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MEETING ON THE ASSESSMENT AND FURTHER DEVELOPMENT OF THE WHO PROGRAMMES ON STREPTOCOCCAL DISEASES AND MENINGOCOCCAL INFECTION

(Geneva, 10-13 October 1988)

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The Meeting for the Assessment and further Development of the WHO Programmes on Streptococcal Diseases and Meningococcal Infection was held in Geneva from 10 to 13 October 1988. Dr T. Bektimirov, Assistant Director-General, opened the meeting on behalf of the Director-General. Dr J. Rotta was elected Chairman, Professor K. Prakash, Vice-Chairman, and Professor E.L. Kaplan, Rapporteur.

The purpose of the meeting was to review current worldwide trends in meningococcal and streptococcal infections and their sequelae, to identify and evaluate specific approaches towards better control of these diseases, to evaluate and recommend improved and new laboratory technology for their diagnosis, and to make recommendations to WHO for initiating and coordinating particular projects in the field.

I. STREPTOCOCCAL DISEASES

1. Group A streptococcal infections and their sequelae

Throughout the past decade WHO collaborative studies have been successfully carried out to provide guidance for the development of programmes for the control of group A streptococcal (GAS) infections and rheumatic fever/rheumatic heart disease. However, group A streptococcal infections and rheumatic fever still constitute a major public health problem in developing and developed countries alike. In the countries of the developing world, knowledge about the epidemiology of GAS infections is incomplete, although some progress has been made over the last decade.

In November 1983 an extensive review of the group A streptococcal disease complex was undertaken by an Advisory Committee[1] which made important recommendations regarding the orientation of public health programmes. Many of these programmes have since been implemented.

There have been reports of an increasing number of cases in which certain antibiotics (e.g., penicillin) have failed to eradicate the group A streptococcus from the upper respiratory tract.[2] The reasons for this are not fully understood. There are also indications in some countries of increasing resistance of group A streptococci to other antibiotics (e.g., erythromycin).

Rheumatic fever and rheumatic heart disease continue to be a major cause of cardiovascular mortality in the world today. Rheumatic heart disease, according to the 1988 report of a WHO Study Group on Rheumatic Fever and Rheumatic Heart Disease[3] remains, in the developing countries, the most common cardiovascular cause of death in the first four decades of life and accounts for approximately half of all cardiovascular disease. There is reason to believe that the urbanization that is occurring in the developing world, will lead to further increases in the number of streptococcal infections with these nonsuppurative sequelae. Rheumatic fever incidence figures of 20 per 100,000 population per year are not uncommon and a recent report has indicated a 10% prevalence of rheumatic heart disease in some Brazilian schoolchildren.[4] High incidence and prevalence figures are also available for other countries.[3] Streptococcal infections occur primarily in urban areas, and less is known about their incidence in rural areas in developing countries.

One of the most significant indications that these infections are still a major public health problem is the recent resurgence of rheumatic fever in North America.[5] Of special interest in assessing the current trends of streptococcal infections in the world today and in planning their control is the fact that the mid-1980s outbreak of rheumatic fever in the United States occurred in middle class populations with ready access to medical care. Furthermore, the majority of the preceding streptococcal upper respiratory tract infections were mild or even asymptomatic. This indicates that while

antibiotics are effective in both primary and secondary prevention, they are not the complete solution. Intensified research of the GAS biology with ultimate understanding of the pathogenesis of the nonsuppurative sequelae of this infection is required for more effective control measures. An understanding of the pathogenesis may contribute to the development of a vaccine that may ultimately be a more effective method for the control of rheumatic fever.[6]

Although accurate and complete data are not available from all the developing countries, it is clear that GAS skin infections (pyoderma) are also a major problem especially among children. Skin infections due to group A beta-haemolytic streptococci even reach 20% or more of children during certain seasons of the year. The occurrence of acute glomerulonephritis is associated with the spread of nephritogenic GAS serotypes in the community.

Epidemiological studies have shown that organisms that infect skin are primarily of the higher M types when compared with M types isolated from throat infections. As a general rule, it may be said that these streptococci are less rich in M protein than respiratory tract strains.

2. Other streptococcal infections

2.1 Group B streptococcal infection

Although many forms of group B streptococcal (GBS) infection (e.g., bacteraemia, pneumonia and urinary tract infections) have been reported in adults, GBS remains one of the most important causes of neonatal infection in North America and Europe. The mortality in newborns with this infection may be up to 50%. No clear understanding of the relationship has been demonstrated between the prevalence of maternal rectal or vaginal carriage and the incidence of the disease in adults and infants.

Reports from Denmark[7] and Great Britain[8] indicate an incidence of infection (0.3 per 1,000 live births) of about one tenth of that found in North America, although the carriage rates do not appear to be significantly different. However, very little is known about the epidemiology of infection and colonization in humans in the developing countries of the world; the magnitude and extent of the problem have not been studied.

The results of cultures from GBS carriers vary from less than 5% using direct plating of vaginal samples, to over 30% when several samples and enrichment broth techniques have been used. Mother-infant transmission is probably linked to the heaviness of vaginal carriage. The percentage of vertical transmission reported in many European and American countries is about 50%.

The distribution of GBS serotypes causing neonatal disease has varied with geographic location. Serotype III predominates in many areas with a very significant mortality.

Although both chemoprophylaxis and immunoprophylaxis have been proposed for the prevention of GBS infections, supporting data remain incomplete. Treatment with antibiotics such as penicillin or ampicillin of <u>all</u> carrier women during pregnancy or of neonates from carrier mothers would be both impractical and expensive. Furthermore, attempts to eradicate the GBS carrier state in women during pregnancy have frequently failed. Chemoprophylaxis during labour may be more effective in protecting infants. Passive immunoprophylaxis with immunoglobulin of women in labour or neonates at birth has been suggested but important limitation for this is the selection of patients and the cost. Since neonatal immunity to GBS infection correlates with the maternal type-specific group B antibody levels, active immunization of the women with one or more of the four capsular polysaccharides has been proposed for prevention. However, its usefulness has not been confirmed.

2.2 Beta-haemolytic streptococci of groups C and G

Beta-haemolytic streptococci of Lancefield groups C and G are also causes of morbidity and mortality in humans, but less is known about their epidemiology, pathogenesis and control.

In addition to group A streptococci, beta-haemolytic streptococci of groups C and G are also frequently isolated from the upper respiratory tract. Laboratory methods for serological confirmation of these infections are not widely available, making the study and understanding of these diseases more difficult.

It is recommended that attention be directed to further understanding of the epidemiology and pathogenesis of these serologic groups of beta-haemolytic streptococci, including the development of appropriate laboratory methods for confirmation of these infections.

2.3 Non-beta-haemolytic streptococci

The role of non-beta-haemolytic streptococci (e.g., viridans streptococci, enterococci) in infective endocarditis has been recognized for many years. Recently, however, the importance of these organisms in the pathogenesis of serious and life-threatening infections in immuno-compromised patients has also become evident. Relatively little is currently understood about the epidemiology and pathogenesis of many of these infections. More attention to these infections is thus required.

3. Current status in diagnostic technology of streptococcal infections

Since bacteriological identification procedures require laboratory equipment and trained personnel, competent use and widespread availability of conventional diagnostic technology remains an unattained goal in providing optimal primary health care in a very large percentage of the world's population.

In order to reduce the problems and enhance the laboratory's role in clinical and epidemiological assessment of streptococcal infections, newer technologies have recently been introduced. For group A streptococcus, the role of the clinical/diagnostic laboratory is essential also to the control of the nonsuppurative sequelae of these infections, specifically acute rheumatic fever and acute post-streptococcal glomerulonephritis. As a possible substitute for direct culture of streptococci on blood agar plates, a number of rapid antigen detection methods are commercially available. Detection of the group specific polysaccharide of the group A streptococcus by one of several methods (e.g., latex agglutination, co-agglutination, ELISA) is most frequently employed. These rapid non-culture techniques (usually requiring 3-10 minutes) were devised to substitute for conventional techniques in specific situations. However, the conventional culture techniques should always be available at regional and central levels of the health care system (e.g., in laboratories attached to hospitals, etc.). A WHO Collaborative Study reported high sensitivity and specificity of the co-agglutination technique in patients with acute group A streptococcal pharyngitis with more than 100 colonies on a culture plate. However, in asymptomatic patients with fewer colonies of streptococci on the culture plate, the test was less sensitive.[9] In some instances with some of the commercially available reagents sensitivities have ranged only between approximately 60-90%, leading some laboratory directors to conclude that a positive rapid antigen detection test for group A streptococci likely represents a valid positive result, but a negative test requires a simultaneous culture because of the decreased sensitivity.

Improved technologies for rapid bacteria detection tests are likely to result in more sensitive tests to further improve the rapid tests and reduce the costs. Development of rapid antigen detection tests have been less successful for other beta-haemolytic streptococci such as groups B, C, G, etc. Tests for these groups have not undergone extensive trials and most are not commercially available.

Of equal diagnostic importance in the management of GAS infections, particularly in the diagnosis of their nonsuppurative sequelae (acute rheumatic fever, recurrent rheumatic fever, acute post-streptococcal glomerulonephritis), is the determination of antibodies to streptococcal antigens. The classical antibody determination has been the anti-streptolysin O (ASO) test. It is readily available, generally reproducible and standard ASO sera are available for standardization of laboratory results.

However, since it has been shown that determination of more than one antibody is advantageous in certain instances (e.g., streptococcal pyoderma), other tests have been introduced into clinical use.

Of particular interest is the anti-deoxyribonuclease (anti-DNase B) test which has many advantages over the ASO test. However, owing to the lack of an international anti-DNase B standard, difficulty in interpretation has occurred in some countries.

Recently considerable attention has been paid to the Streptozyme[R] test. This test, employing a simple agglutination technique, measures several streptococcal antibodies simultaneously. The WHO collaborative studies have revealed that at the present time the test is insufficiently reliable and cannot replace the ASO and anti-DNase B tests.[10] The concept of the test, however, is sound. To be able to measure antibodies to several group A streptococcal antigens simultaneously would be a distinct advantage. However, a more standardized, easier to read reagent is required.

Antibody to the group-specific polysaccharide can be helpful in documenting a recent GAS infection. However, new information has to be provided on the titration technique of antibody to GAS polysaccharide, the dynamics of the antibody development and the antibody in various clinical patterns of streptococcal disease in man. Such information would encourage more widespread use of the antibody test in routine clinical practice and also in epidemiological studies.

Essential to the understanding and, therefore, to the ultimate control of GAS infections is not only the identification, but also the further characterization of the streptococcal isolates. The ability to carefully characterize (type) streptococcal isolates has usually been confined to larger reference laboratories and has included typing by M-protein, by T-agglutination pattern, and by characterization of the opacity factor (OF) present in the approximately 27 serotypes of the organism that make the opacity factor.

A major obstacle to the more widespread use of streptococcal typing has been the lack of sufficient supplies of typing sera. The T-typing sera are the only commercially available ones. However, T-typing alone is usually insufficient. Neither M-sera nor OF-sera are available in sufficient supply.

Newer technology has made possible research advances in the laboratory identification of GAS, which have facilitated the wider use of the other typing techniques. These include immuno-diffusion techniques for M-typing and micro-techniques for OF typing.

4. Conclusions and recommendations

1. The accurate identification of streptococcal isolates is necessary to the ultimate control of streptococcal infections and their sequelae. Therefore, it is recommended that basic methods for the identification of streptococci be refined, including the development of new techniques, the availability of larger batteries of high-quality typing sera, and the upgrading of methods for the identification of non-groupable streptococci pathogenic to man.

2. The rapid non-culture technique for diagnosis of GAS infection has distinct clinical and public health advantages. Development of new more reliable reagents with greater sensitivity for detection of smaller numbers of bacteria is crucial to attain this goal.

It is also recommended that rapid non-culture tests be developed for haemolytic streptococci of Lancefield groups B, C and G.

3. Reliable, well-standardized streptococcal antibody tests are essential in the diagnosis of nonsuppurative sequelae of GAS infections. There is an urgent need for these reagents. It is recommended that standardized sera for use in the anti-streptolysin O and anti-deoxyribonuclease B determination be produced in sufficient amounts for distribution to appropriate reference laboratories (Annex 1).

4. Owing to difficulties in the distribution of selected biological reagents required for laboratory diagnosis of GAS infections, it is desirable that such reagents be made with appropriate quality control at regional and/or national level. It is recommended that an appropriate programme be developed to assist in the production and quality control testing by WHO Streptococcal Collaborating Centres.

5. To ensure the optimal functioning of the national streptococcal reference laboratories and to transfer new diagnostic technology, appropriate training (including training courses and printed manuals) is required. It is recommended that resources be made available to accomplish this goal, and that planning be undertaken under the direction of WHO.

6. Measurement of antibody to the group-specific polysaccharides of GAS has been shown to be a reliable method for confirming GAS infection. Preliminary observations suggest that it may have prognostic significance in patients developing rheumatic valvular heart disease. It is recommended that these aspects be carefully evaluated and that the reagents and methodology for utilization of this antibody test be made available to streptococcal reference laboratories (Annex 2).

7. Essential to the understanding of the epidemiology and pathogenesis of the non-suppurative sequelae of GAS infection is the accurate identification/typing of GAS isolated from patients with streptococcal infections and their non-suppurative sequelae and from epidemiological studies. Furthermore, such data may prove useful in the ultimate development of GAS vaccine. As there is a paucity of available anti-M and anti-opacity factor (OF) sera, it is recommended that enhanced efforts be continued to produce typing sera for distribution to the WHO collaborating centres and reference/research laboratories involved in the WHO programme (Annex 3).

8. A new improved micro-technique for OF typing has been developed which is much less labour-intensive and is adaptable to the use of human sera. It is recommended that a collaborative study be undertaken to document its efficacy and applicability for OF typing and epidemiological field studies (Annex 4).

9. Group B beta-haemolytic streptococci are known to be a major cause of neonatal meningitis and sepsis in many countries, but little is known about the epidemiology of these infections in developing countries. It is recommended that epidemiological studies be encouraged and coordinated by WHO to investigate the incidence of these group B streptococcal infections.

10. The mainstay of primary and secondary prevention of rheumatic fever is penicillin and erythromycin treatment of GAS infection. Because there is evidence of antibiotic treatment failure, it is recommended that surveillance of GAS for their sensitivity to these antibiotics be undertaken on a global basis. It is recommended that WHO initiate and supervise such a collaborative effort.

II. MENINGOCOCCAL INFECTION

1. Introduction

Neisseria meningitidis (meningococcus) is a major cause of bacterial meningitis throughout the world. Endemic attack rates in developed countries range from 1-10 per 100,000 persons and in developing countries are generally greater than 20 per 100,000 persons per year.[11,12] Epidemic disease has a worldwide distribution as well, with attack rates that may exceed 500 per 100,000 persons. The meningococcal disease burden is especially severe across sub-Saharan Africa where large outbreaks occur with a periodicity of 8 to 10 years.[13,14]

The case fatality rate of meningococcal disease commonly ranges between 5 and 20%, depending on the epidemic/endemic situation and the health infrastructure available. However, mortality figures do not completely describe the consequences of meningococcal infection. Neurologic sequelae may occur including seizures, mental retardation, hearing loss, and learning disability. The rate of neurologic complications may be 2 to 3 times the mortality rate. Non-neurologic complications also occur but the rate is less well defined. Since meningococcal disease most commonly occurs in children and young adults and the majority of mortality and morbidity occurs in this group as well, years of life lost and the impact to society of this illness is great.

Significant progress has been made during the past several decades in understanding the epidemiology and microbiology of meningococcal disease as well as its treatment and prevention. In addition to the descriptive epidemiology, there is now some understanding of risk factors for the disease and the occurrence of outbreaks. New microbiological methods have resulted in the characterization of protein antigens, thereby improving the understanding of the host immune response to the organism. Effective antibiotics for treatment and prophylaxis are available throughout the world. Vaccines effective in preventing disease caused by serogroups A, C, Y and W-135 are also available. Despite all these advances, however, epidemics continue to occur, with considerable morbidity and mortality.

The purpose of this report is to briefly review some of the recent developments related to meningococcal disease and to make recommendations for improving surveillance, diagnosis, treatment and prevention. Because most meningococcal disease occurs in developing countries, some of the recommendations will be more applicable to the situation in those countries. Furthermore, some recommendations can be acted on immediately while others require implementation over a longer term; some recommendations refer to programmes still under development.

2. Surveillance

Accurate meningococcal disease surveillance is important for the purposes of evaluating the burden of this disease within the field of purulent meningitis, identifying risk factors for illness, promptly detecting the occurrence of epidemics, applying appropriate therapy, and assessing the need for immunoprophylaxis with vaccination. There are several components to effective surveillance:

- Disease must be correctly diagnosed (section 4).
- Case reports, including epidemiological information, must be collected and analysed; and where possible:
- Isolates characterized serologically and antibiotic sensitivity determined.

Annex 5 shows an outline of an appropriate surveillance system for meningococcal infection. Current surveillance efforts fall far short of this ideal at each of the four levels in most parts of the world. It should be a highest priority of the WHO programme on meningococcal disease to improve surveillance at all levels in order to approach this objective. Specific actions to facilitate attainment of this goal should include:

(a) Identification of regional centres with epidemiological and microbiological expertise and sufficient resources to provide support to countries in the region. These centres, similar to the WHO Meningococcal Collaborating Centres in France and the Netherlands should be identified based on their interest and capabilities.

(b) Identifying and promoting the development of national centre(s) for diagnosis and surveillance of meningococcal disease and all bacterial meningitis. To accomplish this, a survey should be conducted to assess the capabilities and interest of laboratories and institutions (Annex 6).

(c) Provision of information and education to governments, laboratories and institutions concerning the importance of meningococcal disease surveillance.

(d) Promoting the development of improved techniques for transporting specimens or isolates.

(e) Support the completion of a serotyping system for meningococci, and conduct a pilot study of the prevalence of different serotypes coordinated by the regional centres.

(f) Publish an annual or semi-annual WHO meningococcal disease report for dissemination to all levels in the surveillance effort.

While the group realizes the difficulties of these tasks, initial establishment of a system will generate momentum for later improvements. However, in order to interpret results of surveillance it must be assured from the beginning that epidemiological data collected be accurate and representative.

3. Typing of meningococcal isolates

One of the most exiting new developments in the study of meningococcal disease is the ability to characterize strains beyond the level of serogroup. Strain typing is important for epidemiological surveillance, vaccine development and in efforts to better understand the virulence of certain strains, the immune response to infection and the risk factors for the development of invasive disease.

Several typing systems are currently being developed.[15] Serotyping, characterizing strains on the basis of protein antigens, can be done in a rapid and reproducible fashion using monoclonal antibodies.[16] This technique is highly relevant to epidemiological studies, investigations on pathogenesis, and vaccine development. Because this system is still being developed, antibodies are not currently available for distribution.

Clonal analyses based on differences in electrophoretic mobility of isoenzymes are being used in several laboratories. These systems are able to characterize the genotype of all isolates, even those that are non-typable and could be useful in epidemiological investigations.[17,18]

Other typing systems, such as those involving restriction endonuclease digest patterns of chromosomal DNA or ribosomal RNA are less well developed and cannot be fully assessed at this time.

Based on the analysis of these typing systems, it is proposed that:

(a) Support, including funding, be provided for completion of a serotyping system.

(b) A single centre, or two regional centres be established to maintain hybridoma cell lines and produce monoclonal typing antibodies.

(c) Typing antibodies be provided to the regional surveillance centres for use as described above. Typing antibodies could be made available to interested national laboratories.

(d) Vaccine development (using protein antigens to define serotypes) is encouraged.

(e) Further development of other investigational typing systems (ET, rRNA, DNA) is encouraged for use in epidemiological investigations.

4. Diagnosis of meningococcal infection

Common bacterial causes of meningitis after the neonatal period include *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. The relative importance of each of these organisms depends on the country, the season, the age group, etc., and whether meningococcal disease is endemic or epidemic. Diagnosis of meningococcal infection is based on the epidemiological situation, clinical presentation and culture of the organism or detection of meningococcal antigen in the laboratory. Establishment of a "case definition" is important for surveillance and epidemiological investigation. Adherence to a strict case definition, however, should not be used as a guide for therapy. Suggested definitions for suspected, probable and confirmed meningococcal disease are shown in Annex 7.

Antigen detection techniques, while available for groups A and C meningococci, are expensive, their shelf life is short and sensitivity may be less than direct staining methods (methylene blue or Gram stain). However, the use of this technique in the initial stage of an outbreak can be helpful to confirm these serogroups as the causal agent. During meningococcal disease outbreaks in developing countries, the benefit of antigen detection is largely outweighed by its cost and the difficulty in accurately performing these tests. Additionally, encouragement to improve laboratory facilities in developing countries will be of greater importance for diagnosis of meningococcal as well as other infections.

5. Antibiotic sensitivity testing and treatment

Treatment of bacterial infections must be based on knowledge of the sensitivity of the organism to antibiotics. Selection of a single antimicrobial agent from among all effective antibiotics depends on efficacy, cost and ease of administration. Since the precise identification of the responsible bacteria from a patient with meningitis is not always obtained, the antibiotic susceptibility for all of the three major causative bacteria (*S. pneumoniae*, *H. influenzae* and *N. meningitidis*) needs to be considered, especially during periods of endemic disease.

Disk sensitivity testing for meningococcus is only standardized for penicillin; furthermore accurate testing cannot always be done on a local level. Sensitivity testing of a representative sample of isolates can be done most accurately at a designated reference centre in each country. The antibiotics tested should include penicillin, chloramphenicol, sulfonamide, rifampicin, ceftriaxone (or other second and third

generation cephalosporins), co-trimoxazole and a quinolone. Testing should utilize agar dilution or disk diffusion methods using Mueller-Hinton without blood as a base.[19] To detect strains with decreased sensitivity to penicillin, a 2 IU tablet should be used for selected strains in addition to the 10 IU disk.[20] Determination of resistance mechanisms (beta-lactamase, acetylation of chloramphenicol) is less important in immediate therapy and can be performed later in a reference laboratory or collaborating centre.

During periods of epidemic meningococcal disease in developing countries, a single intramuscular (IM) dose of chloramphenicol in oil has proved to be effective therapy.[21,22] Unfortunately, this agent is not available in all countries. Preliminary studies suggest that one or several intramuscular doses of ceftriaxone may also be an effective therapy.[23] Penicillin, ampicillin and chloramphenicol are effective therapy but require intravenous (IV) administration and multiple doses. Sulfonamides are also effective if the organism is known to be sensitive. However, because of high rates of resistance, sulfonamides should not be used unless sensitivity is known. Oral administration of chloramphenicol may be appropriate in certain situations when intravenous therapy is not possible.

In non-epidemic situations, where organisms other than meningococci are relatively more prevalent, empiric therapy for cases where no organism has been identified (by direct examination, culture or antigen detection) needs to take into account local resistance patterns, particularly the frequency of ampicillin resistant H. influenzae. Further studies are required to assess whether a single dose of chloramphenicol in oil is sufficient to treat H. influenzae or S. pneumoniae infections.

It is suggested that:

(a) More widespread distribution and use of chloramphenicol in oil should be promoted for use during outbreaks in developing countries. In countries where the antibiotic is not available, efforts should be made to obtain it.

(b) Further studies should be conducted to evaluate the effectiveness of other agents that can be administered IM and require few doses (as for example ceftriaxone).

(c) Sensitivity testing in developing countries should be done at one or several national laboratories in a standardized fashion. It is important that the testing techniques used are capable of detecting strains with decreased sensitivity to penicillin.

6. Immunoprophylaxis

The best way of limiting the spread of meningococcal disease outbreaks is vaccination. Effective polysaccharide vaccines exist for two of the major meningococcal serogroups, A and C. Immunogenic polysaccharide vaccines are available for serogroups Y and W-135. The limitations of current vaccines are poor immunogenicity and a short protection period, especially in young children.

Vaccines of certain serotypes of group B are currently being developed. Immunogenicity studies and limited efficacy studies have been undertaken for several B:15 vaccines. The serotype specificity of group B vaccines underscores the importance of continued surveillance, including serotyping of isolates in areas where group B meningococcus is a major cause of disease.

Serotyping may be important for the development of improved group A and C vaccines as well. Conjugation of a protein antigen with the polysaccharide antigen, or the use of an oligosaccharide antigen is likely to improve immunogenicity and duration of protection following immunization.

It was therefore concluded that:

(a) Priority should be given to vaccination as the primary means of controlling epidemic meningococcal disease.

(b) The development, testing and application of a group A/C-protein conjugate vaccine should be a priority.

(c) Because of its implications for vaccine development and utilization, worldwide surveillance including serotyping of representative strains is important.

(d) Development of special group B meningococcal vaccines and more broad-based vaccines using protein and/or oligosaccharide antigens are encouraged under the WHO Programme for Vaccine Development for Encapsulated Bacteria.[24]

7. Chemoprophylaxis

Household contacts of patients with meningococcal disease and contacts in closed populations (e.g. military) are at increased risk of developing this infection. In developed countries, chemoprophylaxis is usually recommended for these individuals. In some countries, a few antimicrobial agents such as minocyclin, spiramycin, and most commonly rifampicin are used as chemoprophylaxis of meningococcal infection. These agents are expensive and compliance may be a problem because of their three-dose regimens.

Two other antimicrobial agents have recently been investigated. A single IM injection of ceftriaxone proved more effective than rifampicin during a recent outbreak of group A meningococcal disease.[25] This agent has not been well evaluated for infection caused by other serogroups. Preliminary studies also indicate efficacy of a single oral dose of ciprofloxacin in adults.[26] A disadvantage of these regimens is their high cost. It should be remembered that neither penicillin nor chloramphenicol is effective in chemoprophylaxis.

Despite the availability of effective chemoprophylaxis, its use is not recommended except in very limited circumstances. Because most persons who develop meningococcal disease do so following exposure to a carrier rather than a case, chemoprophylaxis has no role in the control of epidemics. Similarly, isolation of cases is of no usefulness. Close contacts of patients with meningococcal disease should be aware of the symptoms of the illness and, if symptoms such as fever develop, should immediately seek medical care.

8. Detection of outbreaks and epidemic preparedness

Although much is known of the epidemiology of meningococcal disease, prediction of outbreaks is still not possible. Therefore, improved surveillance and early detection of outbreaks is essential for their control. As stated previously, diagnosis of meningitis should be improved through clinical training of health workers, identification and development of microbiology laboratories. Routine reporting of meningitis cases by hospitals in small cities to district or national centres will allow the timely detection of an increased incidence of disease in local areas. When local increases are observed, surveillance efforts should be intensified and if the number of cases continues to increase, public health authorities should mobilize resources for treatment and prevention and neighbouring areas/countries should be notified.

Currently no threshold for an outbreak can be defined. Any analysis of increased local rates of disease needs to take into consideration the epidemiology of meningococcal disease in the region including seasonality, occurrence of recent outbreaks, disease in neighbouring countries and characteristics of affected patients. Further study of the

epidemiology of meningococcal disease needs to be done to improve the ability to predict the occurrence of epidemics. Existing raw data on epidemics in Africa needs to be examined carefully to gain additional insight into factors related to the occurrence of outbreaks.

Once an epidemic begins, rapid treatment and vaccination are essential in limiting mortality and morbidity. Obtaining sufficient antibiotics and vaccine rapidly may be difficult. Stockpiles are not currently maintained despite the long-term stability of these agents.

When availability of vaccine is limited, its effective utilization depends on knowledge of the epidemiology of meningococcal disease and the immunogenicity of vaccination. Intervention should be concentrated where rates of disease are greatest, for example in young children. Following a single dose, group A vaccine is immunogenic in children older than 6 months of age and group C vaccine in those older than 24 months. Following a booster dose group A vaccine is immunogenic in children aged between 3 and 6 months.[27]

It was noted that:

(a) Surveillance for meningococcal disease should be improved as described above.

(b) Detection of the early stages of epidemic disease can best be undertaken by health care facilities capable of confirming the clinical diagnosis.

(c) Regular reporting on a frequent (preferably weekly) basis from these hospitals to the district or national level and timely analysis of data are necessary.

(d) WHO has a key role to play in the following areas:

- Development and dissemination of a manual for health workers on surveillance, diagnosis and treatment of bacterial meningitis.

- Issuing alerts and educating health officials at the onset of the dry season in countries where periodic seasonal outbreaks occur.

- Stockpiling vaccine (A and C bivalent) and appropriate antibiotics at regional centres and overseeing distribution to areas with epidemic disease.

(e) As soon as a major outbreak of meningitis due to serogroup A or C meningococcus is detected, emergency mass immunization of the whole population is recommended because these measures have proved to reduce dramatically the spread of epidemic. When vaccine availability is limited, however, priority should be given to children in the youngest age groups. The minimum dose proved immunogenic for groups A and C polysaccharide vaccine is 25 ug.[27]

9. Long-term prevention

Routine immunization of children in high risk areas is not currently feasible. Strategies for preventing outbreaks depend on improved understanding of the epidemiology of meningococcal disease. Potential risk factors including environmental and immunologic factors need to be further characterized by multicentre and multidisciplinary studies.

Improved vaccines also need to be developed which can be used in younger children (allowing incorporation in the Expanded Programme on Immunization in some areas) and afford immunity of longer duration.

10. Conclusions and recommendations

1. Surveillance for meningococcal disease should be improved by establishing a multilevel system involving reporting from local health centres to the district/national level and from there to the regional reference centres. Timely reporting and analysis of data are important in order to detect outbreaks at their earliest stage and to mobilize resources to control the spread of illness. It is recommended that WHO encourage the development of a network of national/regional reference centres.

2. Diagnosis of meningococcal infections should be based on both clinical and laboratory criteria that correspond to a universal case definition used by public health workers for surveillance purposes.

3. Antimicrobial sensitivity testing for meningococci is necessary for both an effective treatment and a surveillance system. This testing should be done by using appropriate methodology at national/regional centres. It is recommended that WHO promote and coordinate surveillance of antimicrobial resistance of meningococci circulating in various geographical areas.

4. Therapy with a single intramuscular dose of chloramphenicol in oil is an effective treatment in outbreak situations where cost and ease of administration are important considerations. When empiric therapy is given in non-epidemic circumstances, its efficacy against other common causes of bacterial meningitis needs to be considered.

5. The development of meningococcal strain typing systems should be encouraged. Because serotyping with monoclonal antibodies is rapid and reproducible, and because of its relevance to vaccine development, completion of this typing system should be given high priority and be supported by WHO.

6. Chemoprophylaxis is expensive and of no benefit in controlling epidemics. Therefore, its use should be reserved for limited circumstances (see Section 7 "Chemoprophylaxis").

7. Immunoprophylaxis by vaccination is the best method for limiting the spread of meningococcal outbreaks. During outbreaks priority for vaccination should be given to those at greatest risk of disease, especially young children. Because vaccine availability is often limited, WHO should maintain a stockpile of bivalent A/C vaccine for distribution during outbreaks as necessary. Development of conjugated A and C vaccines immunogenic in younger children with a longer duration of protection as well as group B vaccine should be encouraged.

8. Efforts to better understand the epidemiology of meningococcal infection (including risk factors for disease and prediction of epidemics) should be encouraged through analysis of existing data and selection of additional data from epidemic situations.

III. MANAGEMENT OF MENINGOCOCCAEMIA WITH SEPTIC SHOCK

1. Anti-J5 therapy

The structure of the central part of endotoxin (lipopolysaccharide, LPS), the core, is common among Gram-negative bacteria, including *Neisseria meningitidis*. It has, therefore, been postulated that antibodies against endotoxin core might protect against a wide range of Gram-negative bacteria. It has been shown that high levels of antibodies to the core LPS are present in antisera collected after immunization of rabbits with

rough mutants such as the J5 mutant of E. coli 0111 (J5 antiserum), but levels of these antibodies in humans are much lower. While some investigations have shown that antibodies against endotoxin core protect animals from Gram-negative sepsis and death, others have not been able to show such protection.

At the present time, the possibility of passive immunization with polyclonal or monoclonal anti-core LPS antibodies remains an interesting possibility. Although clinical studies suggest that the administration of J5 antiserum protects in Gram-negative bacteraemia and septic shock,[28] several questions remain as to:

- The precise epitope(s) of endotoxin core against which such antibodies are directed.

- Whether these anti-core LPS antibodies cross-react with LPS from different strains, such as LPS from smooth Gram-negative bacilli or N. meningitidis.

- Whether these antibodies have the ability to interfere with LPS recognition by the immune system in vitro and in vivo in experimental animal models and in clinical studies. The demonstration of such interference would be of crucial importance since it is now recognized that the harmful effects of LPS are mainly mediated by the immune system.

More basic research is necessary to strengthen the hypothesis that interference with LPS offers a means of addressing the septic shock and meningococcaemia syndromes. A number of clinical trials with polyclonal and monoclonal antibodies are currently under way and should yield information about the clinical efficacy of this therapeutic approach.

2. Anti-tumour necrosis factor (TNF) therapy

Animal models have shown that the immune system has the ability to produce cytokines that can mimic the clinical picture of septic shock. In clinical trials, the severity of the disease was correlated with TNF levels measured at admission.[29,30] In three different animal models, anti-TNF antibodies have been found to be protective against endotoxaemia or bacteraemia.[31] The interplay of cytokines (e.g., TNF) needs further study to elucidate the pathology associated with meningococcal meningitis and fulminant septicaemia.

3. Recommendations

1. The chemical structure of meningococcal LPS should be further analysed and its antigenic determinants should be better characterized.

2. The role of cytokines in the pathogenesis of meningococcal diseases should be studied. It is recommended that a workshop for the critical evaluation of available TNF assays be organized by WHO.

3. The role of anti-core lipopolysaccharide antibodies and anti-cytokine antibodies in the treatment of meningococcal infections and shock should be carefully studied. After sufficient evidence is available, additional experimental animal and ultimately clinical trials should be carried out.

4. Owing to the very significant morbidity and mortality of meningococcal disease and because these infections are widespread in many parts of the world, WHO should promote and coordinate studies on the feasibility of this therapy.

ANNEX 1

PRODUCTION OF STANDARDIZED ANTIBODY FOR ANTI-DEOXYRIBONUCLEASE B

The purpose of this study is to prepare reference streptococcal anti-DNase B which would serve as standard material, be produced in sufficient quantities and supplied to national streptococcus reference laboratories to enable them to prepare and adapt their own anti-deoxyribonuclease B reference materials.

The project should have two parts:

(a) Preparation of the international antibody reference material.

(b) Trial of its suitability in reference laboratories.

It is recommended that the following methods be used in the project:

- Macromethod for high accuracy quantitation of anti-DNase B reference materials, using spectrophotometric measurement of end point.

- Micromethod for routine assay of human sera for anti-DNase B content.

In both methods the methylgreen DNA will be used as substrate.

Deoxyribonuclease types A, B, C and D are to be separated and purified, and antibodies to them raised in rabbits. The nuclease B is to be made in large quantity for immunization purposes in order to prepare enough samples of standard antibody. It is proposed that either goats or horses are used for this purpose.

The WHO Collaborating Centres in Lyon, Minneapolis and Prague will participate in the first phase of the project (preparation of antigen and antibody). It is recommended that at least three or four national laboratories (in addition to the above-mentioned Collaborating Centres) participate in the second stage (i.e., field trial of the new reference antibody to prepare the local standards, the suitability of local standards, to standardize the antigen and to attain comparability of results in routine measuring of the antibody in human sera).

ANNEX 2

STANDARDIZATION AND UPGRADING OF THE TEST FOR ANTIBODY TO GROUP A STREPTOCOCCUS POLYSACCHARIDE

New information has been provided on the titration technique of antibody to group A streptococcal polysaccharide, the dynamics of the antibody development, and the antibody levels in various clinical patterns of streptococcal disease in man. These findings suggest greater usage of the test in routine diagnostic services. The objectives of this study are to develop the technique so as to ensure technical reproducibility and biological significance.

1. Proposed technology

Currently, antipolysaccharide assay can be carried out by the following methods:

- Radio-immune-assay.
- Immunoenzyme assay.
- Passive haemoagglutination.
- Agglutination of group A streptococci treated by proteinase.
- Latex agglutination reaction.

The test should not be too sophisticated for clinical diagnosis at intermediate and peripheral health laboratory services.

Two tests were recommended:

(a) ELISA (immunoenzyme) method, which should be used as a reference test.

(b) Latex agglutination technique, which is to be evaluated together with the immunoenzyme method with the prospect of introducing it into clinical laboratories in developing countries.

2. Standardization of antigen

For comparability of results and clinical diagnosis, a standardization of reagents is necessary with regard to:

- A method of antigen production.
- Purity control (absence of protein M or peptidoglycan).
- Controlling structure, conformation, molecular weight and rhamnose level.
- Criteria of antigen stability and conservation procedure.

3. Reagents

The reagents should be made available in each of the collaborating laboratories. For the study itself, however, the reagent should preferably be prepared in one centre and distributed to the collaborators. The other alternative is to pool the reagents (prepared at the collaborating laboratories) and distribute them to the collaborators.

4. Field trials of technology

Field trials should be carried out on the following topics:

Normal population

Healthy children of 5 to 15 years of age with no evidence of upper respiratory infections and no preceding infection in the last two months.

- Number of children to be studied: 50-100

- Blood sample to be taken: one sample

Symptomatic upper respiratory tract infections (URTI)

with positive culture for group A streptococcus.

- Age group: 5 to 15 years

- Number of children to be studied: 50-100

- Blood samples to be taken: first sample to be taken within 48 hours after examining the patient. Second sample to be taken 3 to 4 weeks after first sample. If possible, third sample should be taken 3 to 4 weeks after second sample (optional).

Acute rheumatic fever

Blood should be taken at onset of acute rheumatic fever diagnosed according to modified Jones' criteria. Three blood samples are to be taken at the same intervals as patients in the URTI group.

Documented rheumatic heart disease

- Number of children to be studied: 50

- Blood samples to be taken: several blood samples to be taken periodically over several months' interval. It must be noted when the most recent attack of RF occurred.

Pyoderma

Same criteria and procedure as for the URTI group.

Acute glomerulonephritis

Same criteria as for acute rheumatic fever group.

Pharyngitis due to streptococcus groups C and G (optional). Same criteria and procedure as for the URTI group.

Antibodies to streptolysin O and DNase B should be titrated on all the blood samples in parallel with the antipolysaccharide assays.

5. Participating laboratories

The WHO Collaborating Centres in Lyon, Minneapolis, New Dehli and Rome as well as the National Reference Centres in Kuwait and Leningrad.

ANNEX 3

WHO COLLABORATIVE STUDY OF M AND OF TYPING SERA

This project was initiated in 1987 with the participation of five WHO Collaborating Centres, located in Lyon, Minneapolis, New Delhi, Prague and Rome. The objective of the project was to make available larger quantities of M and OF typing sera for the more common serotypes of group A strepococci recovered in laboratories around the world. It was also decided to specifically include those serotypes most frequently recovered from patients with documented acute rheumatic fever.

At the time this report was written, typing sera had been prepared in either rabbits or guinea pigs for 25 of the 35 serotypes originally selected. These sera are currently being examined in the respective laboratories for type-specificity. Before release it is necessary that each typing serum also be examined in the WHO Collaborating Centres in Minneapolis and Prague.

Although the amount of confirmatory work necessary in these laboratories is considerable, this aspect is crucial to the success of the project. It was estimated that the production of the typing sera in animals will be completed in early 1989, but that evaluation of the sera before release will take at least an additional six to eight months. When distributing sera, priority should be given to laboratories that are currently mapping the occurrence of various serotypes in the world.

GROUP A STREPTOCOCCAL TYPING SERA PRODUCED TO DATE IN ANIMALS (RABBIT, GUINEA PIG), OR AVAILABLE HUMAN SERA AS A PART OF THE WHO COLLABORATIVE STUDY*

Serotype	Sera produced or available		
	M-type	OF-type (animal)	OF-type (human)
1	x		
2	x	x	x
3	x		
4		x	x
5	x		
6	x		
9		x	x
11			x
12	x		
13	x		
15	x		
18	x		
19	x		
22	x		x
24	x		
25		x	x
28			x
33	x		
48		x	x
49		x	x
52	x		
54	x		
57	x		
58		x	x
59			x
60		x	x
61		x	x
62		x	x
63		x	x
64?			x
66		x	x
68			x
73		x	x
74	x		
75			x
76		x	x
77		x	x
78		x	x
79		x	x
81			x

* Some of the OF typing sera were produced in the National Streptococcal Centre, Leningrad, USSR

ANNEX 4

MICROTECHNIQUE FOR SERUM OPACITY FACTOR (OF) CHARACTERIZATION OF GROUP A BETA-HAEMOLYTIC STREPTOCOCCI

In addition to determination by T-antigen identification and M-protein typing, currently 27 types of group A streptococci elaborate a serum opacity factor that is type specific. Serotypes that make the OF are types that are usually difficult to characterize by M-typing, making characterization by OF typing necessary. Previous techniques for OF typing have been labour-intensive and have required the use of sera made in guinea pigs, which is difficult to produce.

A new microtechnique has been developed that is less labour intensive, may be determined qualitatively (with the eye) or quantitatively (using an ELISA reader), and is adaptable to the use of human sera, thus eliminating the need for production of OF antisera in guinea pigs.[32]

This technique thus far has proven reliable and reproducible, and it is felt that it can be easily transferred to similar laboratories for characterization of streptococcal isolates.

The purposes of the WHO collaborative study are:

- To confirm the feasibility of the technique in several laboratories.

- To develop a large pool of human sera for OF typing by collecting sera in several different geographic locations, confirming the presence of type specific OF antibodies, and pooling the serum for distribution to laboratories around the world. Laboratories involved will be the WHO Collaborating Centres in Minneapolis (USA), New Delhi (India), Prague (Czechoslovakia) and the National Streptococcal Centre in Leningrad (USSR).

ANNEX 5

SURVEILLANCE SYSTEM FOR MENINGOCOCCAL DISEASE

1. Local

1.1 Diagnosis

A. Clinical, using case definition (Annex 7)

B. Microbiological

(a) Microscopy
(b) Culture when available
(c) Antigen detection when available

1.2 Collection and reporting of epidemiological data

1.3 Transporting CSF and/or isolate to hospital laboratory and the national centre for confirmation and further studies

2. National centre(s)

2.1 Confirmation of diagnosis

A. Application of clinical case definition
B. Confirmation of microbiological diagnosis

2.2 Compilation of epidemiological data

2.3 Determination of serogroup of isolate

2.4 Determination of antibiotic sensitivity of a representative sample of isolates

2.5 Feedback of results to local level

2.6 Regular communication of summary data to regional centre

2.7 Transportation of selected isolates to regional/WHO collaborating centre(s) for further studies

2.8 Training of local health workers and laboratory personnel

3. Regional

3.1 Compilation of epidemiological data

3.2 Serotyping of isolates

3.3 Where indicated, studies of resistance mechanisms and more extensive antibiogrammes

3.4 Feedback of results to national level

3.5 Regular communication of summary data to WHO

3.6 Facilitation of national centre activities

A. Training of epidemiologic and laboratory personnel
B. Providing grouping antisera
C. Providing transport media
D. Other technical support as needed

4. World Health Organization

4.1 Compilation of epidemiological data

4.2 Regular dissemination of data to regional/national levels

4.3 Provision of support to regional/WHO collaborating centres

4.4 Coordination and support of vaccine development

ANNEX 6

INVENTORY OF LABORATORY FACILITIES FOR THE DIAGNOSIS OF ACUTE BACTERIAL MENINGITIS (*Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*)

1. Level A: minimal requirements (dispensaries or peripheral health centres)

(a) Sampling material

- Availability of needles for lumbar puncture in related clinical centres.
  - children
  - adults
- Sterile, screw-topped tubes for transport of CSF
  - transport medium
  - containers

(b) Direct examination

- Centrifuge (optional)
- Gram-stain reagents
- Methylene blue stain
- Microscope

2. Level B: referral centres

In addition to the same equipment as at level A, culture facilities should be maintained, including:

- Incubators
- Chocolate agar (Columbia agar + 5-10% sheep blood)
- Plating loops

3. Level C: confirmatory laboratory

(a) *Neisseria meningitidis*

- Oxidase disk test
- Biochemical test
- Serogrouping (slide agglutination)
- Serotyping (optional)

(b) *Streptococcus pneumoniae*

- Optochine disk test
- Agglutination

(c) *Haemophilus influenzae*

- Blood agar + culture of *S. aureus* (satellite test)

(d) Antibiogram (optional)

ANNEX 7

SUGGESTED CASE DEFINITION OF MENINGOCOCCAL MENINGITIS AND ACUTE MENINGOCOCCAEMIA*

1. Suspected cases

1.1 In patients over one year of age

- sudden onset AND
- fever $\geq$ 38.5°C

AND

at least three of the following signs:

- headache
- vomiting
- stiff neck
- petechial rash
- hypotension (systolic blood pressure $<$ 80 mm Hg)
- convulsions and/or coma
- epidemic situation

1.2 In infants under one year of age

at least two of the following signs:

- acute fever
- irritability (unconsolable), lethargy
- convulsions
- bulging fontanelle
- petechial rash
- hypotonia

OR

only one of the above-mentioned signs

AND

at least two out of the following signs:

- vomiting
- stiff neck
- epidemic situation

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* To be applied by qualified health personnel at the first referral health facility

2. Probable cases

Suspected cases

AND

Cloudy or purulent CSF

AND

- either petechial rash
- or positive CSF direct examination

3. Confirmed cases

Probable cases

AND/OR

- either A or C antigen detected in CSF
- or positive cultivation of CSF/blood/skin lesion

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