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STREPTOCOCCUS PNEUMONIAE AND HAEMOPHILUS INFLUENZAE TYPE B CARRIAGE IN INFANTS PRESENTING TO ZOLA COMMUNITY HEALTH CENTRE FOR ROUTINE IMMUNIZATION.

Nontombi Marylucy Mbelle

A short report submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Masters in Medicine.

Johannesburg 1999

DECLARATION

The experimental work described in this thesis was conducted under the supervision of Professor Keith Klugman, in the Department of Clinical Microbiology and Infectious Diseases, School of Pathology at the University of the Witwatersrand , Medical Research Council and the South African Institute for Medical Research Pneumococcal Diseases Research Unit Johannesburg.

I declare that this short report is my own, unaided work. It is being submitted for the masters in Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other academic institution.

Nontombi Marylucy Mbelle

30th day of June, 1999

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The community of Zola and Emndeni in Soweto for allowing their children to participate in this study.

The community nursing sisters of Zola Community Health Center and Dr. Ngoakoana Khomo for their efforts in making this and subsequent studies of this nature in Soweto possible.

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In particular the unique Professor Hendrick Koornhof for his constant encouragement in all my endeavors.

My Mom, Nobuntu, Mzamo and friends for their constant support, in particular Lesley whose selfless belief in me enabled me to complete this work.

DEDICATION

To uTata – Reginald Daka Mbelle

ABSTRACT

Background

Acute respiratory tract infections are the most common cause of illness and death in the pediatric population worldwide. It is estimated that 70 – 80% of severe pneumonias in Africa are caused by *S.pneumoniae* (the pneumococcus) followed by *H. influenzae* type b. Surveillance reveals that drug resistance is increasing worldwide, South Africa not being an exception. This has considerably complicated the management of infections caused by both the pneumococcus and *H. influenzae* type b (Hib).

It is widely accepted that colonization of the nasopharynx even briefly precedes middle ear infection and invasive pneumococcal disease. Early onset of colonization after birth has been associated with early onset of middle ear infections. Furthermore, colonized children are able to transmit these organisms to other children.

Carriage of pneumococci commonly occurs in young children. The carriage of resistant pneumococci is usually limited to those serotypes carried in children. New conjugate vaccines may be able to reduce colonization of these serotypes.

This study was undertaken to determine the serotypes and susceptibility of pneumococci and *H. influenzae* type b, and the proportion of healthy children colonized at Zola Community Health Centre (ZCHC) in Soweto.

Methods

A total of 278 children were recruited into the study. Epidemiological data were obtained verbally using a questionnaire. Nasopharyngeal swabs were taken from healthy infants at birth, 6, 10, 14 weeks, 9 and 18 months and at 5 years of age. Swabs were immediately plated on Bacitracin heated blood (BHB) agar plates followed by 5% sheep blood agar plates on site. Agar plates were incubated in 5% carbon dioxide for the isolation of *S.pneumoniae* and *H.influenzae*. Identification, antibiotic susceptibility and serotyping were done by routine laboratory methods.

Findings

Fifty six percent (156) of children were female and 43.9% (122) male. Newborns were excluded from the analysis since no bacteria were isolated. Pneumococcal and *H. influenzae* type b carriage rates overall were 43.5% (121) and 10.4% (29) respectively. Early pneumococcal carriage was evidenced by 27.5% and 7.8% carriage of pneumococci and *H. influenzae* type b respectively at 6 weeks. Carriage was highest at 9 months (63.5%) and 5 years (17.5%) for *S. pneumonia* and *H.* influenzae type b respectively. Overall, 18.7% (52) of subjects carried antibiotic resistant pneumococci. Forty four percent of all pneumococcal carriers were resistant to one or more antimicrobials. Six percent (7) of all isolates were resistant to all classes of antibiotic tested. Of the children carrying pneumococci, 5.4% (15) of children carried both pneumococci and *H. influenzae* type b concurrently. Antibiotic resistant *H. influenzae* type b were carried by 2.2% (6) of all subjects at a later age from 9 months. The odds of carriage of pneumococci were 2.18 times higher in children < 2 years of age than children 2 years or older (CI = 1.02 - 4.76) p = 0.04. The odds of carrying pneumococci was 1.92 times higher in carriers with siblings than those without (CI = 1.13 - 3.29) p=0.01. The odds of carrying resistant pneumococci was 2.67 times higher in children with siblings than those without (CI =1.06 - 6.82) $p < 0.05$.

No association was found between children carrying *H. influenzae* type b and *S. pneumoniae*. The most frequently carried pneumococcal serotypes were 6A and 19F. Eight children carried multiple serotypes. The serotypes of the antibiotic resistant strains were 4, 6A, 14,19F and 23F. Nasopharyngeal carriage varied in subjects that were sampled at different time points. While 9.5% of the sampled children carried pneumococci at 6 weeks of age, carriage was highest (30.1% and 27.6%) at the 14 week and 9 month time point.
Serotypes 4, 14, 19F and 23F found in the CRM_{197} nonavalent pneumococcal vaccine accounted for 50% of globally resistant strains and 52% of the penicillin resistant isolates.

Serotype 6A accounted for 43.9% of the globally resistant strains and 47.2% of the penicillin resistant isolates.

Interpretation

Surveillance of nasopharyngeal carriage of *S. pneumoniae* and *H. influenzae* in healthy children at Zola community health centre (ZCHC) indicates a high level of H. influenzae type b carriage, penicillin-resistant pneumococcal carriage and early colonization of the nasopharynx. A large portion of penicillin-resistant pneumococcal serotypes carried are the paediatric serotypes; 52.3% of which are the serotypes contained in an experimental nonavalent conjugate pneumococcal vaccine whose efficacy is being tested in Soweto.

The ultimate introduction of *H. influenzae* type b followed by pneumococcal conjugate vaccines in this and other South African communities may decrease the incidence of invasive disease and interrupt carriage within this community.

PRESENTATIONS

Mbelle, NM, Nadan, S, Wasas A, Huebner, RH, Klugman, KP. *Streptococcus pneumonia* and *H. influenzae* type b carriage in infants presenting for routine immunization. (6th Joint Congress of the STD and Infectious Diseases Societies of Southern Africa, Cape Town September 1997).

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CHAPTER 1

INTRODUCTION

1.1 A SOUTH AFRICAN OVERVIEW OF PNEUMOCOCCAL AND H. INFLUENZAE TYPE B DISEASE.

A high incidence of pneumococcal pneumonia was noticed among new recruits to the gold mines in South Africa, and Lister in 1917 successfully showed that pneumonia could be prevented among new recruits in South African mines using a killed suspension of pneumococcus types 1, 2, and 3 administered subcutaneously (Lister 1917). As a result of these studies, vaccination using whole cell preparations of available serotypes was introduced as a routine to the mining population in Johannesburg South Africa and elsewhere in Africa (Ordman 1935).

These findings preceded the use of sulphonamides in 1938, and the successful treatment of pneumococcal pneumonia with penicillin reported by Tillet (Tillet et al 1944). A dramatic reduction in the case fatality rate to between 5% and 8% was noted. With the successful treatment of pneumococcal disease with penicillin, interest in pneumococcal disease waned since the serotyping of isolates was no longer required to affect treatment choice. Some researchers noticed however that while pneumococcal disease was well managed in adults, children, especially those younger than 2 years of age and neonates continued to present with severe pneumococcal disease (Johnson et al 1964, Burke et al 1971). The greatest burden of disease continues to be in young children to this day.

Reports of pneumococcal resistance to available antimicrobials appeared soon thereafter. While initial strains were mutants induced *in vitro*, the isolation of similar strains in humans were subsequently identified and described in the literature (Hansman et al 1967).

Highly penicillin-resistant pneumococci were first isolated in Durban, South Africa in 1977 (Appelbaum et al 1977). Multidrug resistant pneumococci were first isolated and identified in Soweto, South Africa in 1978 (Jacobs et al 1978).

In the face of the rising incidence of pneumococcal strains resistant to one or more antimicrobial agents, it became apparent that the simplest and potentially most effective means of managing the disease was by prevention. As a consequence, one of the earliest vaccine studies using polyvalent polysaccharides was conducted in South African miners in 1976. Hexa - and tridecavalent vaccines prepared by Eli Lilly were tested and found to reduce bacteremic pneumonia infections significantly (Austrian et al 1976). In the following year a Merck Sharp and Dohme preparation of the 6 valent and 12 valent vaccines was also found to reduce pneumonia infections in South African mine recruits. (Smit et al 1977). The experience of the use of polyvalent polysaccharide vaccines has been to increase the host's resistance to serious pneumococcal infection. The use of 23 valent vaccines has led to the prevention of pneumococcal infection by vaccine serotypes and there has also been no apparent replacement by disease caused by other pneumococcal types. (Butler et al 1993). While much is known about *H. influenzae* type b disease worldwide and in developing countries such as The Gambia (Adegbola et al 1994) and Papua New Guinea (Leach et al 1994), it was only in1989 that the susceptibility (Liebowitz et al 1989) and in 1994 that the epidemiology of invasive disease was documented in South Africa (Hussey et al 1994).

1.2 EPIDEMIOLOGY

1.2.1 Pneumococcal disease

It has been estimated that of the 15 million deaths worldwide each year, 12.9 million of these occur in children <5 years of age. Of these 4.3 million are due to acute respiratory tract infection (Leowski 1986). Several studies done have shown that the pneumococcus is the

most frequent cause of severe pneumonia among hospital admissions in children (Shann et al 1986). Only 24% of children in a lung aspirate study had evidence of viral infection. In one third of these, viruses were isolated in the absence of respiratory symptoms. Bacteria, not viruses were found more often in children who had died as reported in several studies (Shann 1984, Ikeogu 1988) In a study performed in The Gambia, *S.pneumoniae* followed by *H. influenzae* type b was the commonest bacterial cause of pneumonia (Adegbola et al 1994). In South Africa, the burden of *H. influenzae* type b disease has not been established. *S.pneumoniae* has been recently estimated in HIV negative children at 70/100 000/year and in HIV infected children at 1844 cases per 100 000/year (Jones et al 1998).

These figures can only be an underestimation since blood cultures are insensitive, and therefore underestimate the incidence of disease. Transthoracic puncture is more sensitive but highly invasive and can thus not be used to accurately estimate the incidence of pneumococcal disease.

Nasopharyngeal carriage too while thought to precede invasive disease, is a poor indicator of disease since many carriers do not succumb to disease and serotypes isolated from sterile sites may not be found in the nasopharynx. It is also unhelpful for predicting disease in communities where carriage rates are high. No serological assays are presently available to diagnose active pneumococcal disease.

1.2.2 *H. influenzae* type b disease

The epidemiology of *H. influenzae* disease in South Africa has been described in detail by Hussey and colleagues in a study done in Cape Town (Hussey et al 1994). The epidemiology differs in some respects from that seen in industrialized countries as well as from other developing countries. Meningitis and pneumonia are the most common clinical presentations. Epiglottitis, as in other developing countries does not occur at all. The overall case fatality

rate was 9.2% being highest for septicaemia (40%). Serotype b was responsible for 86.5% of all invasive disease. Non-type b strains were isolated in children with pneumonia, septicaemia and mastoiditis. As in The Gambia (Bijlmer et al 1990) and Senegal (Cadoz et al 1981) , 97.3% of all meningitis cases were due to *H. influenzae* type b.

1.3 PATHOGENESIS

1.3.1 Pneumococcal disease

It is assumed that nasopharyngeal carriage precedes pneumococcal disease. The factors leading to invasion and pneumococcal disease are however not fully understood since carriage of serotypes normally known to cause severe disease can occur in the absence of disease. Many workers have investigated factors which may lead to the breakdown of host defenses and the development of pneumonia and bacteraemia. Some of these follow.

It is still not clear whether low temperatures necessarily facilitate pneumococcal invasion of the lower respiratory tract. Observations of miners in the United States and Britain showed an increased rate of infections in factory workers exposed to a wide range of temperatures. It has been shown experimentally that cold immersion lowers pulmonary antibacterial activity presumably by it's effect on the cellular metabolism of the alveolar macrophage but possibly by altering pulmonary blood flow (Green et al 1965). An antecedent viral infection is also thought to be an important predisposing factor. Virus induced damage is thought to enable bacteria to multiply in the alveoli. It has been observed in humans that many episodes of pneumonia are preceded by an upper

respiratory tract infection. In Africa however, studies by Maynard and other workers found the preceding upper respiratory tract infection to be an exception rather than the rule (Maynard 1913). In another study, Fekerty showed that although 50% of subjects with pneumococcal pneumonia gave evidence of a recent upper respiratory tract infection,

laboratory evidence of respiratory virus aetiology could only be obtained in 10% (Fekerty et al 1971). The diagnosis of viral disease is known to be difficult especially as serological tests which are more useful than isolation of the virus may not be available for many species of respiratory virus.

Overcrowding has also been suggested as a predisposing factor of pneumococcal disease in developing countries. It is thought that this together with indoor pollution of the air by smoke contributes to heavy nasopharyngeal colonization that occurs in many children in developing countries in the first few months of life (Montgomery et al 1990). Patients with dysgammaglobulinaemias are also known to have a high incidence of recurrent pneumococcal infections. Persons with IgA antibody deficiency are known to suffer repeated respiratory tract infections. The role and relevant importance of secretory antibody in defense against pneumococcal infection has however not been defined (Chipps et al 1978).

Functional asplenia is also known to predispose individuals to recurrent pneumococcal disease. The spleen is known to selectively clear pneumococci from the blood stream and then generate the early antibody response to them. Children with sickle cell disease have an impaired ability to activate the alternate complement pathway. These individuals also present with recurrent pneumococcal infections (Brisno 1971).

1.3.2 *H. influenzae* type b disease

The pathogenicity of *H. influenzae* is determined largely by the polysaccharide capsule of which there are 6 antigenically and biochemically distinct types (a-f). Serotype b is known to be the most significant cause of invasive disease (Turk and May 1967). The capsular antigen of the type b polysaccharide consists of a repeating polymer of ribosyl and ribitol phosphate (PRP). This repeating polymer has been shown to be the major virulence factor of *H. influenzae* type b. Antibody to PRP capsule has been shown to enhance activation of

complement, opsonisation, phagocytosis and killing in human studies. It has been shown to be both therapeutic and protective (Ward et al 1988).

1.4 MICROBIAL CARRIAGE

1.4.1 Pneumococcal carriage

Although any of the 90 serotypes can cause disease (Henrichsen 1995), there is a preponderance of strains that commonly not only colonize the nasopharynx but also cause invasive disease (Austrian et al 1978). It has been suggested that these variations in virulence between pneumococcal types are due to an inability of virulent types such as 1, 2, 4 and 8 to activate the alternate complement pathway and thus evade opsonisation in the pre-antibody phase of infection (Fine 1975). Shifts in prevalence of certain serotypes are noted over time among the more frequent types. The patterns may also differ between Western and developing countries (Sniadack et al 1995).

Certain types such as 1, 2, 3 and 5 rarely colonise the nasopharynx but often cause severe disease (Bruyn et al 1992). They also tend to spread epidemically (Bruyn et al 1992). Some groups have also been shown to be more prevalent in young children. The most common groups/types causing infections in children are 4, 6, 7, 9, 14, 19F and 23F (Sniadack et al 1995). Children are often asymptomatic carriers of types/groups. Other serogroups/types can however also be carried. Serotype/groups 6, 14, 19 and 23 often demonstrate antimicrobial resistance. This is probably as a result of frequent antibiotic exposure of the strains that are most often carried (Klugman et al 1990). Clones have thus been identified in types 14, 6, 19 and 23; some of which have spread extensively, within communities and from country to country. Antigenicity of the capsular polysaccharide varies and is poorest for serotype 6B, 19F and 23F (Klein 1987, Douglas et al 1983). Capsular gene cassette transformation,

leading to a change in capsule specificity, is a common event in such antibiotic resistant clones but probably also in natural clones as well (Coffey et al 1991).

1.4.2 *H. influenzae* type b carriage

As with pneumococcal disease, colonization of the nasopharynx is thought to precede invasive disease and enable transmission to susceptible individuals. *H. influenzae,* particularly non-encapsulated strains colonise the upper respiratory tract without causing illness. Children are colonised early, with virtually all children being colonised by the age of 5 years (Moxon and Wilson et al 1991). It is well recognised that *H. influenzae* type b strains also colonise the nasopharynx but less frequently than non type b haemophili. Evidence suggests that colonisation rates in developing countries are higher and occur at a younger age than in countries such as the United States of America (Bijlmer et al 1989). A study done in Papua New Guinea found that 10% of healthy children <5 years of age were colonised with *H. influenzae* type b (Gratten et al 1984). In the Gambia, the prevalence rates of colonisation were found to be as high as 33% during the first 5 years of life (Bijlmer et al 1989). Close contact in settings such as day care centers, orphanages and household has been shown to increase the rate of colonisation to as high as 50% (Granoff and Ward 1984).

1.5 ANTIMICROBIAL RESISTANCE.

1.5.1 Pneumococcal resistance

Penicillin resistance is now becoming a worldwide phenomenon. The distribution of penicillin-resistant pneumococcal strains have been reported and summarised in several publications including a recent review by Klugman (Klugman et al 1997). The latter paper documents the frequency of isolation of strains of pneumococci with MIC's >0.12μg/ml (intermediate and high level resistance) around the world. The lowest levels recorded were in

Norway, Sweden, Holland, Germany, Italy, India, Finland, and the United Kingdom. Canada, Iceland and parts of Australia had levels of penicillin-resistant pneumococci in the 10 to 25% range. The highest levels of penicillin resistant pneumococci were found in USA, most of South America, Portugal, Spain, South Africa, and France. It is likely however that with more active surveillance of pneumococcal isolates worldwide, the incidence of antibiotic resistant pneumococci is likely to be higher than previously thought in countries in Eastern Europe, Asia and the rest of Africa. In the USA, the rates of penicillin-resistant pneumococci have risen rapidly since 1990. A survey of 1527 isolates in the USA showed that 24% of pneumococci isolated were resistant to penicillin; 10% of which had high level resistance (MIC≥2mg/ml) (Doern et al 1996). In South Africa, a three fold increase in penicillin resistance of pneumococci (4.9% to 14.4%) was reported from 1979 – 1990 (Wasas et al 1998). This global increase in the resistance of pneumococci to penicillin, has had a significant impact on the management of pneumococcal disease, especially in the treatment of otitis media and meningitis. Strains of pneumococci resistant to penicillin are often resistant to other β–lactams, trimethoprim – sulfamethoxazole, macrolides and other classes of antimicrobials. In some communities, a smaller but still substantial proportion of isolates are resistant to multiple drugs. The susceptibility of these intermediate and high level penicillin resistant isolates of pneumococci has led to tests for susceptibility to other antimicrobials especially with the emergence of resistance to third generation cephalosporins. Of the penicillin intermediate and high level resistant isolates tested, cefpirome was the most active cephalosporin. (Klugman et al 1997, Martinez – Beltran et al 1995).

1.5.2 *H. influenzae* type b resistance

As with the pneumococcus , surveys of antimicrobial susceptibility indicate that there has also been a steady increase in the prevalence of antibiotic resistance among *Haemophilus*

Norway, Sweden, Holland, Germany, Italy, India, Finland, and the United Kingdom. Canada, Iceland and parts of Australia had levels of penicillin-resistant pneumococci in the 10 to 25% range. The highest levels of penicillin resistant pneumococci were found in USA, most of South America, Portugal, Spain, South Africa, and France. It is likely however that with more active surveillance of pneumococcal isolates worldwide, the incidence of antibiotic resistant pneumococci is likely to be higher than previously thought in countries in Eastern Europe, Asia and the rest of Africa. In the USA, the rates of penicillin-resistant pneumococci have risen rapidly since 1990. A survey of 1527 isolates in the USA showed that 24% of pneumococci isolated were resistant to penicillin; 10% of which had high level resistance (MIC≥2mg/ml) (Doern et al 1996). In South Africa, a three fold increase in penicillin resistance of pneumococci (4.9% to 14.4%) was reported from 1979 – 1990 (Wasas et al 1998). This global increase in the resistance of pneumococci to penicillin, has had a significant impact on the management of pneumococcal disease, especially in the treatment of otitis media and meningitis. Strains of pneumococci resistant to penicillin are often resistant to other β–lactams, trimethoprim – sulfamethoxazole, macrolides and other classes of antimicrobials. In some communities, a smaller but still substantial proportion of isolates are resistant to multiple drugs. The susceptibility of these intermediate and high level penicillin resistant isolates of pneumococci has led to tests for susceptibility to other antimicrobials especially with the emergence of resistance to third generation cephalosporins. Of the penicillin intermediate and high level resistant isolates tested, cefpirome was the most active cephalosporin. (Klugman et al 1997, Martinez – Beltran et al 1995).

1.5.2 *H. influenzae* type b resistance

As with the pneumococcus , surveys of antimicrobial susceptibility indicate that there has also been a steady increase in the prevalence of antibiotic resistance among *Haemophilus*

influenzae type b isolates. Rates of antimicrobial resistance in South African children are however reported to be lower than those reported in other studies from industrialized and developing countries (Hussey et al 1994 Kayser et al 1990 and Weinberg et al 1990). In the United Kingdom, there was an increase in ampicillin resistance from 1.6% to 7% between 1977 and 1992 (Powell et al 1992). In the United States the increase between 1984 and 1986 was 21% to 31.7% (Doern et al 1988).

Ampicillin resistance was detected in 10.8% of clinical isolates of children hospitalized in the metropolitan area of Cape Town. All but one of these strains were β–lactamase producing ; Resistance to erythromycin and cotrimoxazole were found in 87.2% and 20.4% of all strains (Hussey et al 1994).

As expected, antibiotic resistant strains are more prevalent in closed communities as demonstrated in a study done in children hospitalized for tuberculosis. Penicillin, cotrimoxazole and erythromycin resistance was 44%, 83% and 85% respectively. Such isolates were also found to be universally resistant to rifampicin. These untoward findings would impact on prophylaxis to prevent secondary cases of invasive *H.influenzae* type b infection (Cartwright et al 1991).

1.6 MANAGEMENT OF PNEUMOCOCCAL AND *H. INFLUENZAE* TYPE B INFECTION.

1.6.1 Strategies in antimicrobial therapy

The clinical manifestations of pneumococcal and *H. influenzae* type b infections are wide and varied in severity. *S. pneumoniae* causes a larger proportion of acute otitis media cases than any other agent and is the least likely to resolve without treatment (Barnett and Klein 1995). The steady increase in drug resistant pneumococcal and *H. influenzae* type b infections, raises complex clinical and public health issues such as which first line drugs can be chosen

that are efficacious , accessible and cheap. In response to this, a reassessment of treatment protocols has taken place (Dowell et al 1999). Amoxicillin remains the best oral antimicrobial agent because it has the best pharmacokinetic and pharmacodynamic properties. It is safe, efficacious and inexpensive. Recent information indicates that high doses of amoxicillin (70-90mg/kg/day) given as amoxicillin –clavulanate in two doses daily may achieve middle-ear fluid concentrations that are sufficient to eliminate penicillin resistant pneumococci, β–lactamase producing *H.influenzae* and *Moraxella catarrhalis* (Seikel et al 1997). Alternative therapy for treatment failures (defined as lack of clinical improvement after 3 days of therapy) includes cefuroxime, cefprozil or intramuscular ceftriaxone. Cefuroxime has been used extensively and is recommended as an alternative to amoxicillin (Dowell et al 1999). Cotrimoxazole and macrolides have also been used as first or second line agents of acute otitis media. Surveillance data indicates that resistance to these agents in the community is higher than previously thought. Moreover cross resistance has been shown to occur between these two drugs and the β–lactam agents in use. No approval currently exists for the use of the newer fluoroquinolones in children.

The eradication of meningeal pathogens requires antibiotic concentrations in the cerebrospinal fluid that exceed the minimal bactericidal concentration by at least a factor of ten (Tauber et al 1984). Resistance to β–lactams, including extended spectrum cephalosporins and to chloramphenicol - agents widely used for the treatment of meningitis (Bradley and Conner 1991), results in delayed sterilization of the cerebrospinal fluid in children with meningitis. While penicillin-resistant pneumococci with intermediate resistance may respond to either ceftriaxone or cefotaxime, organisms with high level resistance require a combination of ceftriaxone and cefotaxime with vancomycin for sufficient sterilization of the cerebrospinal fluid (Klugman et al 1995). In areas where reports of treatment failures with extended spectrum cephalosporins have been reported, the recommended regimen for treatment of

meningitis is ceftriaxone or cefotaxime with vancomycin (Friedland et al 1993). The use of rifampicin in combination with extended spectrum β–lactams is contentious. Conflicting reports on antagonism and synergy of β–lactams with rifampicin have been reported in the literature (Brandot 1994, Klugman 1995).

Studies done on the treatment of community acquired pneumonia showed that the outcomes were the same whether penicillin resistant or sensitive pneumococcal pneumonias were treated with penicillin or ampicillin in standard dosage (Friedland and Klugman 1992, Tan et al 1992). It is however not known whether highly active β–lactam antibiotics such as cefotaxime, ceftriaxone or imipenem will be more efficaceous than penicillin or amoxicillin for treating pneumonia caused by highly resistant pneumococci. In such cases, vancomycin and imipenem should be considered for therapy.

1.6.2 Vaccines and the management of pneumococcal and *H. influenzae* type b disease.

The existing polysaccharide vaccines are of limited value in infants and young children because they are weak immunogens and do not stimulate a T cell dependent response (Stein 1992). The immunogencity of polysaccharides can be improved by conjugating the specific polysaccharide antigen with a protein carrier. This changes the nature of the antipolysaccharide response to T cell dependent from T cell independent (Avery 1929, Bradley – Mullen 1980). The covalent coupling of protein carriers with polysaccharides has been successfully used with *H. influenzae* type b vaccines (Ward and Cochi 1988). The methodology employed can vary the number of polysaccharides included in the vaccines, the carriers used and the nature of linkage between polysaccharide and protein. The protein carriers usually used in current vaccines are diphtheria and tetanus toxoids (Eskola 1985), outer membrane protein complexes of Neisseria meningitides B and a non toxic mutant form of diphtheria toxin CRM_{197}. (Ahonkhal 1991, Madore, 1990). All these *H. influenzae* type b

conjugate vaccines have been shown to be immunogenic and efficacious in preventing invasive *H. influenzae* type b disease even though the magnitude of the antibody response may differ with each conjugate. Some infants with a low antibody response may be protected against disease when exposed to *H. influenzae* type b because conjugate vaccination primes infants providing them with the ability to mount a satisfactory serum antibody response when they encounter the organism (Granoff et al 1993). There are currently four PRP protein conjugate vaccines some of which are licensed for use in older children and some in younger infants. They differ in the size of the use in polysaccharide, the protein conjugate used and the linkage used between the two. The PRP outer membrane protein conjugate (PRP-OMP) (Merck Sharp and Dohme) is immunogenic after a single dose in children. The booster response to a second dose is however negligible. (Weinberg and Granoff 1988). The protective efficacy in a placebo-controlled randomised double blind study among United States Navajos was 93% both after the first dose at 6-8 weeks and before the second dose 1 month later (95% CI: 67% - 99%)(Santosam et al 1991). The PRP – CRM_{197} conjugate (Praxis/Lederle) on the other hand was found to be highly immunogenic after the first dose in children > 15 months old but a poor response was obtained in younger children (Anderson 1983). The booster response was however high in most individuals (Anderson 1983, Madore et al 1990). An efficacy trial of this vaccine showed 100% efficacy after 3 doses (Black et al 1991). The PRP tetanus toxoid conjugate (Merieux) requires 3 doses to induce high antibody levels in infants < 6 months of age (Hessel et al 1989). In The Gambia, a PRP - T Hib conjugate vaccine study showed the vaccine to be effective in preventing pneumonia and meningitis due to *H. influenzae* type b after 3 doses (Mulholland et al 1997). The PRP-diphtheria toxoid conjugate (Connaught) had a poor response in young infants in Alaska, (Ward et al 1990) but good response in Finnish children (Eskola et al 1990). It is therefore only licensed for use in children 15 – 60 months of age. All of these conjugate vaccines with

the exception of PRP-OMP use protein antigens also found in DTP vaccine. It has been suggested that an enhanced immune response is achieved in previous or simultaneous DTP immunised recipients of these *H. influenzae* type b conjugate vaccines. The magnitude of the PRP antibody response is influenced by a variety of factors, including the age of the individual, type and duration of exposure, and the methods of antibody measurement. The serum concentration of PRP antibody required for protection is not known but has been estimated to be 0.15 to 1.0mg/ml (Kayhty et al 1983).The successful conjugation of polysaccharide to protein carriers in the *H. influenzae* type b conjugate vaccines has facilitated the development of conjugate pneumococcal vaccines using similar principles. The four main vaccine manufacturers have developed pneumococcal conjugate vaccines using the same approach. The difference is the polyvalent nature of pneumococcal conjugate vaccines. The choice of antigens used in these pneumococcal polysaccharide conjugate vaccines are based primarily on the predominant serotypes causing disease in the population targeted for vaccination (Sniadack et al 1995).

Seven serotypes; 14, 6B,19F, 18C, 23F, 4 and 9V account for approximately 80% of clinical isolates the United States of America (Butler et al 1995). These serotypes also tend to be those resistant to antimicrobials (Klugman 1990). While the polysaccharide is the immunogenic moeity in most serotypes, the oligosaccharide of 18C is used in Lederle vaccine preparation to achieve an adequate immune response (Mbelle et al press). Similarly, the cross reaction between serotype 19A and 19 F is limited and therefore a vaccine containing serotype 19F need not necessarily protect an individual from infection with serotype 19A (Penn et al 1982). On the other hand the close antigenic relatedness of serotype 6A and 6B is expected to afford cross protection of 6A on inclusion of 6B in a vaccine formulation. The immunogenicity of 5 and 7 valent pneumococcal conjugate vaccines has been demonstrated in studies done in The Gambia, Finland and the United States of America (Leach et al 1996,

Ahman et al 1996 and Rennels et al 1998). Subsequently, a heptavalent conjugate pneumococcal vaccine has been shown to be highly effective in preventing invasive disease in young children in the United States of America (Black et al 1998).

Polyvalent conjugate vaccines may cover a large proportion of serotypes causing invasive disease. A nine valent pneumococcal conjugate vaccine containing serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F would cover >85% of the serotypes of pneumococci isolated from the blood or CSF of pediatric patients admitted to hospital in Soweto, South Africa (Mbelle et al in press). Data on vaccine coverage suggest that the benefits in preventing invasive disease decrease with increasing valency of these polyvalent vaccines. Although the global use of polyvalent conjugate pneumococcal vaccines may reduce the high rates of sickness and death in children worldwide; species - wide, protein - based vaccines would offer more widespread protection against the pneumococcus. The prime vaccine candidates are enzymes and toxins as well as surface proteins whose exact functions are not known. Pneumococcal candidate vaccines studied to date include neuraminidase, autolysin, pneumolysin, pneumococcal surface protein A (PspA) and pneumococcal surface adhesin A(PsaA) (McDaniel et al 1991 , Paton et el 1991) . Immunisation of mice with inactivated pneumolysin toxoid results in enhanced survival when challenged with pneumococci (Paton et al 1983). PspA is a surface protein present in all clinically relevant pneumococcal strains. While PspA's from different pneumococcal strains vary serologically, many PspA antibodies cross react with PspA's from unrelated strains (McDaniel et al 1991). In addition, active immunisation of mice with PspA generates a protective immune response against diverse pneumococcal strains (McDaniel et al 1991). Other approaches include conjugates using pneumolysoid, pertussis toxin and salmonella protein as carriers. Small peptides have also been coupled to pneumococcal polysaccharide based on their ability to stimulate T-cells.

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Invasive pneumococcal disease has been identified as an important opportunistic infection in both adults and children infected with the human immune virus (Mao and et al 1996, Jones et al 1998). The incidence of pneumococcal bacteremia increased by 36.9 fold in HIV seropositive pediatric hospital admissions to Soweto, South Africa compared with HIV seronegative children (Jones et al 1998). Recurrent pneumococcal infections in such individuals have prompted a number of investigators to examine the antibody response in HIV infected individuals following exposure to various immunogens. Results indicate that the antibody response in HIV infected individuals to T independent polysaccharide antigens is defective (Janoff et al 1988). Young children with acquired immunodeficiency syndrome responded poorly to both tetanus conjugated *H. influenzae* type b vaccine (Act Hib) and polysaccharide pneumococcal vaccine (Pneumovax 11) (Gibb et al 1995). No correlation with age adjusted CD4 counts was demonstrated. It is not known if a similarly disappointing result will be obtained with conjugated pneumococcal vaccines. Long term follow up of HIV infected adults immunised with pneumococcal vaccines revealed a lesser serological response 3 years after immunistion compared to healthy uninfected individuals (Len et al)

1.7 OBJECTIVES.

A comparative study was undertaken to:

i)Establish the carriage and frequency of specific serotypes of *S. pneumoniae* and *H. influenzae* type b in young children in Soweto. This study can form the basis of future studies on the impact on carriage of conjugate pneumococcal or Hib vaccines.

ii)Identify the age of colonization of infants by sampling the nasopharynx at birth; 6, 10 and 14 weeks; 9 and 18 months and at 5 years of age. It has been shown that early colonization with pneumococci is associated with early onset of otitis media .

iii)Determine the rates of antibiotic resistance of isolates since it is known that the introduction of vaccines have the potential to decrease the carriage of resistant strains. The association of the carriage of resistant strains with isolation of similar isolates from sterile sites has been demonstrated .

iv)Determine the epidemiological factors that may influence the carriage of pneumococci or *H. influenzae* type b.

This study preceded an immunogenicity trial of a 9 valent pneumococcal conjugate vaccine in which all subjects also received Tetramune (DTP – Hib - CRM197).

CHAPTER 2

SUBJECTS AND METHODS

2.1 STUDY SITE

The study was carried out at the immunization clinic of the Zola Family Health Center between April and June 1996. This primary health clinic serves a population of approximately 75 000 comprising the townships of Zola and Emndeni in Soweto. Vaccine coverage at this and other clinic sites in Soweto is high being 96%, 95%, 93% for the first, second and third dose of DPT vaccine given respectively (Kapongo 1997).

2.2 SUBJECTS

Subjects were recruited from consecutive infants and children presenting for routine. immunization at 0, 6, 10, 14 weeks, 9 and 18 months as well as at 5 years of age. All subjects recruited at birth were neonates born at Chris Hani Baragwanath Hospital. All clinic clients were routinely fully examined by a community health nurse and deemed healthy before appropriate vaccines were given. Subjects were immunized at 6, 10 and 14 weeks of age with oral polio (Poliorol trivalent, Biocine, Italy), hepatitis b (Hepacine, Cheil - Sugar Organization, Korea) and diphtheria – tetanus - pertussis (DTP Merieux –Pasteur Merieux, Lyon France) vaccines routinely. The two injections of 0.5ml were given in either thigh. At birth subjects were immunized with oral polio and BCG. (Japanese strain State Vaccine Institute, Pinelands, Cape Town).

2.3 ENROLLMENT

Permission to do the study was obtained from the Committee for Research on human subjects of the University of the Witwatersrand. Following immunization, infants and their guardians were referred to the study room for recruitment. Verbal informed consent was obtained from

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each guardian using the preferred language of communication. Pertinent epidemiological data were obtained using a questionnaire filled in by the guardian. The nasopharynx of each recruit was sampled using a calcium alginate swab (Calgiswab type 1, Spectrum, USA) inserted through the nostril, using the pernasal route.

2.4 BACTERIOLOGY

Nasopharyngeal swabs were plated directly onto BHB (Oxoid Columbia base, UK) agar followed by blood agar plates (SA vaccines, RSA) with 5% sheep blood containing 5mg/ml gentamicin (Jacobs, et al 1978). All plates were incubated overnight at 35°C to 37°C in 5% CO_2. A presumptive identification of *S. pneumoniae* was based on the morphology of colonies and the presence of α - hemolysis. Confirmatory tests included inhibition by optochin, bile solubility and capsular typing for the quellung reaction using antipneumococcal serum (Statens Seruminstitut, Copenhagen). Five colonies with different morphology were picked, serotyped and tested for antibiotic susceptibility . Susceptibility testing of the antibiotics penicillin, chloramphenicol, tetracycline, cotrimoxazole, erythromycin, clindamycin and rifampicin were determined by disk-diffusion method on 5% sheep blood Mueller Hilton agar plates (5% lysed horse blood used for cotrimoxazole) and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS). Oxacillin discs were used to predict penicillin susceptibility. Isolates exhibiting inhibition zones for any antibiotic were further tested according to the minimum inhibitory concentration (MIC) methodology and interpreted according to the NCCLS criteria. The identification of *H. influenzae* type b was based on satellitism around a staphylococcal streak, a negative α –aminolevulinic porphyrin test and the requirement for both haemin (X factor) and NAD (V factor). The identification was confirmed by serotyping using standard agglutination with type specific antisera (Murex Diagnostics, Dartford UK). Susceptibility to ampicillin,

chloramphenicol and cotrimoxazole was determined by disk- diffusion method using *Haemophilus* test medium and interpreted according to the NCCLS criteria. β–lactamase production was identified using β- lactamase media in which phenol red (0.01% SA Vaccines RSA) was used as an indicator. Resistant strains were identified using antimicrobial impregnated discs, the zone size measured and susceptibility interpreted according to the NCCLS criteria. Antimicrobial susceptibility was confirmed according to minimum inhibitory concentration methodology and interpreted according to the NCCLS criteria. Antibiotic resistance was defined as a minimum inhibitory concentration at or above the intermediate breakpoint. ATCC strains from the College of Pathologists were used for quality control. Multidrug resistance was defined as resistance to three or more of the 6 classes of antimicrobial : cotrimoxazole, erythromycin, chloramphenicol, tetracycline, clindamycin and rifampicin tested.

2.5 STATISTICAL ANALYSIS

The Epi Info statistical package (Version 6, USD, Stone Mountain, USA) was used to analyse the data. The relative risks and their 95% confidence intervals were calculated using the Epi Info version 6. Age adjusted ratios and relative risks were calculated and stratified where possible. Subjects whose ages had not been documented were excluded from the study. All newborns in the study were excluded from any analysis because no bacteria were isolated from the nasopharynges of any of them.

CHAPTER 3

RESULTS

3.2 SOCIODEMOGRAPHIC DATA

Of the 300 children we had hoped to recruit into the study (50 for each scheduled visit), a total of 278 children were enrolled. While the majority of children came within a short range of their scheduled visit, compliance decreased with subsequent visits. The mean age of recruitment was 15 months. The age range of the study subjects was 1 month to 12 years. Of these, 56% (156) were female and 44% (122) male. Sixty per cent (164) had at least one sibling, the majority of whom had a single sibling 62% (112). Only 15% (40) of the children, most of whom were >5 years of age attended a day care center. Information regarding the use of antibiotics was available in 98% (274) subjects. Of these, 20% (55) of parents/guardians gave a history of prior antibiotic use in the child in the past month. Information on hospitalization was obtained in 99% (275) subjects, of whom only 5% (13) had been hospitalized.

3.3 CARRIAGE

Pneumococcal and *H. influenzae* type b carriage rates were 44% (121) and 10% (29) respectively. Five per cent (15) of all the children carried both pneumococci and *H. influenzae* type b. Pneumococcal and *H. influenzae* type b carriage varied between different age groups. Carriage rates for pneumococci were however higher than for *H.influenzae* type b for all age groups. (Table 1). No association was found between *H. influenzae* type b and pneumococcal carriage OR 1.16 (CI = 0.41 - 3.28) p = 0.746.

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Table 1 Carriage of *S. pneumoniae* and *H. influenzae type b* in different age groups

Age Group[a]	*S.pneumoniae*		*H. influenzae* type b	
	Total (%)	Resistance[b](%)	Total(%)	Resistance[c](%)
6 weeks	14/51 (27.5)	6/14 (42.9)	4/51 (7.8)	0/4 (0)
10 weeks	13/45 (28.9)	6/13 (46.2)	1/45 (2.2)	0/1 (0)
14 weeks	26/49 (53.1)	11/26 (43.3)	4/49 (8.2)	0/4 (0)
9 months	33/52 (63.5)	19/33 (57.6)	8/52 (15.4)	2/8 (25)
18 months	23/41 (56.1)	11/23 (47.8)	5/41 (12.2)	2/5 (40)
5 years	12/40 (30.0)	4/12 (33.3)	7/40 (17.5)	2/7 (28.6)

15 children carried both *S. pneumoniae* and *H. influenzae* type b.
(a) indicates time point of clinic visit.
(b) penicillin resistance
(c) antibiotic resistance

3.3.1 *Haemophilus influenzae* type b

3.3.1.1 *H. influenzae* type b resistance patterns

The carriage rate of *Haemophilus sp* was 28% (77), 38% (29) of which were *H. influenzae* type b. The carriage of *H. influenzae* type b varied from 2.2% (1) at the 10 week visit to 17.5% (7) at the 5 year visit (Table 1). Of the 6 isolates of *H. influenzae* type b with decreased susceptibility to antibiotics; 3 (10.3%) and 4 (13.8%) were resistant to ampicillin and cotrimoxazole respectively. Only 1 (3.4%) of the isolates had decreased susceptibility to chloramphenicol. Two isolates had decreased susceptibility to ampicillin and either chloramphenicol or cotrimoxazole (Table 2). No β–lactamase activity was reported in any of the ampicillin-resistant isolates identified .

3.3.1.2 Risk factors for *H. influenzae* type b carriage.

There was no significant association between the number of siblings and carriage of *H. influenzae* type b. Neither the age nor attendance at a day care center were significant risk factors for carriage. Hospitalization was also not found to be a significant risk factor for carriage. There was no significance detected regarding the reported use of antibiotics one month prior to nasopharyngeal sampling and *H.influenzae* type b carriage.

3.3.2 Streptococcus pneumoniae

Pneumococci were isolated from the nasopharyngeal swabs of 43% (121) children who had presented for routine immunization at the clinic and were recruited into the study. Carriage rates at 6,10, 14 weeks; 9 and 18 months and at the 5 year time points were 27.5%, 28.9%, 53.1%, 63.5%, 56.1% and 30.0% respectively (Table 1).

3.3.2.1 Pneumococcal Resistance Patterns

The overall rate of penicillin resistance was 36/121 (29.7%); that of cotrimoxazole 35/121 (28.9%) and tetracycline 19/121 (15.7%). Erythromycin resistance was found in 9/121 (7.4%) of all isolates and rifampicin resistance in 1/121 (0.8%) (Table 2). Only six percent (7) isolates were sensitive to all 5 classes of antibiotics tested. Single drug resistance was found in 27 (22.3%) of isolates. Of these resistance to cotrimoxazole was the most frequent 17 (14%) followed by penicillin 7 (5.8%) and tetracycline 3 (2.5 %).

Table 2 Carriage of antibiotic resistant strains of *S. pneumonae* and *H. influenzae* type b in Zola / Emndeni children.

		S. pneumoniae	*H. influenzae* type b
		No (%)	No (%)
Positive cultures		121	29
Resistance to	Pen/Ampicillin	36 (30)	3 (10)
	Cotrimoxazole	35 (29)	4 (14)
	Chloramphenicol	6 (5)	1 (3)
	Erythromycin	9 (7)	
	Tetracycline	19 (16)	
	Clindamycin	8 (7)	
	Rifampicin	1 (1)	

Of the penicillin resistant pneumococci, 29 (24.0%) were resistant to at least one other class of antibiotic. Fifteen (12.4%) of all isolates resistant to penicillin were also resistant to 2 other classes of antibiotics. Multidrug resistance was identified in 16 (13.2%) of all pneumococcal isolates (Table 3).

The serotypes of the penicillin resistant strains were 4, 6A, 14, 19F, 23F. Serotypes 6A and 19F accounted for 27/36 (75%) of all penicillin resistant isolates. Seventeen (14.0%) of penicillin resistant isolates were serotype 6A. Thirty five (37.1%) and 19 (15.7%) of all isolates were resistant to cotrimoxazole and tetracycline respectively. (Table 2). Of the 16 multidrug resistant isolates identified, 7 (43.8%) were of the 6A serotype; 4 (25.0%) of serotype 19F; 2 (12.5%) of serotype 23F and 3 (18.8%) of serotype 14 (Table 3.) *In vitro* susceptibility showed that the majority 34 (94%) of penicillin resistant pneumococci had intermediate resistance. (Table 4). All isolates resistant to erythromycin and tetracycline had

high level resistance. The one isolate resistant to rifampicin was also resistant to 3 other drugs. (Table 3). Of the globally resistant isolates; serotypes 4, 14, 15, 34, 6A,19F and 23F were identified. Rifampicin resistance was identified in a single isolate (0.8%) that happened to be multiple resistant. (Table 3).

3.3.2.2 Pneumococcal Serotypes

Five colonies of pneumococci of different morphology were picked off, identified and serotyped. Multiple serotypes were isolated from 2.9% (8) children. These were not restricted to specific age groups. (Table 5). Serotype 6A was the most common serotype isolated from children carrying pneumococci. The predominant serotypes with >10 isolates were 6A, 19F and 23F. Isolates of serotypes 19F, 23F, 4 and 14 account for 43.8% of the serotypes in the 9 valent pneumococcal conjugate vaccine. If cross reactivity of 6A and 6B is considered, vaccine coverage increases to 73.6% (Table 6).

Table 3 Antibiotic resistance patterns and serotypes of *S. pneumoniae* nasopharyngeal isolates from Zola / Emndeni children.

Pattern of resistance	No. of isolates (n = 57)							
	Serotypes							Total
	6A	19F	23F	4	14	15	34	
Pen	3	3		1				7
Cotr	8	3	3	1		1	1	17
Tet					2	1		3
Pen, Cotr	7	4		3				14
Pen, Chlor, Tet		1						1
Pen, Tet, Cotr		1						1
Pen, Chlor, Tet, Rif		1						1
Tet, Ery, Clind, Cotr		1						1
Pen, Tet, Ery, Cotr			1					1
Pen, Chlor, Tet, Cotr			1		3			4
Pen, Tet, Ery, Clind,Cotr	7							7

Pen – Penicillin
Cotr – Cotrimoxazole (Trimethoprim – Sulfamethoxazole)
Tet – Tetracycline
Ery – Erythromycin
Chlor – Chloramphenicol
Rif – Rifampicin
Clind – Clindamycin

Table 4 In vitro susceptibility of nasopharyngeal isolates of *S. pneumoniae* with decreased susceptibiiity.

Antimicrobial Agent	No. of isolates inhibited		Range of MIC'S[1]
	Intermediate	High	
Penicillin	34	2	0.120 – 4.00
Erythromycin	0	9	1. 00 – 64.00
Clindamycin	0	8	32.00 – 4.00
Chloramphenicol	-	6	8.00 – 16.00
Tetracycline	0	19	8.00 – 64.00
Rifampicin	0	1	8.00
Cotrimoxazole	14	21	1/19 - 8/152

1. Minimum inhibitory concentration based on NCCLS standards.

Table 5 Nasopharyngeal carriage of multiple serotypes from Zola / Emndeni children in Soweto.

Age	Serotypes
4 months	4, 19F
4 months	6A, 19F
4 months	23F, 21
10 months	6A, 15
16 months	18, 23F
20 months	6A, 24
6 years	34, 24

Table 6 Pneumococcal serotypes isolated from the nasopharynx of Zola / Emndeni children.

Rank	Serotype	No. (%) n = 121
1	6A	36 (29.8)
2	19F	25 (20.7)
3	23F	11 (9.1)
4	4	9 (7.4)
5	14	8 (6.6)
6	15	7 (5.8)
7	21	4 (3.3)
8	34	4 (3.3)
9	11	3 (2.5)
	other*	14 (11.5)

Above represent pneumococci isolated at initial sampling.
* other serotypes encountered were 1, 7, 16, 8, 10, 20, 20, 29 and 9.

3.3.2.3 Risk factors for pneumococcal carriage with antimicrobial resistant pneumococci.

The odds of carriage were 2.18 times higher in children less than 2 years of age than children 2 years and older (P = 0.04). The odds of carriage were 1.92 times higher in children who had siblings than in children who did not (p = 0.01). The adjusted odds from multivariate analysis (unconditional multiple logistic regression model) for age was 2.5, siblings 2.2 and had a p value < 0.05. There was no association between the use of antibiotics prior to nasopharyngeal sampling or hospitalization and carriage. Gender and attendance at a day care center were also not significantly associated with carriage. Carriage of resistant pneumococci was not associated with antibiotic use, gender, age, attendance of day care centers or

hospitalization. The odds of carrying resistant pneumococci were 2.67 times higher in carriers with siblings than those without (CI = 1.06 - 6.82) $p<0.05$. There was no significant difference in carriage of the penicillin-resistant pneumococci in children <2 years (48/91) 52.7% and those ≥2 years (8/25) 32% OR 2.37 (CI=0.85 – 6.73) p=0.06.

3.3.2.4 Pneumococcal carriage in twins.

Six sets of twins were enrolled in the study. In two of these fully sensitive pneumococci were isolated from one twin in each set. In one set, neither twin carried pneumococci or *H. influenzae* type b. In two other sets both twins carried multiply resistant pneumococci, one twin of which also carried *H. influenzae* type b. Two sets of twins had subsequent visits although the interval between sampling times was not known.

CHAPTER 4

DISCUSSION

One hundred and eighty five (66.5%) of all children entered into the study were <1 year of age and 227 (81.7%) <2 years of age. The majority of subjects were from households with one sibling. Of the subjects entered into the study, 60% had one sibling. Information regarding antibiotic use was obtained in 98% of subjects however it is not clear if guardians were sure that the medication given for conditions such as mild upper respiratory infections was symptomatic or antibiotic therapy. Most guardians were aware of the possibility of hospitalization of the subjects, although hospitalization was rare in this group.

The carriage of pneumococci and *H. influenzae* type b in these infants was 43.5% and 10.4% respectively. Five percent of the children carried both pneumococci and *H. influenzae* type b. No association was found between carriage of pneumococci and *H. influenzae* type b. This study found that the carriage rates of *Haemophilus* species and *H. influenzae* type b were 28% and 10.4% respectively. The *H. influenzae* type b results were comparable to other surveys done in healthy children which showed carriage rates of 25-80% for all *H. influenzae* and 2-15% for *H influenzae* type b. (Hussey 1994, Mpairwe 1970, Moxon 1991). In a study performed in healthy Cape Town children, carriage rates were 46% and 5% for all *H. influenzae* and for *H. influenzae* type b respectively. The geographic and seasonal variation in carriage of *Haemophilus influenzae* type b has been described and may account for this slight variation noted (Cochi and Ward 1991). The season of sampling in the Cape Town study was not stated. Antibiotic resistance in these healthy children was higher than found in the Cape Town study. (Hussey et al 1994). Resistance to ampicillin/penicillin and cotrimoxazole was 10.3% and 13.7% respectively. In the cohort of healthy children Cape Town study, 4% and 0% of the *H. influenzae* type b isolates were resistant to ampicillin/penicillin and cotrimoxazole respectively. The empiric treatment of invasive *H. influenzae* type b disease

should take these findings into consideration. In none of the children presenting at Zola Community Health Center (ZCHC) were erythromycin resistant *H. influenzae* type b strains identified. This is probably an artifact. No risk factors could be detected for carriage of *H. influenzae* type b in young healthy children at ZCHC. The carriage study done in Kampala, Uganda suggested that *H. influenzae* type b was not readily transmitted even in groups of children with high carriage rates (Mpairwe 1970). Observations have been made that increased transmission probably occurs under conditions of overcrowding, in the presence of a multiplicity of strains and also under conditions where high carriage rates prevail (Leach et al 1994).

There were nevertheless significant rates of carriage of *H. influenzae* type b susceptible and resistant to antimicrobials commonly prescribed at primary health care centers. Coupled with the high incidence of invasive disease documented in a study performed at Cape Town; the above warrant the incorporation of *H. influenzae* type b conjugate vaccines into the routine Extended Programme on Immunization schedule of young children in South Africa (Hussey 1993). Numerous studies have demonstrated the safety and efficacy of haemophilus polysaccharide vaccines conjugated to different protein carriers. Furthermore, other studies have demonstrated a resolution in oropharyngeal carriage of *H. influenzae* type b in children receiving the *H. influenzae* type b conjugate vaccines. (Takala 1991 Barbour 1995). It is thus expected that these vaccines would also reduce the prevalence of *H. influenzae* type b disease and induce herd immunity. This appears to be the case as the incidence of invasive disease in industrialised countries dropped rapidly following immunisation. (Robbins et al 1996, Ward 1994). A reduction in oropharyngeal carriage of *H. influenzae* type b has also been noted in children immunised with Hib conjugate vaccines (Takala et al 1991, Barbour et al 1995). It has been suggested that the herd immunity induced by the conjugated Hib vaccines is primarily due to a reduction or delay in the initial acquisition of Hib (Barbour et al 1995).

Indeed, if the ability to reduce mucosal colonization is a general property of conjugate vaccines, pneumococcal vaccines now being developed may not only prevent invasive disease but also mucosal infections such as otitis media, sinusitis or bronchitis. This study confirms the high rate of nasopharyngeal colonization by pneumococci in healthy children and infants ≤2 years of age. While it is apparent that colonization of infants occurs early (27.5% at 6 weeks of age), it peaked at 9 months (63.5%) and was still high at 18 months (56.1%). Similar findings were reported by Leach and co-workers in a study done in Australia. (Leach et al 1994).Colonization with *S. pneumoniae* and *H. influenzae* type b in aborigional children in Australia was found to occur as early as 10 and 20 days respectively. In addition, an association was found between early colonization and early onset of acute otitis media. Although otitis media and colonization were both age related, the onset of otitis media was better explained by colonization than by age (Leach et al 1994).

Forty three percent (121) of all healthy children recruited into the study carried pneumococci, of which 43% were antibiotic resistant pneumococci (18% of the total). In a recent study carried out in the USA, 44% of healthy infants ≤6 years of age carried pneumococci and 37% of all isolates were resistant to penicillin (Zenni 1995). Similar findings were reported in a study carried out in Israel (Yagoupsky et al 1998). An earlier study done in Soweto in 1986 at 3 day care centers and a children's home in which children <5 years were sampled reported an average carriage rate of 14% of resistant pneumococci. In that study children at the orphanages alone were carriers of pneumococci in 31% of the cases. (Klugman et al 1986). Following worldwide trends this rate is likely to be higher today. Carriage rates in day care centers have been shown to be much higher than in children not attending day care centers (Klugman et al 1986). The significance of carriage of pneumococci in the nasopharynx is that resistance rates may correlate with clinical disease, if open communities are chosen for sampling. (Ratetsky et al 1981).

In a study done in neighbouring Lesotho, pneumococcal carriage in children in all age groups was 62% in rural children and 56% in urban children; rates higher than those reported in this study and data from the United States of America (Doyle et al 1992). The differences were however not statistically significant. Carriage in children ≤36 months was however significantly higher than those>36 months for rural children (Mthwalo et al 1998). The frequency of penicillin resistance among children in the rural areas and in Maseru in neighbouring Lesotho was 2.5% and 6.4% respectively values lower than in South Africa (Mthwalo et al 1998). Penicillin resistance was detected in 18.7% of nasopharyngeal isolates in our study. Interestingly, despite the significant difference in carriage rates of Sowetan and urban Basotho children, the risk factors for carriage of penicillin resistance pneumococci in both groups were comparable. Prior antibiotic use was reported in 20.1% and 21.9% of Sowetan and urban Basotho children respectively; prior hospitalisation in 4.7% and 7.1% and day care center attendance in 14.5% and 19.9% respectively. In addition, antibiotic resistant serotype 23F strains were not encountered in the Lesotho study (Mthwalo et al 1998). These results suggest that children in Soweto are exposed to resistant clones that have been established over a long period of time. While more Basotho children are hospitalised in Maseru, this is probably of short duration. The history of prior antibiotic use has also been shown not to be entirely reliable as it depends on the recall of possibly unfamiliar terms by guardians.

The relatively resistant pneumococcal serotypes isolated in this study are responsible for the majority of serious pneumococcal diseases in children in Soweto and South Africa. (Koornhof, 1993).

The carriage of pneumococci has been correlated with the emergence of clinical disease (Hodges and Maclead 1946, Istre et al 1987). Factors affecting carriage of pneumococci include the age of the subject, season of the year, socioeconomic factors and climate. Of

these, age is probably the most important. Numerous studies have shown that young children are most likely to carry pneumococci (Gray et al 1980) Gray and colleagues have in different studies shown that both age and climate are important epidemiological factors affecting carriage of pneumococci. Their detailed studies followed Alabaman children from birth to 2 years of age and were able to document that the mean age of acquisition of carriage was 6 months. The age at which these children first acquired these pneumococci varied from 4 days (Gray 1980) to 18 months. In other studies done in North America, carriage rates declined from 50% in children 2 years of age to 30% in 7-year old children. Gray also showed that acquisition and carriage rates were higher during the winter. It is not clear whether the weather *per se* directly affects carriage or whether close proximity associated with being indoors in cold weather increases the likelihood of spread. High carriage rates were also encountered among the aborigines in Australia with it's hot temperate climate and low humidity (Hansman 1985) as well as children in Papua New Guinea with it's tropical climate throughout the year. While further information is required on this aspect of pneumococcal carriage, it is likely that the extent of crowding in living conditions is the important factor in carriage rates. Of the pneumococcal serotypes isolated in this present study, groups 6, 19 and 23 were the most predominant. They accounted for 60% of all isolates. These serotypes are some of the most common types of pneumococci isolated from children admitted to hospital with severe illness (Crewe Brown et al 1997).

Type 6,19 and 23 which are commonly carried by Soweto children are the types which are also commonly carried amongst children in North America, Australia and Israel (Mbelle et al 1997, Gray et al 1990, Hansman et al 1985, Yagupskey et al 1998). These serotypes also tend to be those that have decreased susceptibility to antimicrobials. Clustering of decreased susceptibility to antimicrobial drugs among a few pneumococcal serotgroups has been noted worldwide (Klugman et al 1994, Dagan et al 1994). These serotypes are also carried for

prolonged periods by young children increasing the chances of development of further resistance and increasing the likelihood of spread to susceptible individuals. Of the 7 most prevalent serotypes isolated in the United States of America and included in a 7 valent vaccine world wide (4, 6B, 9V, 14, 18C, 19F, 23F), 44% were isolated from the nasopharynx of Zola / Emndeni children. (Sniadack et al 1995). 45% would be present in a 9 valent vaccine containing the additional serotypes 1 and 5 and which would be studied in Soweto children (Mbelle et al in press) . Serotypes 1 and 5 that are included in the 9 valent candidate vaccine to make it globally relevant were rarely isolated from the nasopharynges of the children in this study. Both these serotypes are known to be invasive but rarely colonise the nasopharynx. Serotype 5 was not isolated at all while types 15, 21, 24 and 11 were isolated 6%, 3%, 3% and 2.5% of the time. Other than serotype 15, these types are rarely isolated from the blood and CSF of South African children admitted to hospital with severe disease (Wasas 1998). Similar findings were obtained with type 11 and 15 in Australian children (Hansman 1988). Thus, whilst there is some concordance between common disease types and carrier types there are several notable exceptions.

The risk of carrying penicillin resistant pneumococci was higher in children younger than 2 years of age than those older. These results correlate well with those done by other workers (Dagan et al 1996, Munford et al 1994). The odds of carriage are 1.92 times higher in children with siblings compared to those without OR 1.92 (CI = 1.13 - 3.29 p= 0.01) a result confirmed by other researchers (Dagan et al 1996). Transmission within households is well documented. The introducer of a new serotype into a household is usually a child (Hendley et al 1975). In our study there was no association between the use of antibiotics prior to nasopharyngeal sampling and carriage. These results are at odds with those obtained by several researchers in South Africa, Israel and the United States of America (Klugaman et al 1986Arnold et al 1996, Yagupsky et al 1975). It is possible that the subjects may have

received symptomatic treatment for mild upper respiratory tract infections and not antibiotics as stated by the guardian in the questionnaire. Prior antibiotic use has been shown to be a significant risk factor for carriage of antibiotic resistant pneumococci (Arnold et al 1996). Our results showing the lack of correlation between carriage of resistant pneumococci and attendance at a daycare center are not consistent with other reports (Arnold et al 1996, Yagupsky et al 1998). This is probably because of the low numbers of day care attendees in younger children in this population. In addition, it is likely that fewer resistant clones are in circulation in Soweto than communities in America, Spain and Israel.

Serotypes 4, 14, 19F and 23F found in the CRM_{197} nonavalent pneumococcal vaccine accounted for 50.9% of the globally resistant isolates and 52.8% of the penicillin resistant isolates in this study. Serotype 6A accounted for 43.9% of the globally resistant isolates and 47.2% of the penicillin resistant strains. Isolates from 54 (45%) of the children were serotypes present in the CRM_{197} nonavalent pneumococcal vaccine. Since cross reactivity of subtypes 6A and 6B is expected, serotype 6A would increase the vaccine coverage of these serotypes to 90 (74%). The 5 top ranked serotypes in this study would thus represent 89 (74%) of the total. Serotype 1 was ranked beyond 9 and serotype 5 was not isolated at all. Recent data however suggest that cross reactivity between serotype 6A and 6B may not occur. The data suggested that a 9-valent conjugate vaccine containing serotype 6B was unable to decrease the carriage of serotype 6A in recipients of a 7V conjugate vaccine containing serotype 6B (Mbelle et al in press). The patterns of pneumococcal serotypes isolated from the nasopharynges would need to be monitored to ensure the introduction of appropriate polyvalent pneumococcal conjugate vaccines as part of the Expanded Programme on Immunisation in South Africa

CHAPTER 5

CONCLUSION

Very few data were available of the carriage of *S. pneumoniae* and *H. influenzae* type b in asymptomatic young children in South Africa. This study showed that a significant proportion of children carried both susceptible and resistant organisms in their nasopharynx. The majority of the isolates were serotypes / serogroups normally found in both the nasopharynx and sterile fluids of children. The majority of these would also be found in the 7 valent pneumococcal conjugate vaccine recently shown to be efficacious in preventing pneumococcal bacteraemia (Black et al 1998) . Since serotypes 6A and 6B have an identical chemical composition and are thus likely to be immunologically cross reactive , a 9 valent pneumococcal conjugate vaccine containing serottpe 6B is likely to prevent pneumonia and meningitis caused by the pneumococcus . The exclusive isolation of serotype 6A in this study is puzzling. It may demonstrate the variation in colonization of pneumococci with time. While 6B is commonly isolated currently from blood, 6A was more prevalent in the 1980's. The picking of 5 colonies from a plate based on differing colonial morphology may also lead to errors of interpretation of the predominant serotypes present in the nasopharynx at that time, a feature well described in the literature (Gray et al 1980).

Techniques such as the dot blot may prove more sensitive a tool in the detection of the pneumococcal serotypes cultured from the nasopharynx of sampled children. The ability of this assay to identify the simultaneous carriage of different pneumococcal serotypes in a more objective manner may put to rest the unmasking debate.

Surveillance of pneumococcal isolates referred to the SAIMR from major teaching hospitals in South Africa showed that the major strains resistant to penicillin belonged to serovar 6A as did the initial multiply resistant isolates encountered in Durban in 1978 and briefly in Johannesburg in1998. Serotype 6B is now more predominantly identified than serotype 6A

(Wasas et al 1998). Whether this is due to replacement of serotype 6A by 6B or the unmasking of 6B by factors not known, is not clear.

The results obtained in this study confirm that carriage of resistant pneumococci as well as *H. influenzae* type b in this community is significant. The imminent introduction of conjugate *H. influenzae* type b as part of the EPI programme in South Africa is thus well justified by these results obtained from healthy Soweto infants presenting for routine immunisation. A 9 valent (1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F) pneumococcal vaccine trial comprising most serotypes which were commonly isolated from the nasopharynges of healthy children would hopefully reduce carriage and ultimately the incidence of invasive disease. Such an intervention would thus impact on clinic visits as well as hospital admissions.While replacement in the nasopharynx by non vaccine serotypes has been shown (Mbelle et al 1997) it is hoped that the incidence of pneumonia in vaccinated children will be nevertheless significantly reduced.

CHAPTER 6

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APPENDIX

Subject information sheet

Work sheet with sociodemographic data

Arial view of Greater Soweto

Ethics committee approval

Subjects Information Sheet and Verbal Consent Form

I am Nontombi Mbelle

I am a medical doctor attached to the South African Institute of Medical Research and the University of the Witwatersrand.

Young children can become ill from infections caused by many germs including the bacteria *S pneumoniae* and *H. influenzae* type b. These bacteria can cause mild diseases such as sore throat or ear-ache. Sometimes they cause severe diseases such as infection of the brain (meningitis) and infection of the blood (septiceamia). These bacteria can be found in the throat of healthy children and can be spread from person to person.

The purpose of this study is to find out how commonly these germs are found in the throats of healthy children coming to this clinic for normal immunisation. If you agree to enter the study, I will be able to see how common these bacteria are in the throats of children. I am also hopeful that at the end of this study, I will have found out more about the factors that influence carriage of these bacteria in the throats of children.

If you agree to participate in this study, you will answer some questions about your child and your home. I will also put this swab through your childs nose to see if any of these bacteria are present. This may be a little uncomfortable for your baby.

All information is confidential. The information collected will be available to you, the community through the Faculty of Health, University of the Witwatersrand.

Your participation in this study is voluntary. Your refusal to participate will not in any way affect how you or your child are treated at the clinic.

Date:_____ Guardian:_____

Hospital:_____ Diagnosis:_____

Patient number:_____ Weight:_____

Patient name:_____

Age:_____

Sex:_____

Antibiotics in past month (type/duration):_____

Hospitalization (duration/diagnosis):_____

Reason for current attendance:_____

Day care center attendance:_____

Children in family (under 5 yrs):_____

Pneumo				Haemophilus		
Growth:	Y		N	Bld Agar	Y	N
Number of clones picked:	1	2	3	Bl+B.	Y	N
Stored Robertson's	Y		N	Stored.	Y	N
Optochin	S		R	Sens	Y	N
Bile solubility	Not done		Y	Beta Lactamase	Y	N
Stored -70°C	Y		N	Ala.	Y	N
				Serotype B	Y	N

	Disc Content	Pneumo S	I	R	Zone Diam. mm	Interpretation (Susceptible = S, Intermediate = I, Resistant = R)	E TEST PN MIC
Oxacillin	1ug	> 20	13 - 19	< 13		Ampicillin ≥18 14-17 ≤13 (Penicillin)	
Chloramphenicol	30ug	> 19	15 - 19	< 14		+ ≥25 21-24 ≤20	
Tetracycline	30ug	> 19	14 - 19	< 15			
Erythromycin	15ug	> 20	16 - 20	< 16			
Clindamycin	2ug	> 21	19 - 21	< 19			
Rifampicin	5ug	> 20		< 20			
Other (Specify)							
Cotrimoxazole	25ug	≥ 19	16-15	≤15		+ ≥16 11-15 ≤10	
Amox.							

☐ Fully Sensitive.

☐ Resistant to Pen, Chlor, Tet, Eryth, Clinda, Rif. .....

☐ Serotype: ..... Untypable / Disintegrated / Not typed

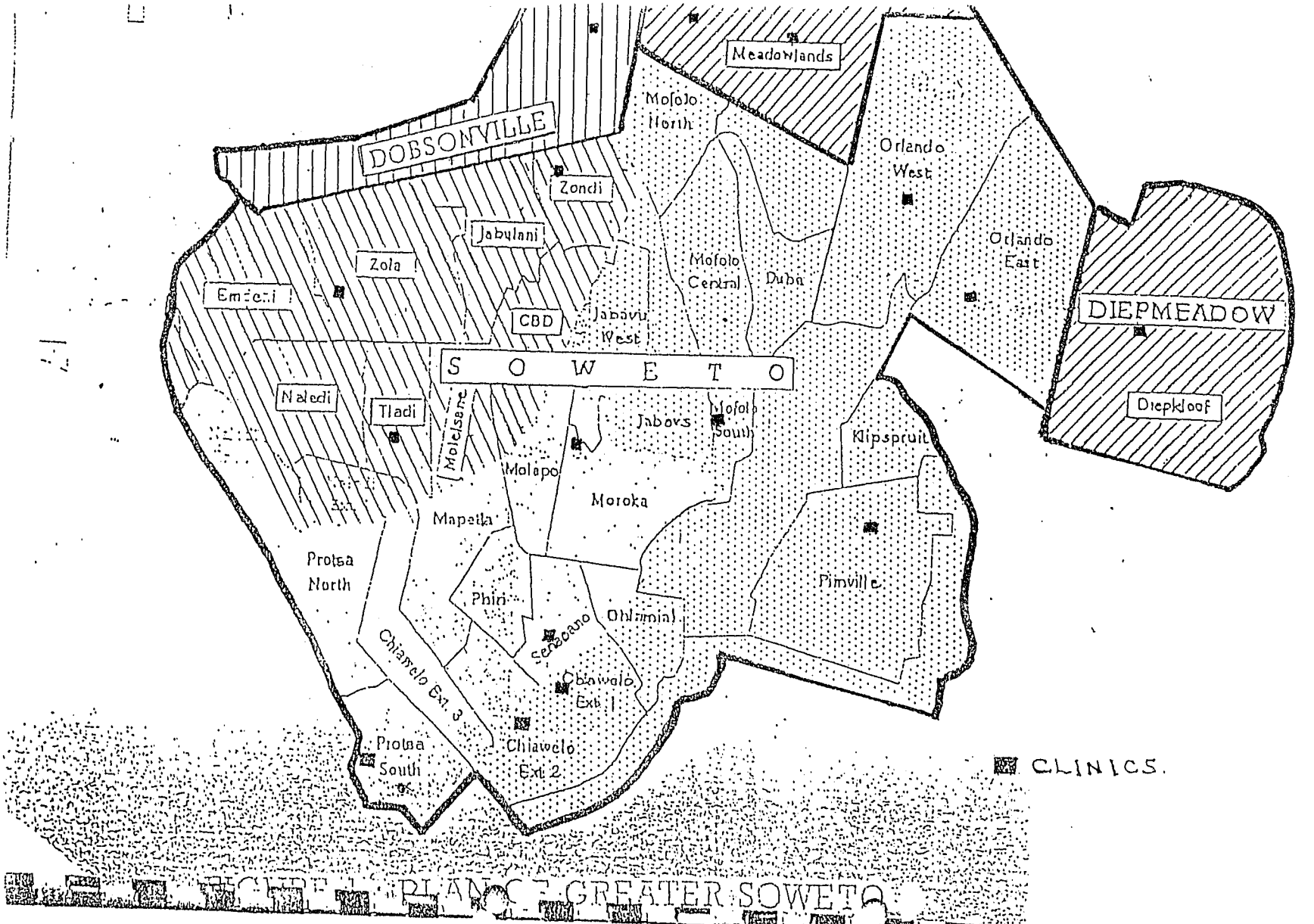


FIGURE 1 PLAN OF GREATER SOWETO

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Mbelle

CLEARANCE CERTIFICATE PROTOCOL NUMBER M 970614

PROJECT	Streptococcus pneumoniae and haemophilus influenzae type B carriage in infants presenting to Zola Community Health Centre for routine immunization
INVESTIGATORS	Dr N M Mbelle
DEPARTMENT	Medical Microbiology, SAIMR
DATE CONSIDERED	970627

DECISION OF THE COMMITTEE

Approved unconditionally

DATE 970627

CHAIRMAN. ..... pp. (Professor P E Cleaton-Jones)

c c Supervisor: Professor K Klugman
Dept of Medical Microbiology, SAIMR

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE....7/7/97.....SIGNATURE .....

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Author Mbelle N M
Name of thesis Streptococcus Pneumoniae And Haemophilus Influenzae Type B Carriage In Infants Presenting To Zola Community Health Centre For Routine Immunization Mbelle N M 1999

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