventing cross-infection of patients in the hospital.

SUMMARY AND CONCLUSIONS

On studying the serological groups of beta-haemolytic streptococci in relation to clinical manifestation of infection at the U.T.H. Lusaka, it was found that group A streptococci were responsible for infections of the upper respiratory tract, pyoderma, infection of burn wounds and other wounds, osteomylitis, and occasionally also septic arthritis and vaginal discharge. Group B streptococci were found as part of the normal microbial flora of the vagina and also were the cause of infections in the genito-urinary tract (mainly in females), puerperal sepsis and neonatal meningitis. Group C streptococci caused infections of the upper respiratory tract and genito-urinary tract. Group D streptococci caused genito-urinary tract infections, infections of wounds and also bacterial endocarditis. Group F streptococci, infections of the genito-urinary tract. Groups A, C, D and F streptococci were also found among the normal flora of the genito-urinary tract, although the highest percentage was of group B. Group G streptococci caused upper respiratory tract infections, infections of wounds and also septic arthritis.

On comparing the results of this research with those of studies in the U.K. and U.S.A., the percentage of streptococcal groups isolated from the various clinical sources and clinical conditions were largely similar, except that

in the U.T.H. Lusaka there was a higher percentage of group A streptococci in infected wounds. Also group B streptococci were absent in the upper respiratory tract and in infected wounds in the U.T.H. study but were found in these locations in the other studies.

On studying the antimicrobial sensitivity pattern of 217 streptococcal isolates, it was found that streptococci of groups A, B, C, F and G were resistant to penicillin G (25%). Penicillin G remains the drug of choice for all groups of streptococci except group D enterococci, with erythromycin for penicillin allergic patients. Group D enterococci were consistently sensitive to ampicillin. All streptococcal groups A, B, C, D, F and G showed high resistance to co-trimoxazole and tetracycline and these antimicrobial agents should be avoided for treatment of infections due to the beta-haemolytic streptococci.

Serological grouping of beta-haemolytic streptococci isolated from clinical specimens is important especially for determining the coice of antimicrobials in treatment and duration of treatment, for tracing the source of infection, and control of infection. However in a busy clinical laboratory, routine serological grouping of beta-haemolytic

streptococci is not practicable and is too expensive. Since group A streptococci are established causative agents of U.R.T.I. followed by rheumatic heart disease which is preventable by treatment with penicillin G. or penicillin V for a period of ten days, bacitracin screening for group A streptococci should be carried out.

The significantly higher percentage of group A streptococci isolated from infected burn wounds, and other wounds in surgical wards at U.T.H. as compared with the percentage isolated in the U.S.A. and U.K. studies is probably due to nosocomial infections.

One of the commonest clinical conditions of patients presenting at the Out Patient Department is upper respiratory tract infection. The general tendency of medical and clinical officers to prescribe tetracycline or co-trimoxazole as alternatives to penicillin should be prohibited, since the main bacterial pathogen in upper respiratory tract infections, namely Streptococcus pyogenes, shows a high percentage of resistance to these two agents. The antimicrobial agents of choice for upper respiratory tract infections should be penicillin G or penicillin V with erythromycin for those patients allergic to penicillin.

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Finally it would be interesting to find out from further studies the true prevalence of group B streptococci causing neonatal meningitis, their relation to maternal carriage, the serotypes involved, and to explore the possibilities of preventing neonatal colonization of "pathogenic" serotypes by chaemotherapy of the pregnant mother.

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